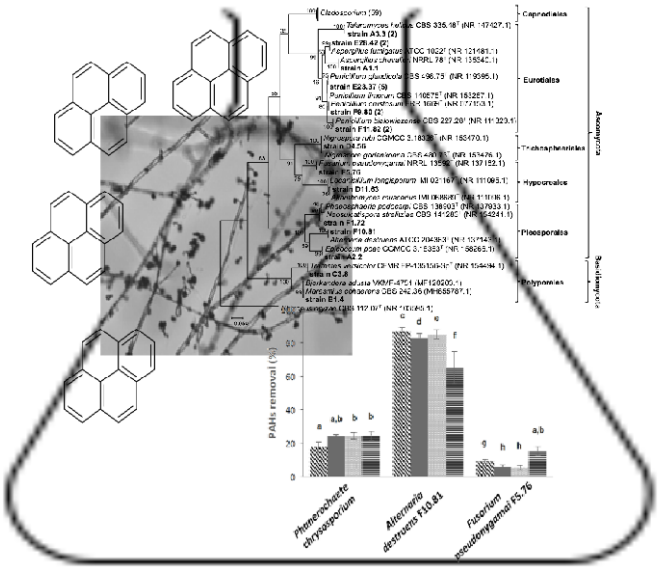
Cultivable diversity of PAH-removing fungi



Highlights

- PAH-removing cultivable fungi diversity revealed in PAH-contaminated marine sediments
- *Alternaria destruens* F10.81 strain exhibited highest capacity for removing PAHs
- Homogeneous pyrene internalization in *A. destruens* F10.81 strain hyphae

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

Joyce Álvarez-Barragán[1], Cristiana Cravo-Laureau[1], Lukas Y. Wick[2], Robert Duran[1*]

[1]Université de Pau et des Pays de l'Adour, UPPA/E2S, IPREM UMR CNRS 5254, Bat. IBEAS, Pau, France

[2]Helmholtz Centre for Environmental Research - UFZ, Department of Environmental Microbiology, Leipzig, 04318, Germany

*Corresponding author: Robert Duran, Université de Pau et des Pays de l'Adour, E2S/UPPA, IPREM UMR CNRS 5254, BP 1155, 64013 Pau Cedex, France. E-mail: robert.duran@univ-pau.fr, tel: (+33)559407468.

Abstract

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

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

Introduction

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

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

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

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

Material and Methods

Culture media

The culture media used in this study were based on the seawater minimal medium (swMM; Brito et al., 2006), which composition was as follow: KCl 0.75 g/L, $CaCl{\cdot}2H_2O$ 1.47 g/L, NH_4Cl 1.5 g/L, $MgSO_4{\cdot}7H_2O$ 6.64 g/L, NaCl 20 g/L, Na_2CO_3 0.265 g/L, 1 mL of trace elements solution (H_3BO_3 300 mg/L, $FeSO_4{\cdot}7H_2O$ 1.1 g/L, $CoCl_2{\cdot}6H_2O$ 190 mg/L, $MnCl_2{\cdot}2H_2O$ 50 mg/L, $ZnCl_2$ 42 mg/L, $NiCl_2{\cdot}6H_2O$ 24 mg/L, $Na_2MoO_4{\cdot}2H_2O$ 2mg/L), 1mL of vitamin solution (biotine 2 mg/L, ρ-aminobenzoate 10 mg/L, thiamine 10 mg/L, pantothenate 5 mg/L, pyridoxamine 50 mg/L, vitamin B_{12} 20 mg/L, nicotinate 20 mg/L), and 100 µL of phosphate buffer 50 mM. The pH was adjusted with HCl to 6.5. Chemicals were purchased from Sigma Aldrich (Germany).

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

Selection and conservation of fungal strains

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

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

Fungi identification sequencing and phylogenetic analysis

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

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

Fungal tolerance to PAHs

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

Fungal PAHs removal rates

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

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

PCR detection of peroxidase and laccase genes

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

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

PAHs internalization and transport

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

Results and discussion

Identification of fungal strains Isolated from coastal sediments

In total, 85 fungal strains were isolated from PAHs contaminated coastal sediments in seawater media containing malt dextrose agar (swMDA). The strains were identified with the complete ITS sequence (including ITS1, 5.8S rRNA gene, and ITS2 regions), which provide accurate identification of fungi species even at the subspecies level (Fajarningsih, 2016). The phylogenetic analysis showed that 83 strains belong to the Ascomycota Phylum and two strains belong to the Basidiomycota Phylum (Fig. 1 and 2). Such result was not surprising since fungi belonging to Ascomycota have been found prevalent in marine sediments (Babu et al., 2010; Birolli et al., 2018; Ravelet et al., 2000) and other environments (Reyes-César et al., 2014). The 85 fungal strains fall into six different Orders: Capnodiales (59 strains), Eurotiales (14 strains), Trichosphaeriales (1 strain), Hypocreales (2 strains), Pleosporales (7 strains) and Polyporales that belong to Basidiomicota Phylum (2 strains).

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

All the Capnodiales were affiliated to two complexes of the *Cladosporium* genus (Fig. 2) defined by a multi-locus phylogeny (Schubert et al., 2007). Among the Cladosporioides complex, the isolated strains were affiliated to species known to be associated with human and animals diseases such as *C. crousii*, *C. welwitschiicola*, *C. austroafricanum*, *C. pini-ponderosae*, and *C. puyae* (Sandoval-Denis et al., 2016), and with marine organisms such as *C. colombiae* (Ravi Theja and Chandra, 2020). Similarly, the isolated strains belonging to the Herbarum complex, *C. rhusicola*, *C. subcinereum*, *C. angustiherbarum* have been described involved in human and animals infections (Sandoval-Denis et al., 2016), while *C. allicinum* was found associated with marine organisms (Poli et al., 2020; Bovio et al., 2019) and several strains related to *C. herbarum* have been described for their ability to degrade PAH in marine sediment (Marco-Urrea et al., 2015; Xiao et al., 2020). Noteworthy, the strain D16.68 is the more distant from *Cladosporium* species (Fig. 2) suggesting that it might represent a novel species within the *Cladosporium* genus, but further phylogenetic analysis based on multi-locus are

234 required to elucidate the taxonomic position. Although *Cladosporium* has been already reported in
235 saline environments (Zalar et al., 2007), in hydrocarbon contaminated sediments (Ravelet et al.,
236 2000) showing as well resistance to metals (Shao and Sun, 2007), it was surprising to obtain mainly
237 strains of this genus. It is likely that members of the *Cladosporium* genus are well adapted to the
238 culture conditions imposed during the screening procedure. The cultural approach owns some
239 limitations. Indeed, the development of conidia is controlled by different factors (Tan et al., 1995),
240 such as the presence of PAHs (Zafra et al., 2015), influencing the selection of cultivable strains. In
241 order to overcome such limitations, the application of different culture conditions will enlarge the
242 diversity of isolated strains.

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

251

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

253 Most of the isolated fungal strains (54 strains, 64%) were able to grow in the presence of at least
254 the presence of one PAH showing their tolerance to hydrocarbons (Fig. 3). Among them, 61%
255 tolerate the presence of benzo[*a*]pyrene, 52% pyrene and 45% fluoranthene. Few fungal strains
256 were able to develop in presence of phenanthrene either alone (19%) or in mixture with other PAHs
257 (14%). Similar results showing high tolerance of fungal strains to pyrene, and low tolerance to
258 phenanthrene and PAHs mixture have been reported in the same range of concentrations (Lee et
259 al., 2014). Toxic effects on fungal growth have been observed with phenanthrene (Lisowska, 2004)
260 and metabolites produced from PAHs mixture (Lundstedt et al., 2003). Interestingly, the tolerance
261 capacity is consistent with the phylogeny (Fig. 3), the members of the same Order showing similar
262 tolerance patterns. Noteworthy, the two groups Cladosporioides and Herbarum within the
263 Capnodiales Order showed distinct tolerance capacities, which further support the classification into
264 two distinct groups.

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

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

In order to further characterize the genetic PAH degradation potential of the isolated fungal strains, the presence of genes encoding manganese peroxidase (MnP), lignin peroxidase (LiP) and laccase (Lac), known to participate in the degradation of PAHs (Ghosal et al., 2016), was examined in their genomes by PCR (Fig. 4). All strains posses at least one of these genes, the LiP2 being the most distributed (82/85 strains, 96%) among the isolated fungal strains (Fig. 4). Noteworthy, when the LiP2 gene was not present, the strain possessed the MnP2 gene. Almost all strains (80/85 strains, 94%) possessed at least a manganese peroxidase gene, MnP2 gene being the most detected (75/85 strains, 88%). Such results were not surprising since most of the peroxidase enzymes are known to be produced in marine environment (Bonugli-Santos et al., 2015). Surprisingly, the Lac gene, found in many marine fungal species (Ben Ali et al., 2020; D'Souza-Ticlo et al., 2009), was detected in only 4 strains, which exhibited the most genetic potential possessing more than 5 of the targeted genes. However, since various types of Lac genes have been described in fungi (Moreno et al., 2017; Yang

et al., 2016), the primers used to detect the presence of Lac genes are probably not well suited for recovering the entire Lac gene diversity. The use of primers targeting broader Lac gene diversity or targeting at least Lac gene detected within the Ascomycota phyla (the major phyla of the isolated strains) is required to better define the presence of Lac genes in the isolated strains. Similar observations can be drawn for the LiP and MnP genes indicating that further efforts are needed for in depth characterization of the genetic potential of the isolated strains.

