



HAL
open science

Screening and metabolic potential of fungal strains isolated from contaminated soil and sediment in the polychlorinated biphenyl degradation

J. Germain, M. Raveton, M.N. Binet, B. Mouhamadou

► **To cite this version:**

J. Germain, M. Raveton, M.N. Binet, B. Mouhamadou. Screening and metabolic potential of fungal strains isolated from contaminated soil and sediment in the polychlorinated biphenyl degradation. *Ecotoxicology and Environmental Safety*, 2021, 208, pp.111703 -. 10.1016/j.ecoenv.2020.111703 . hal-03493947

HAL Id: hal-03493947

<https://hal.science/hal-03493947>

Submitted on 15 Dec 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

Screening and metabolic potential of fungal strains isolated from contaminated soil and sediment in the polychlorinated biphenyl degradation

Germain J, Raveton M, Binet MN, Mouhamadou B*

Laboratoire d'Ecologie Alpine, UMR 5553 CNRS/USMB Université Grenoble Alpes, 38058
Grenoble Cedex 9, France

*Corresponding author. Tel: +33 476 63 54 41

E-mail: bello.mouhamadou@univ-grenoble-alpes.fr Laboratoire d'Ecologie Alpine UMR
5553 CNRS / USMB Université Grenoble Alpes, 38058 Grenoble Cedex 9 ; France

Abstract

Polychlorinated biphenyls (PCBs) are widespread persistent pollutants deleterious for environment and very dangerous for human kind. As the bioremediation of PCB polluted sites by model white-rot fungi is still unsatisfactory, the use of efficient native strains which have the natural capacity to develop on polluted sites may constitute a relevant alternative strategy. In this study, we isolated 12 fungal strains from PCB contaminated soil and sediment, improved the screening method to obtain the most efficient ones in biodegradation and detoxification of PCBs and characterized potential underlying enzymatic activities. Four strains *Penicillium chrysogenum*, *P. citreosulfuratum*, *P. canescens* and *Aspergillus jensenii*, showed remarkable biodegradation capacities, greater than 70%. The remaining PCB-toxicity of their culture, including that of *Trametes versicolor* and *Acremonium sclerotigenum*, which present interesting ecological and metabolic properties, was studied. Only *P. canescens* was able to significantly reduce the toxicity related to PCBs and their metabolites. The enzymatic activities induced by PCBs were different according to the strains, namely laccases in *T. versicolor* and peroxidases in *Ac. sclerotigenum*. Our promising results show that the use of native fungal strains can constitute an effective strategy in the depollution of PCB polluted sites.

Keywords: Polychlorinated biphenyls; Native fungal strains, Biodegradation; Toxicity; Enzymatic activities.

1. Introduction

Remediation of soil contaminated with organic pollutants has become one of the major challenges of our modern society in recent years. Industrial processes, agricultural practices and the use of chemicals in many areas of daily life are responsible for the deliberate or accidental release of potentially toxic molecules into the environment. These organic compounds are found in the atmosphere, in water and in many cases in soils. PCBs are part of these organic pollutants. They are organochlorine compounds consisting of a biphenyl ring substituted by one or more chlorine atoms with 209 possible congeners differing in the number of chlorine atoms and their position on the biphenyl ring. They have been used worldwide for a variety of applications since the 1930s in industry, particularly because of their electrical resistance, low reactivity and are currently at the root of many localized or diffuse contaminations (Liu et al., 2015; Passatore et al., 2014). PCBs belong to the most common and persistent organic pollutants (UNEP, 2017). They are deleterious to environment through their bioaccumulation and biomagnification potential (Fagervold et al., 2007) and very toxic to humans (ATSDR, 2000; Ross, 2004; Svobodová et al., 2009) as for instance the dioxin-like PCBs which were classified as carcinogenic by the International Agency for Research on Cancer (IARC).

Current strategies for the cleanup of PCB-contaminated matrices are based primarily on thermal desorption or incineration at high temperatures (Liu et al., 2015; Stella et al., 2017). Since these methods are expensive, energy-consuming and capable of generating toxic degradation products, alternative biological methods using plant or microorganisms with a real effectiveness and environmentally friendly are needed.

Fungal strains represent interesting biological resources for PCB remediation because of their remarkable ecological and metabolic properties. They represent about 75% of soil microbial biomass, colonize any type of habitat and are able to tolerate extreme environmental

conditions characterized among others by significant changes in pH, temperatures, presence of water stress or environmental pollutants (Harms et al., 2011). They have various low specificity enzymes including extracellular (peroxidases, phenol oxidases such as laccases) and intracellular (cytochrome P450 monooxygenases, nitroreductases) enzymes capable of degrading a wide range of environmental pollutants (Baldrian, 2008; De et al., 2006; Harms et al., 2011). Although they exhibit real effectiveness in PCB removal under laboratory conditions in liquid medium, their use in solid substrates remains unsatisfactory. This stumbling block, observed in both ligninolytic Basidiomycetes fungi and filamentous Ascomycetes fungi, may be due to the very nature of the used fungal strains that is, less effective strains in biodegradation (Elangovan et al., 2019; Tomasini and Hugo León-Santiesteban, 2019), the lack of information regarding their enzymes involved in PCB biodegradation (Harms et al., 2011; Tigini et al., 2009) and the nature of the contaminated substrates including the presence of antagonistic microorganisms regarding the used fungal strains, the concentration of PCBs and their low bioavailability (Baldrian, 2008).

While the issue due to the nature of substrates can be overcome by treating them adequately using appropriate surfactants, by sterilizing them or by adding additional nutrients (Baldrian, 2008; Steffen et al., 2007), the use of efficient fungal strains requires both the isolation of native fungal strains potentially adapted to the substrates to be depolluted, the establishment of effective screening methods and the characterization of enzymes induced by PCBs that may be involved in their biodegradation.

So far, most of the screening methods performed on fungal strains to characterize the most effective ones in PCB biodegradation are essentially based on the disappearance of PCBs in their culture media which may be resulting from PCB biosorption or biodegradation without taking into account the presence of metabolites derived from PCBs or the decrease in toxicity of their culture media. Although some metabolites resulting from the fungal biodegradation of

PCBs have been described in literature (Čvančarová et al., 2012; Kamei et al., 2006), their research in culture media can prove to be tedious (Tigini et al., 2009) and is not facilitated by the fact that they differ according to the strains since the mechanisms of PCB biodegradation, not yet clearly elucidated, seem to depend on the studied strains or the PCB congeners (Chun et al., 2019; Fujihira et al., 2009). Regarding the ecotoxicity of fungal culture media, only few studies have been reported, but they have shown a link between the effectiveness of the fungal strains in degrading PCBs and their capacity to suppress the toxicity associated to PCBs in their culture media throughout the incubation period (Čvančarová et al., 2012; Sadaňoski et al., 2019). Hence, bioassay tests seem to be an interesting alternative to complete and improve the screening method. Moreover, they are easy to develop and have shown in many cases that PCBs adversely affect the development of numerous organisms including species belonging to the Basidiomycetes fungi of the *Ganoderma* genus (Sadaňoski et al., 2018).

The aim of this study was to obtain indigenous efficient fungal strains in the biodegradation of PCBs and to characterize the enzymatic activities potentially involved in their biodegradation. Fungal strains were isolated from PCB polluted sediment and soil and identified using morphological and molecular criteria. Their capacity to degrade PCB congeners was investigated in liquid cultures. The ecotoxicity of the cultures of most efficient strains as well as the laccase and peroxidase activities were assessed in the liquid cultures of the most efficient strains.

2. Materials and methods

2.1 PCBs

The 7 PCB congeners PCB 28, 52, 101, 118, 138, 153, and 180 were obtained from Sigma Aldrich Corp. (St. Louis, MI, USA). A stock solution containing 57.5 µg/mL of each PCB in DMSO (99.99%) was prepared.

2.2 Fungal strains

The strains used in this study were isolated both from PCB-contaminated soil (concentration of 7 PCB indicators: 800 mg/kg, organic matter: 3.61%, pH = 8.21) sampled from a former industrial site (GPS: 46°17'54.0"N 6°18'23.6"E), supplied by SERPOL (groupe SERFIM) and from PCB contaminated sediment (concentration of 7 PCB indicators: 3 mg/kg, total carbon: 10.50%; total nitrogen: 0.28%; pH = 6.9) sampled from the Bourget Lake near Annecy (France, GPS: 45°43'23.5"N 5°52'06.3"E). From the PCB polluted soil, the strains were isolated by the suspension / dilution method (Périgon et al., 2019). Briefly, 1 g of contaminated soil was added to 9 mL of sterile water containing 0.05% of sodium dodecyl sulfate (w/v). After stirring, aliquots of the suspension were serially diluted and spread onto Petri dishes containing malt extract agar (1.5% w/v). Cultures were incubated at 26°C. After isolation, fungal strains were identified by microscopic approach according to their vegetative apparatus and their asexual reproductive features (Booth, 1977a, 1977b; Domsch et al., 1980; Ellis, 1971). Regarding the Bourget lake sediment, a fragment of carpophore developing on this substrate was isolated and cultured onto Petri dishes containing malt extract agar (1.5% w/v). All these strains are conserved in the culture collection of the University Grenoble Alpes (CMPG Collection Mycology Pharmacy Grenoble).