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

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

The removal capacity of *Alternaria destruens* F10.81 and *Fusarium pseudonygamai* F576 was compared with that of *Phanerochaete chrysosporium*, which is the most studied fungi for PAH-degradation (Cao et al., 2020). It serve often as control fungi even for comparing PAH-removal capacity from strains belonging to different phyla (Cao et al., 2020), because it exhibit the capacity

to degrade a broad range of organic compounds (Deschler et al., 1998; Duran et al., 2002), including several PAHs (Pointing, 2001). Under our conditions *P. chrysosporium* presented low rates of PAHs removal (< 30%), just above to that exhibited by *F. pseudoygamai* F5.76 and around 3 times less to that observed for *A. destruens* F10.81 (Fig. 5). In fungi, gene regulation involves complex control mechanisms as those observed for peroxidases genes. It is known that in most fungal strains the LiP, MnP and Lac genes are expressed during the idiophase, the fungal secondary phase, when nitrogen is limited and under the control of complex regulation signals (Junghanns et al., 2005; Kamitsuji et al., 2004; Knop et al., 2015; Duran et al., 2002; Solé et al., 2012), although the expression of MnP genes have been observed under high nitrogen content in fungal genera such as *Pleurotus* and *Trametes* (Kaal et al., 1995; Janusz et al., 2013; Stajić et al., 2006). Thus, the differences observed in removal capacities between the fungal strains are probably due to the medium composition and culture conditions.

It is likely that the seawater medium with high nitrogen content as well as the culture conditions used in our study limited the removal capacities of *P. chrysosporium* (Singh and Chen, 2008) and *F. pseudoygamai* F5.76. In contrast, *A. destruens* F10.81 exhibited removal rates above 80% for all PAHs except for benzo[*a*]pyrene (65% removal; Fig. 5). Such higher PAH removal capacity of *A. destruens* F10.81 suggested that either its genes involved in PAH removal respond to different regulation signals than the other two strains or the PAH removal was performed by other mechanisms. For example, the expression of LiP, MnP and Lac genes has been observed under high nitrogen content in some fungal species (i.e. *Pleurotus ostreatus* and *Trametes trogii*) and even under both high and low nitrogen content for *Dichomitus squalens*, while for other fungal species, such as *P. chrysosporium*, the peroxidase genes are expressed under nitrogen limitation (Janusz et al., 2013; Stajić et al., 2006). The expression of genes involved in PAH-removal even in high nitrogen content might be an asset for the fungal saprotrophic life-style in marine environments where secreted enzymes, such as peroxidases, are likely to be lost by rapid diffusion in the aquatic environment (Richards et al., 2012).

Possible PAH removal has been described through biosorption mechanisms, which include adsorption onto cell surface (Raghukumar et al., 2006) and absorption into the cell (Verdin et al., 2005; Yang et al., 2013). Several studies have demonstrated the capacity of fungi to uptake PAHs (Deng et al., 2010; Wu et al., 2009) and also to transport them along the fungal hyphae (Furuno et al., 2012; Schamfuß et al., 2013). Both strains, *Fusarium pseudoygamai* F5.76 and *Alternaria*

destruens F10.81, were able to uptake and transport pyrene (Fig. 6). Clear pyrene containing vacuoles were observed in *F. pseudoygamai* F5.76 (Fig. 6c,d) while pyrene was homogeneously distributed in *A. destruens* F10.81 (Fig. 6e,f) suggesting that the fungal strains have developed different strategies for PAH uptake. It has been demonstrated that the vacuoles serves for possible storage of PAHs as carbon source and for PAH transport along the hyphae allowing the distribution of PAH within the mycelia network (Darrah et al., 2006; Furuno et al., 2012). Consistently, pyrene was also accumulated into conidia in *F. pseudoygamai* F5.76 (Fig. 6c,d), which represents carbon source reserve for the development of conidia as previously reported (Allaway et al., 1997; Bago et al., 2002). In contrast, the homogenous pyrene distribution in *A. destruens* F10.81 (Fig. 6e,f) suggested a diffusion mechanism. Such different pyrene uptake mechanism probably explains the highest removal capacities of *A. destruens* F10.81 in comparison to *F. pseudoygamai* F5.76 (Fig. 5). However, further studies are required to determine whether higher pyrene absorption or internal degradation by monooxygenease (i.e. cytochrome P450) are the underlying physiological mechanisms of PAH removal in *A. destruens* F10.81.

Conclusion

The exploration of the cultivable fungal diversity of hydrocarbon-contaminated coastal sediments revealed that coastal sediment hide fungal diversity yet unexplored for their metabolic potential, especially regarding PAH removal capacity. A large proportion of the isolated strains (48%), dispatched within 6 fungal genera, exhibited PAH-tolerance with a removal capacity (degradation and/or sorption) above 60%. Such diversity in PAH-removal capacity represents a functional potential for ecosystem recovery exploitable for bioremediation treatments (Harms et al., 2011). However, the mechanism underlying the PAH-removal capacity (degradation and/or sorption) is unclear because it is probably not related to the presence of extracellular peroxidase genes (LiP, MnP and Lac) and it is strain specific. The comparison of two isolated strains exhibiting contrasted removal capacities showed different PAH-uptake behaviour suggesting that the mechanisms by which fungi perform PAH-uptake might determine the efficiency of PAH-removal. *Alternaria destruens* F10.81, the most efficient PAH-remover (above 80%) was able to internalize pyrene homogenously into the hyphae that contrasted with the behaviour of *Fusarium pseudoygamai* F5.76 in which PAH-vacuoles were observed but exhibiting a PAH-removal capacity below 20%. It is likely that *Alternaria destruens* F10.81 owns features well adapted to PAH-contaminated coastal

sediments, which represent potential for the development of a bioremediation process. However, further studies are required to understand the PAH-removal mechanism in order to manage fungal resources to mitigate the effects of PAH contamination.

Acknowledgment

We acknowledge the support of the French/German bilateral program PHC-PROCOPE FungDeg (Elucidating biomarkers and functions of fungal bacterial interactions during the degradation of polycyclic aromatic hydrocarbons at changing environmental conditions; project no. 42512WL). We would like to thank all partners of the FungDeg project and MELODY group for their useful discussions. JAB received a fellowship from CONACyT number 326250/471689 and a mobility grant from the centre of excellence E2S at Pau University.

References

Allaway, W.G., Ashford, A.E., Heath, I.B., Hardham, A.R., 1997. Vacuolar Reticulum in Oomycete Hyphal Tips: An Additional Component of the Ca^{2+}Regulatory System? Fungal Genet. Biol. 22, 209-220. https://doi.org/10.1006/fgbi.1997.1015

Amend, A., Burgaud, G., Cunliffe, M., Edgcomb, V.P., Ettinger, C.L., Gutiérrez, M.H., Heitman, J., Hom, E.F.Y., Ianiri, G., Jones, A.C., Kagami, M., Picard, K.T., Quandt, C.A., Raghukumar, S., Riquelme, M., Stajich, J., Vargas-Muñiz, J., Walker, A.K., Yarden, O., Gladfelter, A.S., 2019. Fungi in the Marine Environment: Open Questions and Unsolved Problems. mBio. 10, e01189-18. https://doi.org/10.1128/mBio.01189-18

Andriani, A., Tachibana, S., Itoh, K., 2016. Effects of saline-alkaline stress on benzo[a]pyrene biotransformation and ligninolytic enzyme expression by *Bjerkandera adusta* SM46. World J. Microbiol. Biotechnol. 32 (3), no. 39. https://doi.org/10.1007/s11274-015-2001-4

Ansari, M.A., Butt, T.M. 2012. Evaluation of entomopathogenic fungi and a nematode against the soil-dwelling stages of the crane fly *Tipula paludosa*. Pest Manag. Sci. 68 (10), 1337-1344. https://doi.org/10.1002/ps.3338

Babu, R., Varadharajan, D., Soundarapandian, P., Balasubramanian, R., 2010. Fungi Diversity in Different Coastal Marine Ecosystem along South East Coast of India. Int. J. Microbiol. Res., 1, 175-178.

Bago, B., Pfeffer, P.E., Zipfel, W., Lammers, P., Shachar-Hill, Y., 2002. Tracking metabolism and imaging transport in arbuscular mycorrhizal fungi, in: Smith, S.E., Smith, F.A. (Eds.), Diversity and Integration in Mycorrhizas. Springer Netherlands, Dordrecht, 189-197. https://doi.org/10.1007/978-94-017-1284-2_18

Bamforth, S.M., Singleton, I., 2005. Bioremediation of polycyclic aromatic hydrocarbons: current knowledge and future directions. J. Chem. Technol. Biotechnol. 80, 723-736. https://doi.org/10.1002/jctb.1276

Bashyal, B.M., Aggarwal, R., Sharma, S., Gupta, S., Singh, U.B., 2016. Single and combined effects of three *Fusarium* species associated with rice seeds on the severity of Bakanae disease of rice. Plant Pathol. 98 (3), 22 https://doi.org/10.4454/JPP.V98I3.001

Ben Ali, W., Chaduli, D., Navarro, D., Lechat, C., Turbé-Doan, A., Bertrand, E., Faulds, C.B., Sciara, G., Lesage-Meessen, L., Record, E., Mechichi, T., 2020. Screening of five marine-derived fungal strains for their potential to produce oxidases with laccase activities suitable for biotechnological applications. BMC Biotechnol. 20, 27. https://doi.org/10.1186/s12896-020-00617-y

Birolli, W.G., de A. Santos, D., Alvarenga, N., Garcia, A.C.F.S., Romão, L.P.C., Porto, A.L.M., 2018. Biodegradation of anthracene and several PAHs by the marine-derived fungus *Cladosporium* sp. CBMAI 1237. Mar. Pollut. Bull. 129, 525–533. https://doi.org/10.1016/j.marpolbul.2017.10.023

Bogan, B.W., Schoenike, B., Lamar, R.T., Cullen, D., 1996. Manganese peroxidase mRNA and enzyme activity levels during bioremediation of polycyclic aromatic hydrocarbon-contaminated soil with *Phanerochaete chrysosporium*. Appl. Environ. Microbiol. 62, 2381–2386. https://doi.org/10.1128/AEM.62.7.2381-2386.1996

Bonugli-Santos, R.C., dos Santos Vasconcelos, M.R., Passarini, M.R.Z., Vieira, G.A.L., Lopes, V.C.P., Mainardi, P.H., dos Santos, J.A., de Azevedo Duarte, L., Otero,

I.V.R., da Silva Yoshida, A.M., Feitosa, V.A., Pessoa, A., Sette, L.D., 2015. Marine-derived fungi: diversity of enzymes and biotechnological applications. Front. Microbiol. 6, 269. https://doi.org/10.3389/fmicb.2015.00269

Bordenave, S., Goñi-Urriza, M., Vilette, C., Blanchard, S., Caumette, P., Duran, R., 2008. Diversity of ring-hydroxylating dioxygenases in pristine and oil contaminated microbial mats at genomic and transcriptomic levels. Environ. Microbiol. 10 (12), 3201-3211. DOI: 10.1111/j.1462-2920.2008.01707.x

Bovio, E., Garzoli, L., Poli, A., Luganini, A., Villa, P., Musumeci, R., McCormack, G. P., Cocuzza, C. E., Gribaudo, G., Mehiri, M., & Varese, G. C., 2019. Marine Fungi from the Sponge *Grantia compressa*: Biodiversity, Chemodiversity, and Biotechnological Potential. Mar. drugs, 17(4), 220. https://doi.org/10.3390/md17040220

Brito, E.M.S., Guyoneaud, R., Goñi-Urriza, M., Ranchou-Peyruse, A., Verbaere, A., Crapez, M.A.C., Wasserman, J.C.A., Duran, R., 2006. Characterization of hydrocarbonoclastic bacterial communities from mangrove sediments in Guanabara Bay, Brazil. Res. Microbiol. 157: 752-762. DOI: 10.1016/j.resmic.2006.03.005

Broda, P., Birch, P.R., Brooks, P.R., Sims, P.F., 1995. PCR-mediated analysis of lignocellulolytic gene transcription by *Phanerochaete chrysosporium*: substrate-dependent differential expression within gene families. Appl. Environ. Microbiol. 61(6), 2358-2364.

Bruneel, O., Pascault, N., Egal, M., Bancon-Montigny, C., Goñi-Urriza, M.S., Elbaz-Poulichet, F., Personné, J.C., Duran, R. 2008. Archaeal diversity in a Fe-As rich acid mine drainage at Carnoulès (France). Extremophiles 12 (4), 563-571. DOI: 10.1007/s00792-008-0160-z.

Bugni, T.S., Ireland, C.M., 2004. Marine-derived fungi: a chemically and biologically diverse group of microorganisms. Nat. Prod. Rep. 21(1), 143-163. https://doi.org/10.1039/b301926h

Cao, H., Wang, C., Liu, H., Jia, W., Sun, H., 2020. Enzyme activities during Benzo[a]pyrene degradation by the fungus *Lasiodiplodia theobromae* isolated from a polluted soil. Sci. Rep. 10 (1), no. 865. https://doi.org/10.1038/s41598-020-57692-6

Cerniglia, C.E., 1997. Fungal metabolism of polycyclic aromatic hydrocarbons: past, present and future applications in bioremediation. J. Ind. Microbiol. Biotechnol. 19(5-6), 324-333. https://doi.org/10.1038/sj.jim.2900459

Cerniglia, C.E., Kelly, D.W., Freeman, J.P., Miller, D.W., 1986. Microbial metabolism of pyrene. Chem. Biol. Interact. 57(2), 203-216. https://doi.org/10.1016/0009-2797(86)90038-4

Cerniglia, C.E., Sutherland, J.B., 2010. Degradation of Polycyclic Aromatic Hydrocarbons by Fungi, in: Timmis, K.N. (Ed.), Handbook of Hydrocarbon and Lipid Microbiology. Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 2079–2110. https://doi.org/10.1007/978-3-540-77587-4_151

Chen, D.M., Taylor, A.F.S., Burke, R.M., Cairney, J.W.G., 2001. Identification of genes for lignin peroxidases and manganese peroxidases in ectomycorrhizal fungi. New Phytol. 152(1), 151-158. https://doi.org/10.1046/j.0028-646x.2001.00232.x

Chen, Q., Hou, L.W., Duan, W.J., Crous, P.W., Cai, L., 2017. *Didymellaceae* revisited. Stud. Mycol. 87, 105-159. https://doi.org/10.1016/j.simyco.2017.06.002

Clemente, A.R., Anazawa, T.A., Durrant, L.R., 2001. Biodegradation of polycyclic aromatic hydrocarbons by soil fungi. Braz. J. Microbiol. 32(4), 255-261. https://doi.org/10.1590/S1517-83822001000400001

Črešnar, B., Petrič, š., 2011. Cytochrome P450 enzymes in the fungal kingdom. Biochim. Biophys. Acta BBA - Proteins Proteomics 1814, 29–35. https://doi.org/10.1016/j.bbapap.2010.06.020

Crous, P.W., Shivas, R.G., Quaedvlieg, W., van der Bank, M., Zhang, Y., Summerell, B.A., Guarro, J., Wingfield, M.J., Wood, A.R., Alfenas, A.C., Braun, U., Cano-Lira, J.F., García, D., Marin-Felix, Y., Alvarado, P., Andrade, J.P., Armengol, J., Assefa, A., den Breeÿen, A., Camele, I., Cheewangkoon, R., De Souza, J.T., Duong, T.A., Esteve-Raventós, F., Fournier, J., Frisullo, S., García-Jiménez, J., Gardiennet, A., Gené, J., Hernández-Restrepo, M., Hirooka, Y., Hospenthal, D.R., King, A., Lechat, C., Lombard, L., Mang, S.M., Marbach, P.A.S., Marincowitz, S., Marin-Felix, Y., Montaño-Mata, N.J., Moreno, G., Perez, C.A., Pérez Sierra, A.M., Robertson, J.L., Roux, J., Rubio, E., Schumacher, R.K., Stchigel, A.M., Sutton, D.A., Tan, Y.P., Thompson, E.H., van der Linde, E., Walker, A.K., Walker, D.M., Wickes, B.L., Wong, P.T.W., Groenewald, J.Z., 2014. Fungal Planet description sheets: 214-280. Persoonia, 32, 184-306. https://doi.org/10.3767/003158514X682395

Crous, P.W., Wingfield, M.J., Richardson, D.M., Le Roux, J.J., Strasberg, D., Edwards, J., Roets, F., Hubka, V., Taylor, P.W.J., Heykoop, M., Martín, M.P., Moreno, G., Sutton, D.A., Wiederhold, N.P., Barnes, C.W., Carlavilla, J.R., Gené, J., Giraldo, A., Guarnaccia, V., Guarro, J., Hernández-Restrepo, M., Kolařík, M., Manjón, J.L., Pascoe, I.G., Popov, E.S., Sandoval-Denis, M., Woudenberg, J.H.C., Acharya, K., Alexandrova, A.V., Alvarado, P., Barbosa, R.N., Baseia, I.G., Blanchette, R.A., Boekhout, T., Burgess, T.I., Cano-Lira, J.F., Čmoková, A., Dimitrov, R.A., Dyakov, M.Y., Dueñas, M., Dutta, A.K., Esteve-Raventós, F., Fedosova, A.G., Fournier, J., Gamboa, P., Gouliamova, D.E., Grebenc, T., Groenewald, M., Hanse, B., Hardy, G.E.S.J., Held, B.W., Jurjević, Ž., Kaewgrajang, T., Latha, K.P.D., Lombard, L., Luangsa-Ard, J.J., Lysková, P., Mallátová, N., Manimohan, P., Miller, A.N., Mirabolfathy, M., Morozova, O.V., Obodai, M., Oliveira, N.T., Ordóñez, M.E., Otto, E.C., Paloi, S., Peterson, S.W., Phosri, C., Roux, J., Salazar, W.A., Sánchez, A., Sarria, G.A., Shin, H.-D., Silva, B.D.B., Silva, G.A., Smith, M.T., Souza-Motta, C.M., Stchigel, A.M., Stoilova-Disheva, M.M., Sulzbacher, M.A., Telleria, M.T., Toapanta, C., Traba, J.M., Valenzuela-Lopez, N., Watling, R., Groenewald, J.Z., 2016. Fungal planet description sheets: 400–468. Persoonia, 36, 316-458. https://doi.org/10.3767/003158516X692185