2.3 DNA extraction and PCR

Total DNA of each strain was extracted using a Fast DNA Spin Kit (QBIogene, Germany) according to the manufacturer's recommendations. The PCRs were carried out according to the conventional protocols using Ampli Taq Gold DNA polymerase (Applied Biosystems, USA) and the primers ITS4 and ITS5 (White et al., 1990) synthesized by Eurogentec (Seraing, Belgium) were used to amplify the fungal ITS regions. Amplifications were carried out in 50 μ L reaction mixtures as described by Mouhamadou et al. (2013).

2.4 Sequencing and sequence analysis

The PCR product of each strain was sequenced by Genewiz (Leipzig, Germany). Comparisons with sequences from the GenBank databases were made using the BLAST search algorithm (Altschul et al., 1990). Phylogenetic analyses were carried out using the neighbor-joining method (Saitou and Nei, 1987), based on Clustal W alignments and the robustness of tree topologies was evaluated by performing bootstrap analysis of 10000 data sets using MEGA 3.1 (Tamura et al., 2007).

2.5 Analyses of PCB degradation

The mycelium of each strain, grown in malt extract (1.5% w/v), was introduced into Erlenmeyer flasks containing 20 mL of modified GS liquid medium (Galzy and Slominski, 1957) supplemented with glucose (5 g/L) and incubated at 26°C on a rotary shaker (100 rpm). After 48 h incubation, the culture of each strain was spiked with 400 μ L of a stock solution of PCB mix giving 1 μ g/mL of each of the 7 congeners and incubated for 5 days at 26°C with agitation (100 rpm). Abiotic cultures (culture medium with only PCBs) and heat killer controls "HKC" (culture medium with PCBs, inoculated with the mycelium treated by 2 autoclaving cycles at 110°C, 10 min) were performed under the same conditions. Each experiment was performed in

triplicate. After 5 days incubation, the mycelium was separated from the culture medium by filtering through Whatman 40 filter. Abiotic controls were analyzed as they were.

2.6 PCB extraction and HPLC analyses

Culture medium and fungal mycelium were extracted separately. Culture medium was extracted three times with 20 mL of hexane:acetone (60:40 v/v) with agitation (250 rpm, at room temperature) for 30 min. To minimize the phenomenon of PCB biosorption, mycelium was first homogenized in the serum bottles with a Potter homogenizer in 20 mL of hexane, vigorously agitated for 10 min and incubated 24 h at room temperature with agitation (250 rpm). The organic phases from culture medium (used for the extraction of PCBs from the liquid medium) and mycelium (used for the extraction of PCBs from mycelium) were evaporated after extraction under vacuum using a rotary evaporator and adjusted to a volume of 5 mL (Mouhamadou et al., 2013). After evaporation of both organic phases, PCBs were analyzed in the “Institut de Chimie Moléculaire of Grenoble”. Aliquots of 1 μ L from culture medium or from mycelium were analyzed using a gas chromatograph (5977-7890B), equipped with a HP-5MS 5 phenyl methyl silox column (ID: 0.25 mm, Length: 30 m, Film: 0.25 mm) and a quadrupole detector (Agilent Technologies USA). The carrier gas was helium. The injector temperature and the transfer line temperature were 250°C and 280°C, respectively. The initial column temperature was 65°C held for 2 min. The gradient setting was 30°C/min to 240°C, held for 2 min at 240°C then 10°C/min to 340 °C. The final temperature was 340°C held for 5 min. The applied method of PCB analysis was validated on the basis of an internal standard, PCB 138 (2,2',3,4,4',5'-hexachlorobiphenyl).

The level of PCBs biodegraded by the fungal strains was determined by comparing the residual PCB level of HKC (residual PCBs of the culture medium and of the killed mycelium) and that of alive mycelium.

2.7 Extracellular enzymatic activities

Laccase and peroxidase activities were monitored in the culture medium of the strains of interest. The mycelium of each strain, grown in malt extract (1.5% w/v), was introduced into Erlenmeyer flasks containing 20 mL of modified GS liquid medium (Galzy and Slominski, 1957) supplemented with glucose (5 g/L) and incubated at 26°C on a rotary shaker (100 rpm). After 48 h incubation, the culture of each strain was spiked with 400 µL of a stock solution of PCB mix in DMSO (99.99%) giving 1 µg/mL of each of the 7 congeners and incubated for 5 days at 26°C with agitation (100 rpm). In parallel, control cultures were carried out by replacing the PCB solution by 400 µL of DMSO (99.99%) or 400 µL of H₂O, respectively. 20 mL of culture medium were harvested from the culture of each strain as in the biodegradation test and concentrated using Macrosep Advance centrifugal filters (VWR) with omega membrane 10K (MWCO 10 kD). Protein content was estimated by the Bradford procedure (Bradford, 1976). Laccase activities were investigated by determining the oxidation of both guaiacol and ABTS (2,2'-Azinobis (3-Ethylbenzthiazoline-6-Sulfonate)) (Abd El Monssef et al., 2016; Karp et al., 2012). For guaiacol ($\epsilon_{470} = 6400 \text{ M}^{-1}\text{cm}^{-1}$), reaction mixtures (300 µL) contained 0.1 M acetate buffer (pH 4), 10 mM of guaiacol in 96% ethanol and 60 µL of an aliquot of the concentrated culture liquid corresponding to 0.6 µg of total protein. For ABTS ($\epsilon_{420} = 36\,000 \text{ M}^{-1}\text{cm}^{-1}$), reaction mixtures (1 mL) contained 1 M acetate buffer (pH 4), 20 mM of ABTS and 20 µL of an aliquot of the concentrated culture liquid. Laccase activities were measured spectrophotometrically every 5 min during 3 h using Varioskan (Thermoscientific, USA) for guaiacol and every 2 min during 16 min using spectrophotometer (Ultrospec 3100 pro, Amersham Biosciences) for ABTS.

Peroxidase activities were determined under the same conditions as those for laccases with ABTS as substrate but in the presence of H₂O₂ (30% v/v) and were calculated by subtracting ABTS oxidation without H₂O₂.

2.8 Ecotoxicity tests

The ecotoxicity tests were performed overall according to the protocol described by Sadañoski et al (2019) with modifications. Each alive or heat-killed fungal strain was incubated in the presence of the 7 PCBs for 7 days under the same conditions as in the biodegradation test. Culture medium and mycelium were extracted with hexane:acetone (60:40 v/v) and hexane, respectively, as described above. Both extracts, combined to obtain the totality of residuals PCBs and their metabolites, were evaporated at room temperature before being suspended in 500 μ L of absolute ethanol. For the toxicity test of products resulting from PCB biodegradation, 100 μ L of extracts in ethanol were spread on the surface of Petri dishes containing 25 mL of malt extract agar (1.5% w/v) and one agar plug (25 mm²) of *Ganoderma lucidum* (BRFM 1601), provided by UMR 1163 Biodiversité et Biotechnologie Fongiques Marseille, France, previously cultivated in malt extract agar (1.5% w/v) was placed in the center of Petri dishes. After 4 days' incubation, we performed four measurements from the center of each dish (*G. lucidum* inoculation point) to the limit of the growth zone and averaged these measurements to estimate the growth rate of *G. lucidum*. In parallel, for each strain, we used two independent controls consisting only of the culture of the alive or heat-killed fungal strain without PCB, performed according to the same protocol described above, allowing us to deduce the toxicity linked only to PCBs and/or their metabolites in ethanol. Finally, the growth rate of *G. lucidum* (determined based on the colony diameter) linked only to PCBs and/or their metabolites was obtained by comparing it with that obtained from a control (solvent control) in which *G. lucidum* was incubated in malt extract agar (1.5% w/v) containing only 100 μ L of ethanol. All these experiments were carried out in four replicates.

2.9 Statistical analyses

Normality of the data was tested by Shapiro-Wilk test. Accordingly, PCB biodegradation was analyzed by Welch test (unequal variances); laccase activity and ecotoxicity tests were analyzed by one-way ANOVA test followed by Tukey test; fungal biomass was analyzed by Kruskal Wallis test and peroxidase activities were analyzed by Kruskal Wallis test followed by pairwise Wilcox test. The analyses of PCB biodegradation by class of congeners according to the strains were assessed by Permanova. All statistical analyses were performed using R package version 3.6.1.

3. Results

3.1 Identification and phylogenetic characteristic of the studied strains

Fungal strains were isolated from PCB contaminated soil by the suspension / dilution method. Eleven strains exhibiting different macroscopic and microscopic features were found and first identified by their morphological characteristics using microscopic observations. Molecular analysis showed that the 11 strains were distributed into six Ascomycota genera *Penicillium*, *Aspergillus*, *Acremonium*, *Stachybotrys*, *Fusarium* and *Trichoderma* thus confirming their taxonomic identification (Fig 1). The carpophore isolated from the Bourget Lake sediment belonged to the Basidiomycota *Trametes versicolor* (Fig 1).