Danilovich, M.E., Ovruski, S.M., Fariña, J.I., Delgado, O.D., 2020. First report on the entomopathogenicity and virulence of *Akanthomyces muscarius* LY 72.14, a Yungas native fungal isolate, for *Anastrepha fraterculus* control. Biocontrol Sci. Techn. 30 (11), 1212-1227. https://doi.org/10.1080/09583157.2020.1802699

Darrah, P.R., Tlalka, M., Ashford, A., Watkinson, S.C., Fricker, M.D., 2006. The Vacuole System Is a Significant Intracellular Pathway for Longitudinal Solute Transport in Basidiomycete Fungi. Eukaryot. Cell 5(7), 1111-1125. https://doi.org/10.1128/EC.00026-06

Deng, Y., Zhang, Y., Hesham, A.E.-L., Liu, R., Yang, M., 2010. Cell surface properties of five polycyclic aromatic compound-degrading yeast strains. Appl. Microbiol. Biotechnol. 86(6), 1933-1939. https://doi.org/10.1007/s00253-010-2477-7

Deschler, C., Duran, R., Junqua, M., Landou, C., Salvado, J.-C., Goulas, P., 1998. Involvement of 3,4-dichlorophenol hydroxylase in degradation of 3,4-dichlorophenol by the white rot fungus *Phanerochaete chrysosporium*. J. Mol. Catal. B Enzym. 5(1-4), 423-428. https://doi.org/10.1016/S1381-1177(98)00056-3

D'Souza, T. M., Boominathan, K., & Reddy, C. A. 1996. Isolation of laccase gene-specific sequences from white rot and brown rot fungi by PCR. Appl. Environ. Microbiol. 62(10), 3739-3744. DOI: 10.1128/AEM.62.10.3739-3744.1996

D'Souza-Ticlo, D., Sharma, D., Raghukumar, C., 2009. A Thermostable metal-tolerant laccase with bioremediation potential from a marine-derived fungus. Mar. Biotechnol. 11(6), 725-737. https://doi.org/10.1007/s10126-009-9187-0

Duran, R., Cravo-Laureau, C., 2016. Role of environmental factors and microorganisms in determining the fate of polycyclic aromatic hydrocarbons in the marine environment. FEMS Microbiol. Rev. 40(6), 814-830. https://doi.org/10.1093/femsre/fuw031

Duran, R., Deschler, C., Precigou, S., Goulas, P., 2002. Degradation of chlorophenols by *Phanerochaete chrysosporium*: effect of 3,4-dichlorophenol on extracellular peroxidase activities. Appl. Microbiol. Biotechnol. 59, 284-288. https://doi.org/10.1007/s00253-002-0988-6

Fajarningsih, N.D., 2016. Internal transcribed spacer (ITS) as DNA barcoding to identify fungal species: a review. Squalen Bull. of Mar. and Fish. Postharvest and Biotech. 11(2), 37-44. http://dx.doi.org/10.15578/squalen.v11i2.213

Fayeulle, A., Veignie, E., Schroll, R., Munch, J.C., Rafin, C., 2019. PAH biodegradation by telluric saprotrophic fungi isolated from aged PAH-contaminated soils in mineral medium and historically contaminated soil microcosms. J. Soils Sediments 19, 3056–3067. https://doi.org/10.1007/s11368-019-02312-8

Fedorak, P.M., Semple, K.M., Westlake, D.W.S., 1984. Oil-degrading capabilities of yeasts and fungi isolated from coastal marine environments. Can. J. Microbiol. 30(5), 565-571. https://doi.org/10.1139/m84-085

Field, J.A., de Jong, E., Feijoo Costa, G., de Bont, J.A., 1992. Biodegradation of polycyclic aromatic hydrocarbons by new isolates of white rot fungi. Appl. Environ. Microbiol. 58(7), 2219-2226.

Furuno, S., Foss, S., Wild, E., Jones, K.C., Semple, K.T., Harms, H., Wick, L.Y., 2012. Mycelia promote active transport and spatial dispersion of polycyclic aromatic hydrocarbons. Environ. Sci. Technol. 46(10), 5463-5470. https://doi.org/10.1021/es300810b

Giloteaux, L., Goñi-Urriza, M., Duran, R., 2010. Nested PCR and new primers for analysis of sulfate-reducing bacteria in low-cell-biomass environments. Appl. Environ. Microbiol. 76 (9), 2856-2865. https://doi.org/10.1128/AEM.02023-09

Ghizelini, A.M., Martins, K.G., Gießelmann, U.C., Santoro, E., Pasqualette, L., Mendonça-Hagler, L.C.S., Rosado, A.S., Macrae, A., 2019. Fungal communities in oil contaminated mangrove sediments – Who is in the mud? Mar. Pollut. Bull. 139, 181-188. https://doi.org/10.1016/j.marpolbul.2018.12.040

Ghosal, D., Ghosh, S., Dutta, T.K., Ahn, Y., 2016. Current state of knowledge in microbial degradation of polycyclic aromatic hydrocarbons (PAHs): A review. Front. Microbiol. 7, 1369. https://doi.org/10.3389/fmicb.2016.01369.

Godoy, P., Reina, R., Calderón, A., Wittich, R.-M., García-Romera, I., Aranda, E., 2016. Exploring the potential of fungi isolated from PAH-polluted soil as a source of

xenobiotics-degrading fungi. Environ. Sci. Pollut. Res. 23(20), 20985-20996. https://doi.org/10.1007/s11356-016-7257-1

Greco, G., Cecchi, G., Di Piazza, S., Cutroneo, L., Capello, M., Zotti, M., 2018. Fungal characterisation of a contaminated marine environment: the case of the Port of Genoa (North-Western Italy). Webbia 73(1), 97-106. https://doi.org/10.1080/00837792.2017.1417964

Grossart, H.-P., Rojas-Jimenez, K., 2016. Aquatic fungi: targeting the forgotten in microbial ecology. Curr. Opin. Microbiol. 31, 140-145. https://doi.org/10.1016/j.mib.2016.03.016

Guermouche M'rassi, A., Bensalah, F., Gury, J., Duran, R., 2015. Isolation and characterization of different bacterial strains for bioremediation of n-alkanes and polycyclic aromatic hydrocarbons. Environ Sci. Pol. Res. 22 (20), 15332-15346. https://doi.org/10.1007/s11356-015-4343-8

Hao, Y., Aluthmuhandiram, J.V.S., Chethana, K.W.T., Manawasinghe, I.S., Li, X., Liu, M., Hyde, K.D., Phillips, A.J.L., Zhang, W., 2020. Nigrospora Species Associated with Various Hosts from Shandong Peninsula, China. Mycobiology 48 (3), 169-183. https://doi.org/10.1080/12298093.2020.1761747

Haritash, A.K., Kaushik, C.P., 2009. Biodegradation aspects of Polycyclic Aromatic Hydrocarbons (PAHs): A review. J. Hazard. Mater. 169(1-3), 1-15. https://doi.org/10.1016/j.jhazmat.2009.03.137

Harms, H., Schlosser, D., Wick, L. 2011. Untapped potential: exploiting fungi in bioremediation of hazardous chemicals. Nat. Rev. Microbiol. 9, 177–192. https://doi.org/10.1038/nrmicro2519

Harrison, I.A., 2009. Biodegradation of PAHs by fungi in contaminated-soil containing cadmium and nickel ions. Afr. J. Biotechnol. 8(21), 5780-5789. https://doi.org/10.5897/AJB2009.000-9465

Janusz, G., Kucharzyk, K.H., Pawlik, A., Staszczak, M., Paszczynski, A.J., 2013. Fungal laccase, manganese peroxidase and lignin peroxidase: Gene expression and regulation. Enzyme Microb. Technol. 52(1), 1-12. https://doi.org/10.1016/j.enzmictec.2012.10.003

Jones, E.B.G., 2000. Marine fungi: some factors influencing biodiversity. Fungal Divers. 4(193), 53-73.

Junghanns, C., Moeder, M., Krauss, G., Martin, C., Schlosser, D., 2005. Degradation of the xenoestrogen nonylphenol by aquatic fungi and their laccases. Microbiology 151(1), 45-57. https://doi.org/10.1099/mic.0.27431-0

Kaal, E.E.J., Field, J.A., Joyce, T.W., 1995. Increasing ligninolytic enzyme activities in several white-rot Basidiomycetes by nitrogen-sufficient media. Bioresour. Technol. 53 (2), 133-139. https://doi.org/10.1016/0960-8524(95)00066-N

Kamitsuji, H., Honda, Y., Watanabe, T., Kuwahara, M., 2004. Production and induction of manganese peroxidase isozymes in a white-rot fungus *Pleurotus ostreatus*. Appl. Microbiol. Biotechnol. 65, 287-294. https://doi.org/10.1007/s00253-003-1543-9

Knop, D., Yarden, O., Hadar, Y., 2015. The ligninolytic peroxidases in the genus Pleurotus: divergence in activities, expression, and potential applications. Appl. Microbiol. Biotechnol. 99(3), 1025-1038. https://doi.org/10.1007/s00253-014-6256-8

Kumar, S., 2004. MEGA3: Integrated software for Molecular Evolutionary Genetics Analysis and sequence alignment. Brief. Bioinform. 5(2), 150–163. https://doi.org/10.1093/bib/5.2.150

Kumar, S., Kaushik, N., 2013. Endophytic Fungi Isolated from Oil-Seed Crop Jatropha curcas Produces Oil and Exhibit Antifungal Activity. PLoS ONE, 8 (2), e56202. https://doi.org/10.1371/journal.pone.0056202