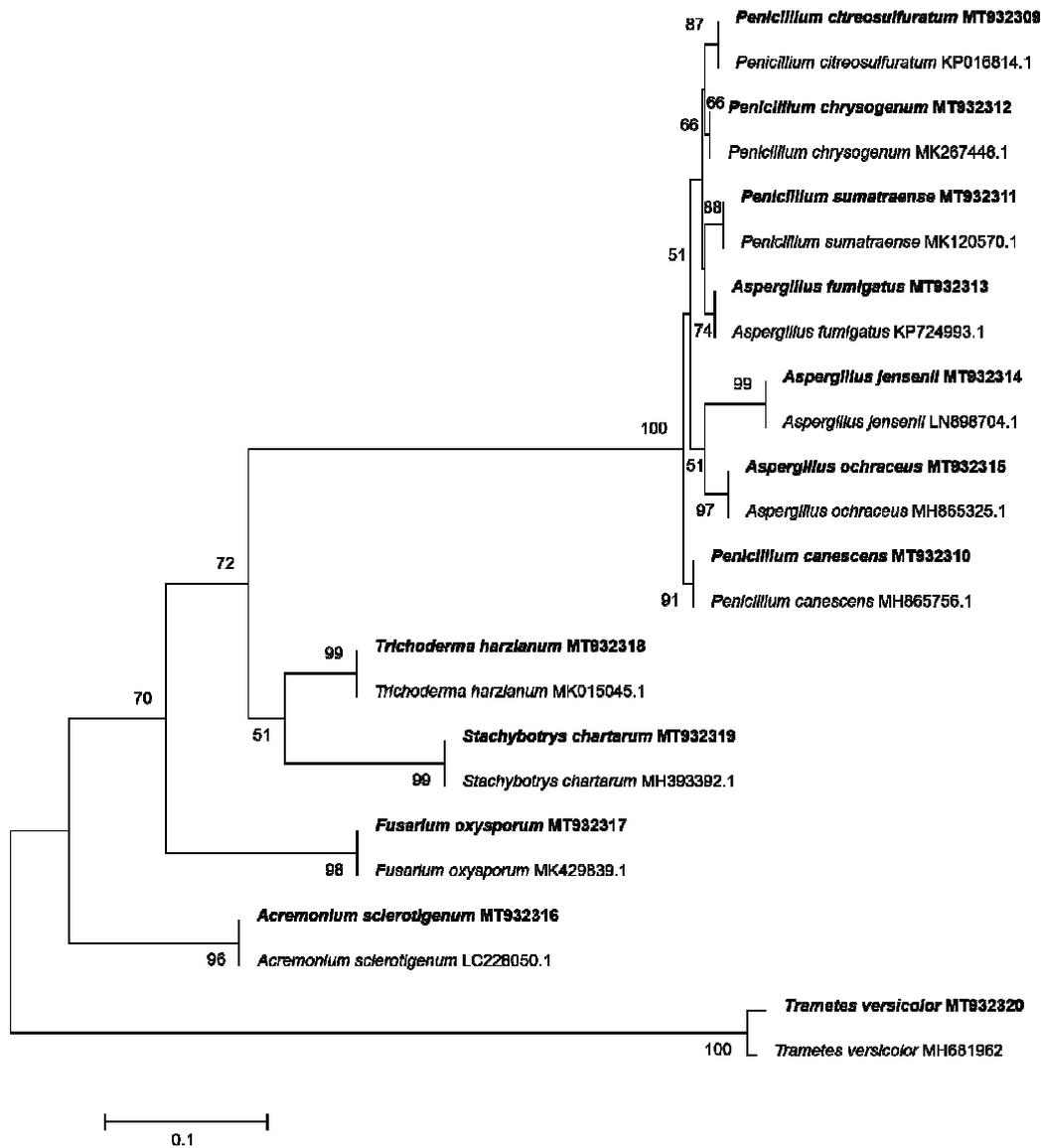


Fig. 1. Consensus trees obtained using the neighbour-joining method based on aligned ITS sequences. Bootstrap values are shown on the branches. The accession numbers of reference sequences which have been recovered from GenBank are indicated. The strains isolated in this study and their accession numbers are indicated in bold.

3.2 Biodegradation potential of the isolated strains

Screening to obtain effective fungal strains in PCB biodegradation was carried out on 12 strains (Table 1).

Strains	Quantity of residual from the culture of live mycellum (μg)		Quantity of residual from heat treated mycellum (μg)		% PCB removal
<i>P. chrysogenum</i>	3.30	\pm 0.17	96.03	= 17.07	96.56**
<i>P. citreosulfuratum</i>	5.63	\pm 3.79	113.94	= 26.13	95.06*
<i>A. jensenii</i>	14.46	\pm 3.13	90.69	= 3.14	84.06*
<i>P. canescens</i>	17.35	\pm 1.76	74.50	= 10.92	76.72**
<i>A. ochraceus</i>	18.13	\pm 0.33	56.70	= 3.41	68.02**
<i>Ac. sclerotigenum</i>	59.97	\pm 17.74	111.74	= 1.45	46.33
<i>T. versicolor</i>	29.56	\pm 3.29	41.04	= 1.44	27.97
<i>P. sumatrense</i>	63.50	\pm 11.00	85.20	= 21.94	25.47
<i>Tr. harzanium</i>	13.13	\pm 1.26	14.80	= 0.78	11.28
<i>F. oxysporum</i>	39.64	\pm 5.04	43.52	= 9.80	8.92
<i>S. chartarum</i>	45.02	\pm 3.42	47.81	= 3.10	5.83
<i>A. fumigatus</i>	64.21	\pm 3.12	40.65	= 10.31	-

Table 1. Quantity of residual PCBs in fungal cultures and corresponding percentage of removal after 7 days of incubation. The results are expressed as means \pm standard deviation (n=3). (Welch test; p-value: * < 0.05 and **: < 0.01).

Results on PCB biodegradation were variable according to the strains. Four strains showed significant levels of biodegradation, greater than 70%. They were *P. chrysogenum*, *P. citreosulfuratum*, *A. jensenii* and *P. canescens*. The biodegradation rate was intermediate between 70% and 20% in *A. ochraceus*, *Ac. sclerotigenum*, *T. versicolor* and *P. sumatrense* and low, not significantly exceeding 20% in *Trichoderma harzanium*, *F. oxysporum*, *S. chartarum* and *A. fumigatus*.

The four strains showing biodegradation rates greater than 70% were considered in our study as effective strains in PCB degradation; they were used for further analyses. We also included *T. versicolor*, a ligninolytic species widely described as able to efficiently degrade PCBs and various environmental pollutants (Cajthaml et al., 2009; Ruiz-Aguilar et al., 2002; Zeddel et al., 1993) and *Ac. sclerotigenum* usually described as plant endophyte fungus (Lo Piccolo et al., 2015; Prathyusha et al., 2015) whose PCB degradation capacity has never been shown.

3.3 Tolerance and biodegradation according to the PCB congeners

The tolerance of each of the six selected strains towards PCBs was assessed by comparing the mycelial dry weight of each strain incubated in presence or in absence of PCBs. There were no statistically significant differences in growth of any fungal strains when grown in the presence or absence of PCBs (Fig 2).

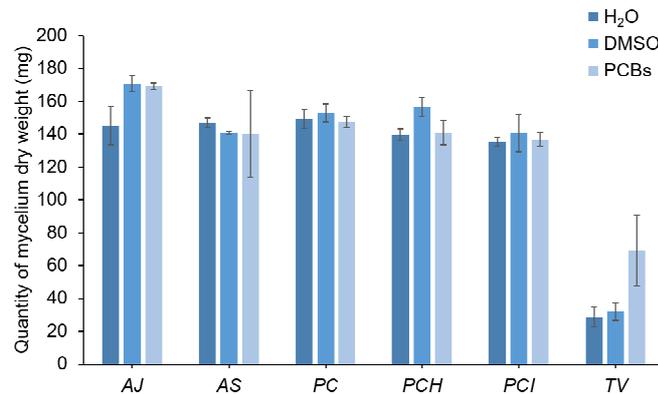


Fig. 2. Mycelium dry mass of six selected strains after 7 days of incubation in presence or absence of PCBs. Bars indicate mean \pm standard deviation (n=4). AJ : *Aspergillus jensenii*, AS : *Acremonium sclerotigineum*, PC : *Penicillium canescense*, PCH : *Penicillium chrysogenum*, PCI : *Penicillium citreosulfuratum*, TV : *Trametes versicolor*.

The biodegradation rate of each class of PCB congeners grouped according to their number of chlorine was determined. Each strain was able to significantly degrade PCBs from tri- to hexachlorinated PCB and there were no significant differences in biodegradation rates according to the class of congeners (Fig 3).

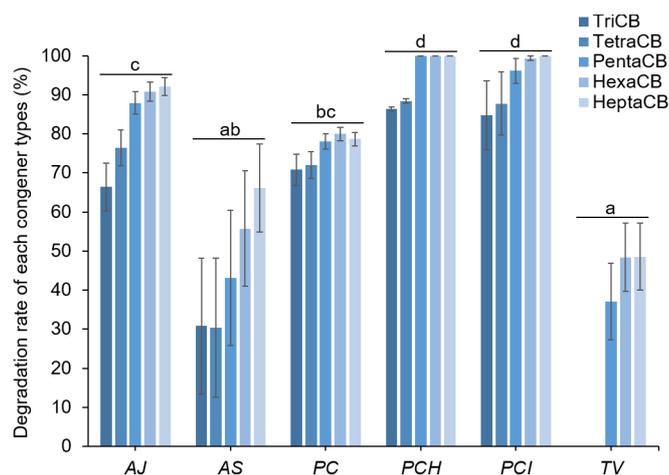


Fig. 3. Percentage of degradation of each type of congeners of six selected strains after 7 days of incubation. Bars indicate mean \pm standard deviation (n=3). Different small letters indicate significant differences between strains but not between type of congeners within strains (Permanova analysis; p-value < 0.05). AJ : *Aspergillus jensenii*, AS : *Acremonium sclerotigenum*, PC : *Penicillium canescense*, PCH : *Penicillium chrysogenum*, PCI : *Penicillium citreosulfuratum*, TV : *Trametes versicolor*.