Lee, Hwanhwi, Jang, Y., Choi, Y.-S., Kim, M.-J., Lee, J., Lee, Hanbyul, Hong, J.-H., Lee, Y.M., Kim, G.-H., Kim, J.-J., 2014. Biotechnological procedures to select white rot fungi for the degradation of PAHs. J. Microbiol. Methods 97, 56–62. https://doi.org/10.1016/j.mimet.2013.12.007

Li, Q., Wang, G., 2009. Diversity of fungal isolates from three Hawaiian marine sponges. Microbiol. Res. 164(2), 233-241. https://doi.org/10.1016/j.micres.2007.07.002

Li, X., Li, P., Lin, X., Zhang, C., Li, Q., Gong, Z., 2008. Biodegradation of aged polycyclic aromatic hydrocarbons (PAHs) by microbial consortia in soil and slurry phases. J. Hazard. Mater. 150(1), 21-26. https://doi.org/10.1016/j.jhazmat.2007.04.040

Lisowska, K., 2004. Microcalorimetry as a possible tool for phenanthrene toxicity evaluation to eukaryotic cells. Thermochim. Acta 411(2), 181-186. https://doi.org/10.1016/j.tca.2003.08.015

Lladó, S., Solanas, A.M., de Lapuente, J., Borràs, M., Viñas, M., 2012. A diversified approach to evaluate biostimulation and bioaugmentation strategies for heavy-oil-contaminated soil. Sci. Total Environ. 435-436, 262-269. https://doi.org/10.1016/j.scitotenv.2012.07.032

Lundstedt, S., Haglund, P., Öberg, L., 2003. Degradation and formation of polycyclic aromatic compounds during bioslurry treatment of an aged gasworks soil. Environ. Toxicol. Chem. 22(7), 1413-1420. https://doi.org/10.1002/etc.5620220701

Marco-Urrea, E., García-Romera, I., Aranda, E., 2015. Potential of non-ligninolytic fungi in bioremediation of chlorinated and polycyclic aromatic hydrocarbons. New Biotechnol. 32 (6), 620-628. https://doi.org/10.1016/j.nbt.2015.01.005

May, R., Schröder, P., Sandermann, H., 1997. *Ex-Situ* Process for Treating PAH-Contaminated Soil with *Phanerochaete chrysosporium*. Environ. Sci. Technol. 31(9), 2626-2633. https://doi.org/10.1021/es9700414

Méjanelle, L., F. Lòpez, J., Gunde-Cimerman, N., Grimalt, J.O., 2000. Sterols of melanized fungi from hypersaline environments. Org. Geochem. 31(10), 1031-1040. https://doi.org/10.1016/S0146-6380(00)00094-2

Mineki, S., Suzuki, K., Iwata, K., Nakajima, D., Goto, S., 2015. Degradation of Polyaromatic Hydrocarbons by Fungi Isolated from Soil in Japan. Polycycl. Aromat. Compd. 35(1), 120-128. https://doi.org/10.1080/10406638.2014.937007

Morales, P., Cáceres, M., Scott, F., Díaz-Robles, L., Aroca, G., Vergara-Fernández, A., 2017. Biodegradation of benzo[α]pyrene, toluene, and formaldehyde from the gas phase by a consortium of *Rhodococcus erythropolis* and *Fusarium solani*. Appl. Microbiol. Biotechnol. 101, 6765-6777. https://doi.org/10.1007/s00253-017-8400-8

Moreno, L.F., Feng, P., Weiss, V.A., Vicente, V.A., Stielow, J.B., de Hoog, S., 2017. Phylogenomic analyses reveal the diversity of laccase-coding genes in Fonsecaea genomes. PLOS ONE 12, e0171291. https://doi.org/10.1371/journal.pone.0171291

Mouton, M., Postma, F., Wilsenach, J., Botha, A., 2012. Diversity and Characterization of Culturable Fungi from Marine Sediment Collected from St. Helena Bay, South Africa. Microb. Ecol. 64(2), 311-319. DOI 10.1007/s00248-012-0035-9

Novotný, Č., Svobodová, K., Erbanová, P., Cajthaml, T., Kasinath, A., Lang, E., Šašek, V., 2004. Ligninolytic fungi in bioremediation: extracellular enzyme production and

degradation rate. Soil Biol. Biochem. 36(10), 1545-1551. https://doi.org/10.1016/j.soilbio.2004.07.019

Orsi, W., Biddle, J.F., Edgcomb, V., 2013. Deep Sequencing of Subseafloor Eukaryotic rRNA Reveals Active Fungi across Marine Subsurface Provinces. PLoS ONE 8, e56335. https://doi.org/10.1371/journal.pone.0056335

Poli, A., Bovio, E., Ranieri, L., Varese, G. C., & Prigione, V., 2020. Fungal Diversity in the Neptune Forest: Comparison of the Mycobiota of *Posidonia oceanica*, *Flabellia petiolata*, and *Padina pavonica*. Front. microbiol. 11, 933. https://doi.org/10.3389/fmicb.2020.00933

Potin, O., Veignie, E., Rafin, C., 2004. Biodegradation of polycyclic aromatic hydrocarbons (PAHs) by *Cladosporium sphaerospermum* isolated from an aged PAH contaminated soil. FEMS Microbiol. Ecol. 51(1), 71-78. https://doi.org/10.1016/j.femsec.2004.07.013

Rafin, C., Potin, O., Veignie, E., Lounes-Hadj, A. Sahraoui., Sancholle, M., 2000. Degradation of benzo[a]pyrene as sole carbon source by a non white rot fungus, *Fusarium Solani*. Polycycl. Aromat. Compd. 21(1-4), 311-329. https://doi.org/10.1080/10406630008028542

Raghukumar, C., Shailaja, M.S., Parameswaran, P.S., Singh, S.K., 2006. Removal of polycyclic aromatic hydrocarbons from aqueous media by the marine fungus NIOCC 312: involvement of lignin-degrading enzymes and exopolysaccharides. Indian. J. Mar. Sci. 35(4), 373-379.

Ravelet, C., Krivobok, S., Sage, L., Steiman, R., 2000. Biodegradation of pyrene by sediment fungi. Chemosphere 40(5), 557-563. https://doi.org/10.1016/S0045-6535(99)00320-3

Ravi Theja, S., Chandra, M., 2020. Molecular characterization of fungi isolated from sea urchin *Stomopneustes variolaris* (Lamarck, 1816) – st. mary's island, west coast of india. Biomedicine, 40 (3) 313-318. https://doi.org/10.51248/.v40i3.18

Reyes-César, A., Absalón, Á.E., Fernández, F.J., González, J.M., Cortés-Espinosa, D.V., 2014. Biodegradation of a mixture of PAHs by non-ligninolytic fungal strains isolated from crude oil-contaminated soil. World J. Microbiol. Biotechnol. 30, 999-1009. https://doi.org/10.1007/s11274-013-1518-7

Richards, T.A., Jones, M.D.M., Leonard, G., Bass, D., 2012. Marine fungi: their ecology and molecular diversity. Annu. Rev. Mar. Sci. 4, 495-522. https://doi.org/10.1146/annurev-marine-120710-100802

Rothermich, M.M., Hayes, L.A., Lovley, D.R., 2002. Anaerobic, sulfate-dependent degradation of polycyclic aromatic hydrocarbons in petroleum-contaminated harbor sediment. Environ. Sci. Technol. 36(22), 4811-4817. https://doi.org/10.1021/es0200241

Pointing, S., 2001. Feasibility of bioremediation by white-rot fungi. Appl. Microbiol. Biotechnol. 57(1-2), 20-33. https://doi.org/10.1007/s002530100745

Sandoval-Denis, M., Gené, J., Sutton, D. A., Wiederhold, N. P., Cano-Lira, J. F., & Guarro, J., 2016. New species of Cladosporium associated with human and animal infections. Persoonia, 36, 281–298. https://doi.org/10.3767/003158516X691951

Schamfuß, S., Neu, T.R., van der Meer, J.R., Tecon, R., Harms, H., Wick, L.Y., 2013. Impact of mycelia on the accessibility of fluorene to PAH-degrading bacteria. Environ. Sci. Technol. 47(13), 6908-6915. https://doi.org/10.1021/es304378d

Scheel, T., Höfer, M., Ludwig, S., Hölker, U., 2000. Differential expression of manganese peroxidase and laccase in white-rot fungi in the presence of manganese or aromatic compounds. Appl. Microbiol. Biotechnol. 54, 686–691. https://doi.org/10.1007/s002530000427

Schubert, K., Groenewald, J.Z., Braun, U., Dijksterhuis, J., Starink, M., Hill, C.F., Zalar, P., de Hoog, G.S., Crous, P.W., 2007. Biodiversity in the *Cladosporium herbarum* complex (Davidiellaceae, Capnodiales), with standardisation of methods for Cladosporium taxonomy and diagnostics. Stud. Mycol. 58, 105-156. https://doi.org/10.3114/sim.2007.58.05

Shao, Z., Sun, F., 2007. Intracellular sequestration of manganese and phosphorus in a metal-resistant fungus *Cladosporium cladosporioides* from deep-sea sediment. Extremophiles 11, 435–443. https://doi.org/10.1007/s00792-006-0051-0

Simister, R.L., Poutasse, C.M., Thurston, A.M., Reeve, J.L., Baker, M.C., White, H.K., 2015. Degradation of oil by fungi isolated from Gulf of Mexico beaches. Mar. Pollut. Bull. 100(1), 327-333. https://doi.org/10.1016/j.marpolbul.2015.08.029

Singh, D., Chen, S., 2008. The white-rot fungus *Phanerochaete chrysosporium*: conditions for the production of lignin-degrading enzymes. Appl. Microbiol. Biotechnol. 81(3), 399-417. https://doi.org/10.1007/s00253-008-1706-9

Solé, M., Müller, I., Pecyna, M.J., Fetzer, I., Harms, H., Schlosser, D., 2012. Differential regulation by organic compounds and heavy metals of multiple laccase genes in the aquatic hyphomycete *Clavariopsis aquatica*. Appl. Environ. Microbiol. 78(13), 4732-4739. https://doi.org/10.1128/AEM.00635-12

Stajić, M., Persky, L., Friesem, D., Hadar, Y., Wasser, S.P., Nevo, E., Vukojević, J., 2006. Effect of different carbon and nitrogen sources on laccase and peroxidases production by selected Pleurotus species. Enzyme Microb. Technol. 38(1-2), 65-73. https://doi.org/10.1016/j.enzmictec.2005.03.026

Syed, K., Doddapaneni, H., Subramanian, V., Lam, Y.W., Yadav, J.S., 2010. Genome-to-function characterization of novel fungal P450 monooxygenases oxidizing polycyclic aromatic hydrocarbons (PAHs). Biochem. Biophys. Res. Commun. 399(4), 492-497. https://doi.org/10.1016/j.bbrc.2010.07.094

Tan, T.K., Teng, C.L., Jones, E.B.G., 1995. Substrate type and microbial interactions as factors affecting ascocarp formation by mangrove fungi. In: Wong YS., Tam N.F.Y. (eds) Asia-Pacific Symposium on Mangrove Ecosystems. Develop. Hydrobiol. 106. https://doi.org/10.1007/978-94-011-0289-6_16

Thompson, J.D., Gibson, T.J., Higgins, D.G., 2003. Multiple Sequence Alignment Using ClustalW and ClustalX. Curr. Protoc. Bioinforma. (1), 2.3.1-2.3.22. https://doi.org/10.1002/0471250953.bi0203s00

Trincone, A., 2010. Potential biocatalysts originating from sea environments. J. Mol. Catal. B Enzym. 66(3-4), 241-256. https://doi.org/10.1016/j.molcatb.2010.06.004

Valentín, L., Feijoo, G., Moreira, M.T., Lema, J.M., 2006. Biodegradation of polycyclic aromatic hydrocarbons in forest and salt marsh soils by white-rot fungi. Int. Biodeterior. Biodegrad. 58(1), 15-21. https://doi.org/10.1016/j.ibiod.2006.04.002

Verdin, A., Lounès-Hadj Sahraoui, A., Newsam, R., Robinson, G., Durand, R., 2005. Polycyclic aromatic hydrocarbons storage by *Fusarium solani* in intracellular lipid vesicles. Environ. Pollut. 133(2), 283-291. https://doi.org/10.1016/j.envpol.2004.05.040

Wu, Y.-R., He, T.-T., Lun, J.-S., Maskaoui, K., Huang, T.-W., Hu, Z., 2009. Removal of Benzo[a]pyrene by a fungus Aspergillus sp. BAP14. World J. Microbiol. Biotechnol. 25(8), 1395–1401. https://doi.org/10.1007/s11274-009-0026-2

Xiao, R., Guo, Y., Zhang, M., Pan, W., Wang, J.J., 2020. Stronger network connectivity with lower diversity of soil fungal community was presented in coastal marshes after sixteen years of freshwater restoration. Sci. Total Environ. 744, no. 140623. https://doi.org/ 10.1016/j.scitotenv.2020.140623

Yang, J., Wang, G., Ng, T.B., Lin, J., Ye, X., 2016. Laccase Production and Differential Transcription of laccase genes in *Cerrena* sp. in response to metal ions, aromatic compounds, and nutrients. Front. Microbiol. 6, 1558. https://doi.org/10.3389/fmicb.2015.01558

Yang, S., Hai, F.I., Nghiem, L.D., Roddick, F., Price, W.E., 2013. Removal of trace organic contaminants by nitrifying activated sludge and whole-cell and crude enzyme extract of *Trametes versicolo*r. Water Sci. Technol. 67(6), 1216-1223. https://doi.org/10.2166/wst.2013.684

Zafra, G., Absalón, A.E., Cortés-Espinosa, D.V., 2015. Morphological changes and growth of filamentous fungi in the presence of high concentrations of PAHs. Braz. J. Microbiol. 46(3), 937-941. https://doi.org/10.1590/S1517-838246320140575

Zalar, P., de Hoog, G.S., Schroers, H.-J., Crous, P.W., Groenewald, J.Z., Gunde-Cimerman, N., 2007. Phylogeny and ecology of the ubiquitous saprobe *Cladosporium sphaerospermum*, with descriptions of seven new species from hypersaline environments. Stud. Mycol. 58, 157-183. https://doi.org/10.3114/sim.2007.58.06

795 **Figure captions**

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

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

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

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

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

Fig. 6: PAHs internalization in the hyphae of *Alternaria destruens* F10.81 and *Fusarium pseudonygamai* F5.76. (a) Experimental schema for the detection of internal transport of PAHs along the mycelia. Fungi were inoculated in a 1 cm^3 cube of solid seawater minimal media with 10% LB and 20 mg/L of pyrene. The red arrow indicates the direction of the hyphae growth. (b) Macroscopic observation showing the colonization of *Fusarium pseudonygamai* F5.76 of a piece of media from the other. The red arrow indicates the hyphae forming bridges between the two pieces of media. Observation of *Fusarium pseudonygamai* F5. 76 hyphae after colonization by (c) light microscopy and by (d) fluorescence after exposing to DAPI light. The red arrows show the storage of PAHs into conidia. Observation of *Alternaria destruens* F10.81 hyphae after colonization by (e) light microscopy and by (f) fluorescence after exposing to DAPI light. The red arrows show the homogeneous distribution of PAHs into the hyphae. The microscopic observations were performed at a magnification of 160X.

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

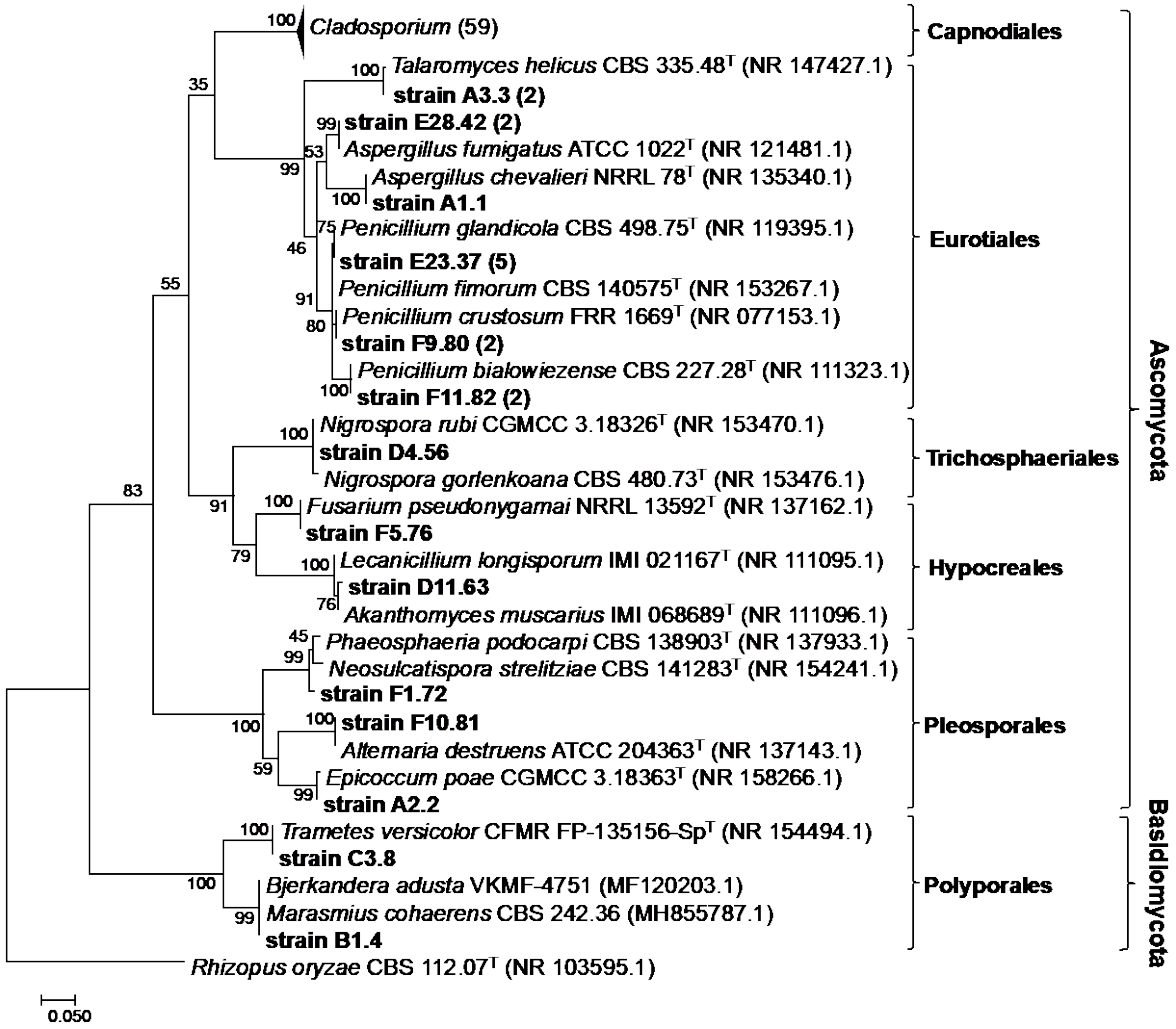
Gene*	Sequence (5'-3')**	Tm (°C)	Size (nt)	Reference
LiP1	LIG1u (F): GCCGCAATTTCTCTTGCTCTTTCCA LIG1d (R): TACATCGAACCACGCGCACGAGATT	57	179/126	Broda et al., 1995
LiP2	LIG2u (F): CATCGCAATTTCGCCCGCCATGGAGGA LIG2d (R): ACCTTCTGAACGAATGGCTTCTGGAGC	57	222/179	Broda et al., 1995
LiP3	LIG3u (F): TATTGCCATCTCTCCTGCTATGGAGGCC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