3.4 Extracellular enzymatic activities

The laccase and peroxidase activities described as involved in the biodegradation of various environmental pollutants were assessed in the culture medium of selected strains. As for laccase activity, for both guaiacol and ABTS substrates, it was only detected in *T. versicolor* culture medium and was strongly expressed, about 10 times greater compared to the controls (enabling to determine the basal activity (H₂O) or that induced by DMSO (Fig. 4A, 4B). Regarding the peroxidase activity, it was detected only in *Ac. sclerotigenum* (Figure 4C) and *P. canescens* (Figure 4D) and solely in the presence of PCBs and in water, respectively.

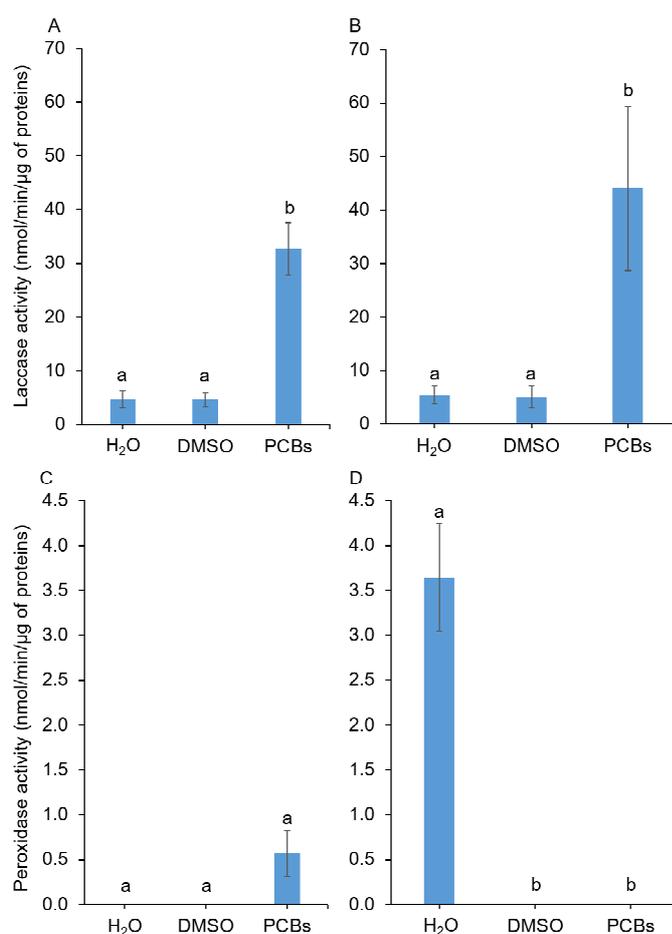


Fig. 4. Laccase and peroxidase activities in liquid media after 7 days of incubation in presence or absence of PCB. A : Laccase activity of *T. versicolor* with guaiacol, B : Laccase activity of *T. versicolor* with ABTS, C : Peroxidase activity of *Ac. sclerotigenum*, D : Peroxydase activity of *P. canescens*. Controls, H₂O and DMSO enable the determination of basal activity or activity induced by DMSO, respectively. Bars indicate mean \pm standard deviation (n=4). Values sharing different small letters are significantly different (Tukey test; p-value < 0.05) for laccase activity and (Wilcox test; p-value < 0.05) for peroxydase activity.

3.5 Toxicity of residual PCBs and their metabolites

The toxicity of the residual PCBs and their metabolites in the cultures of the 6 selected strains were analyzed in solid culture using *G. lucidum* as the sensitive fungal strain. Two strains showed significantly different results (Fig 5). While in *P. canescens*, a higher growth rate of *G. lucidum* with extracts from the alive strain (5.2% higher in comparison to the killed strain) was

obtained suggesting a decrease in the toxicity of PCBs and their metabolites by this strain, in *P. chrysogenum*, the opposite results were obtained with a lower growth rate of *G. lucidum* (9.2% weaker) with extracts from the alive strain. The results obtained in *A. jensenii*, *Ac. sclerotigenum*, *P. citreosulfuratum* and *T. versicolor* did not show significant differences in the growth rate of *G. lucidum* depending on whether the extracts arose from the alive strain or the heat-killed strain (Fig 5).