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

843

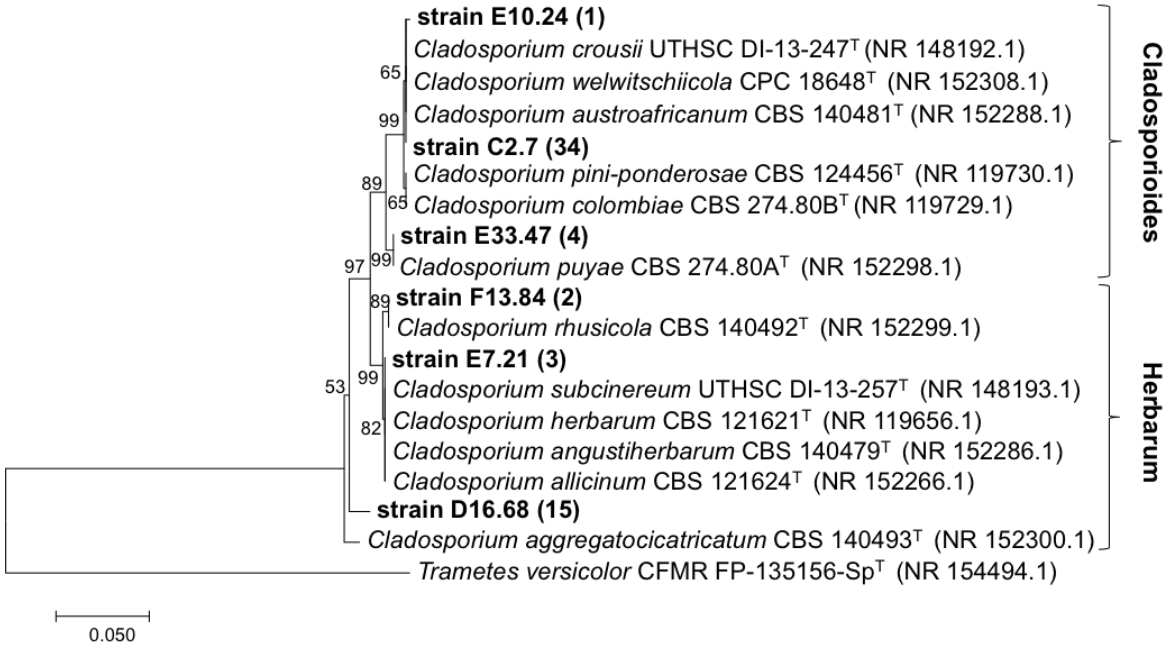
844 **Fig. 1**

845
846

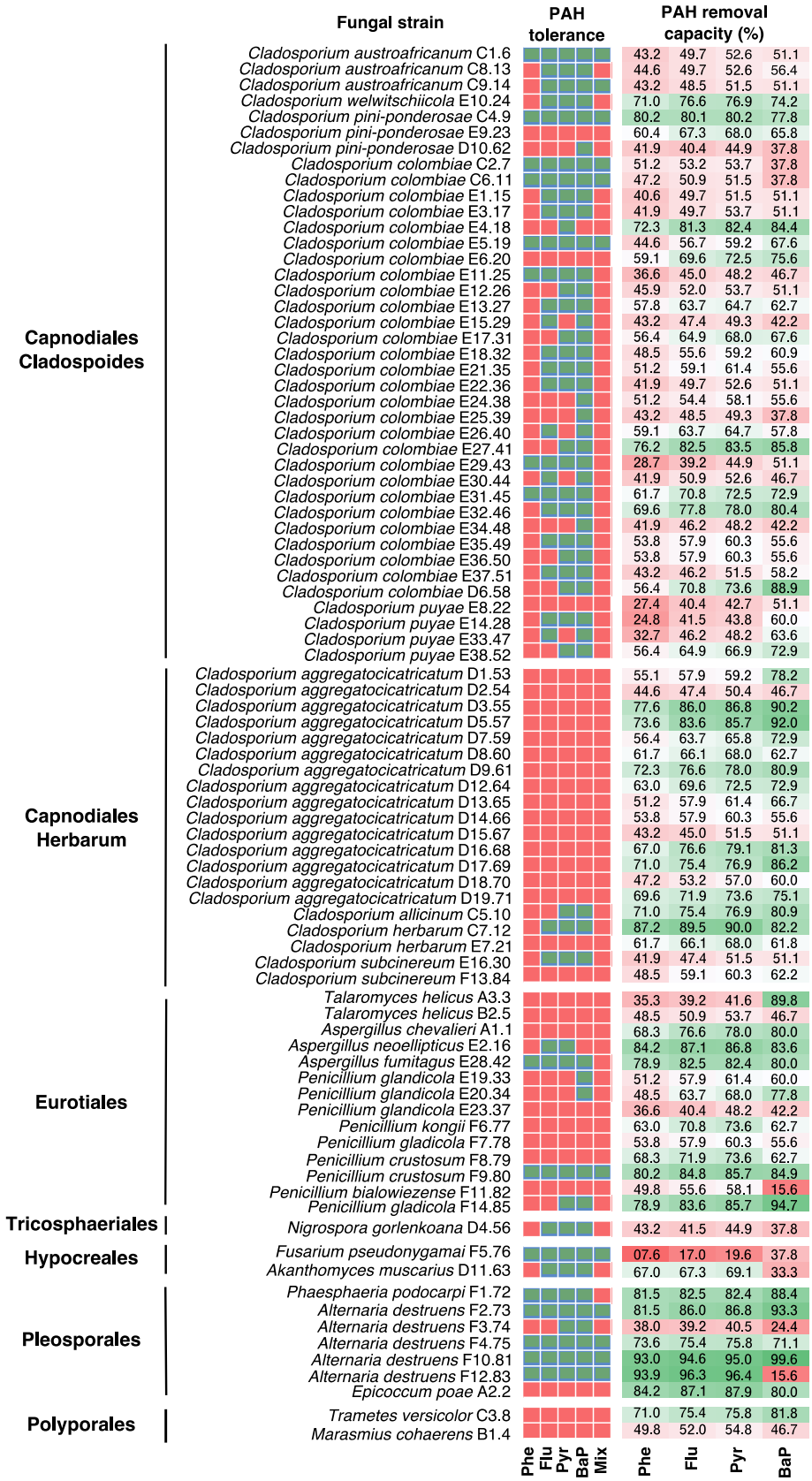


847
848
849

Fig. 2

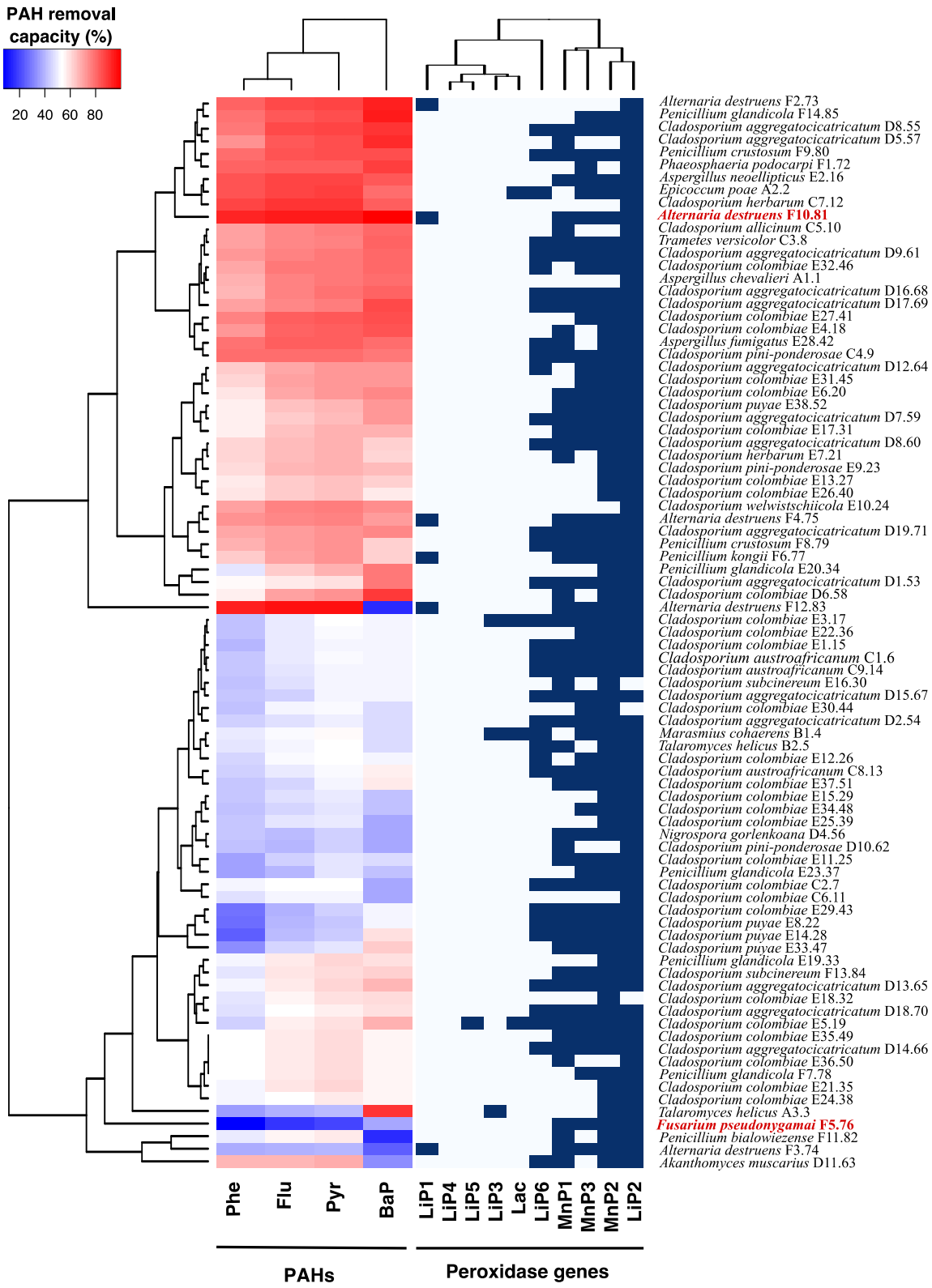

strain E10.24 (1)
Cladosporium crousii UTHSC DI-13-247T (NR 148192.1)
Cladosporium welwitschiicola CPC 18648T (NR 152308.1)
Cladosporium austroafricanum CBS 140481T (NR 152288.1)
strain C2.7 (34)
Cladosporium pini-ponderosae CBS 124456T (NR 119730.1)
Cladosporium colombiae CBS 274.80BT (NR 119729.1)
strain E33.47 (4)
Cladosporium puyae CBS 274.80AT (NR 152298.1)
strain F13.84 (2)
Cladosporium rhusicola CBS 140492T (NR 152299.1)
strain E7.21 (3)
Cladosporium subcinereum UTHSC DI-13-257T (NR 148193.1)
Cladosporium herbarum CBS 121621T (NR 119656.1)
Cladosporium angustiherbarum CBS 140479T (NR 152286.1)
Cladosporium allicinum CBS 121624T (NR 152266.1)
strain D16.68 (15)
Cladosporium aggregatocicatricatum CBS 140493T (NR 152300.1)
Trametes versicolor CFMR FP-135156-SpT (NR 154494.1)
65
99
89
65
97
99
89
53
99
82
Cladosporioides
Herbarum
0.050

857 **Fig. 3**


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

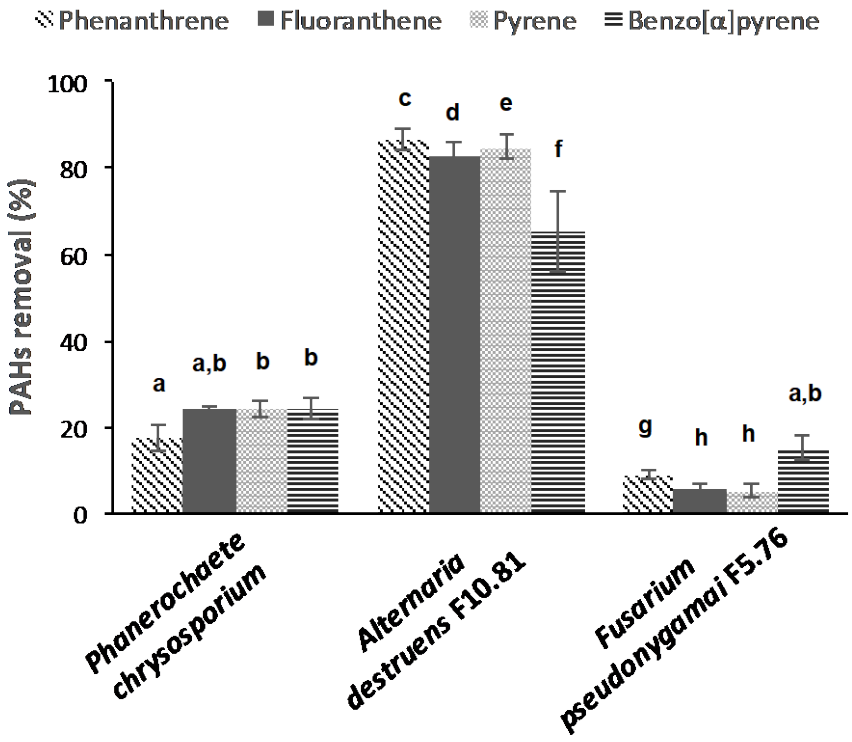
858

859 **Fig. 4**



860
861

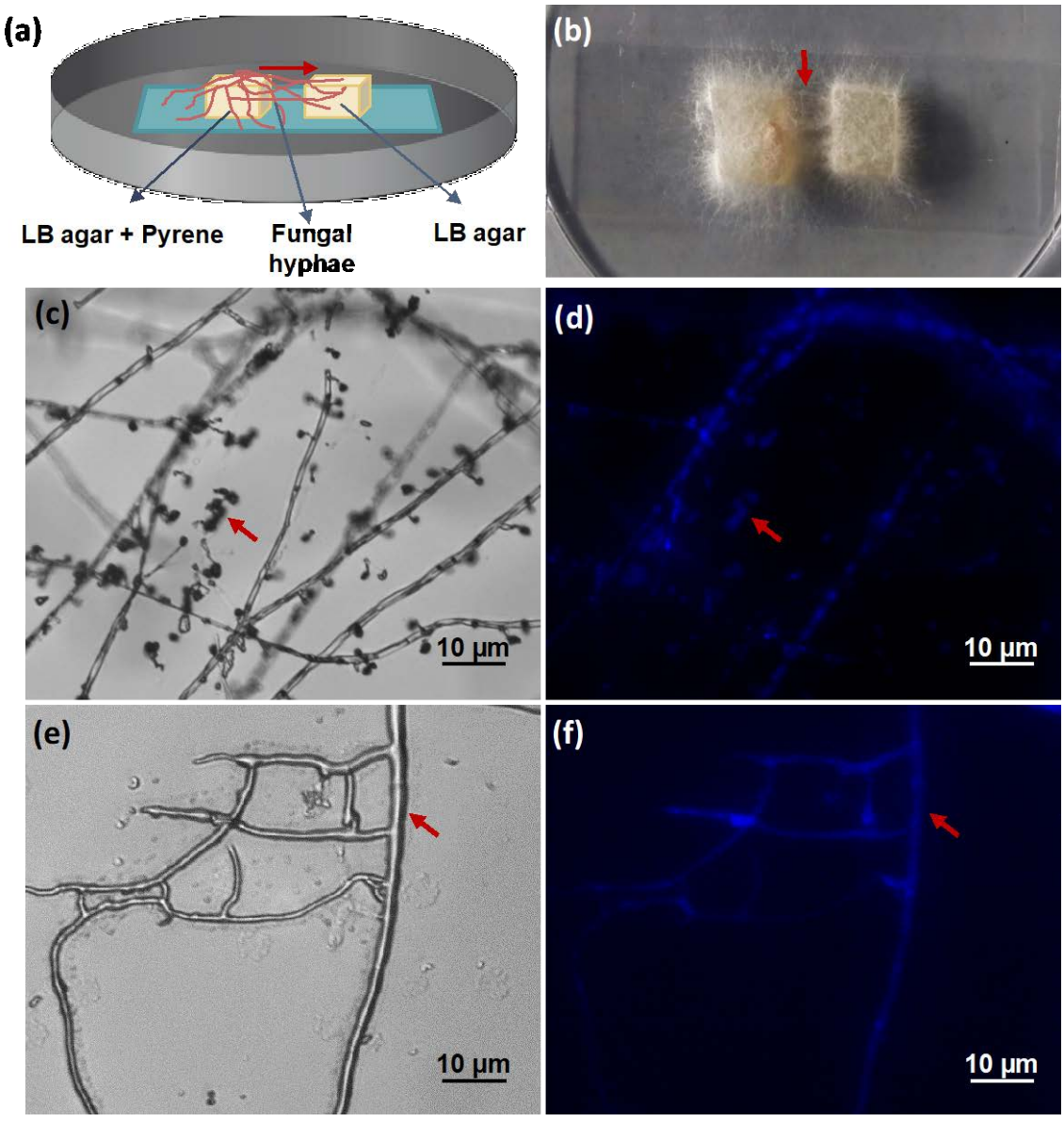
862 **Fig. 5**



863
864
865

866 **Fig. 6**

867
868



869