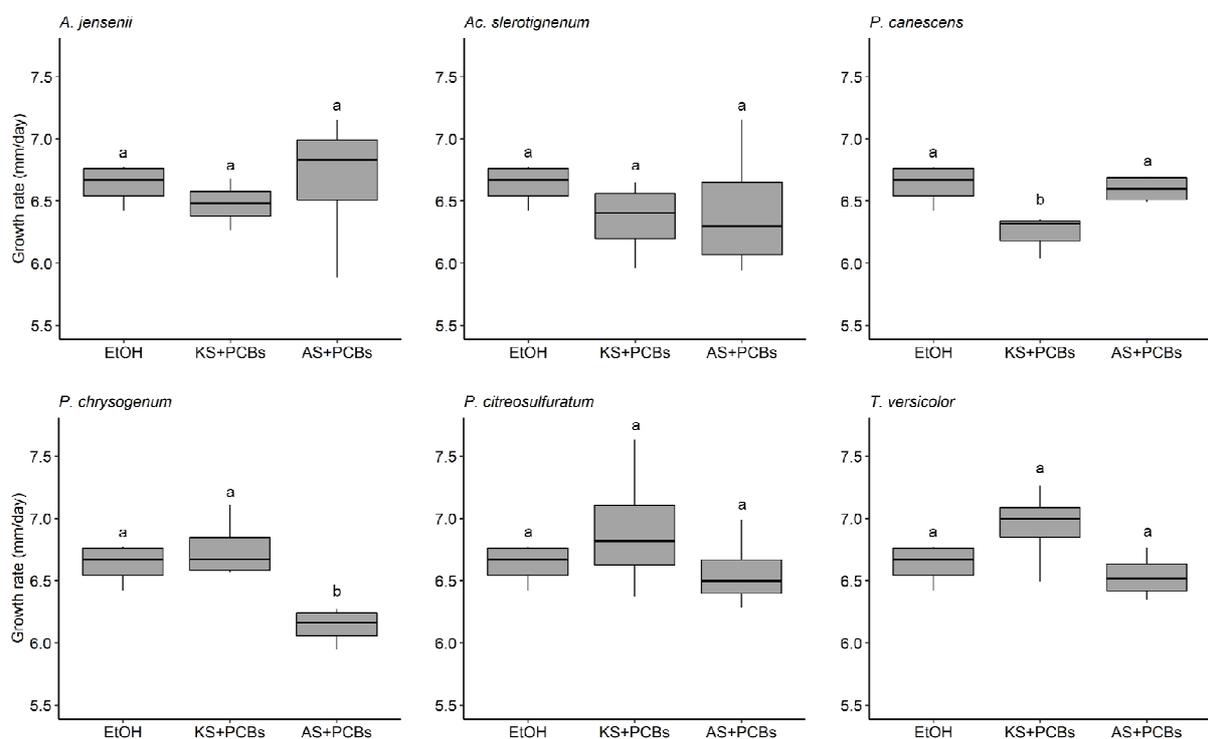


Fig. 5. Box-plots showing the toxicity of the culture of the six selected strains. The lower and upper boundaries of each box enclose 25±75% of the data. The line within the box shows the median value, the bar lines above and below the boxes indicate minimum and maximum values. EtOH: ethanol; KS + PCB: Killer strain incubated with PCBs; AS + PCB: Alive strain incubated with PCB. Different small letters indicate significant differences between values (Tukey test; p-value < 0.05).

4. Discussion

While ligninolytic fungi have been thoroughly studied in PCB mycoremediation and have shown a high capacity for biodegradation in liquid medium, their application to contaminated soils has remained unsatisfactory (Baldrian, 2008; Chun et al., 2019; Harms et al., 2011). The use of native strains able to grow in the polluted substrate could constitute an interesting alternative if they were endowed with a real capacity to degrade PCB and detoxify polluted sites under appropriate environmental conditions. In this respect, we isolated fungal strains from PCB polluted soil and sediment and improved a screening protocol to characterize the most effective ones in the biodegradation of PCBs both by quantifying the residual PCBs in the culture medium and in the mycelium of each strain (Mouhamadou et al., 2013), the latter being caused by the PCB biosorption, by developing and using heat-killed controls to better taking into account the biosorption process (Tigini et al., 2009) and by supplementing the screening methods with the ecotoxicity tests of the culture of the most efficient fungal strains (Sadañoski et al., 2018). No study to our knowledge has combined such strategies for characterizing efficient native fungal strains in the PCB biodegradation. Eleven species of Ascomycetes were obtained from soil reflecting a low fungal diversity of the PCB-polluted soil as reported elsewhere (Mouhamadou et al., 2013; Tigini et al., 2009). The high concentration of pollutant (>800 mg/kg) and the soil deficiency in nutritive substances may probably explain this low fungal diversity (Baldrian, 2008; D'Annibale et al., 2006; Di Toro et al., 2006).

The ability of the 12 studied strains including *T. versicolor*, isolated from a polluted sediment, to biodegrade PCBs was different and varied considerably between strains. While it was insignificant in *A. fumigatus*, all the PCBs that had disappeared in the culture medium being due to biosorption, it was remarkable in four strains resulting in biodegradation rates greater than 70%. The biodegradation capacity of *P. chrysogenum* displaying here the highest rate of

PCB biodegradation, has already been reported (Tigini et al., 2009) (SM 1) and the variations reported in different studies may be due to the specificity of each strain or to the extraction procedure. Conversely, for the three other species *P. citreosulfuratum*, *P. canescens* and *A. jensenii*, these are innovative results. To our knowledge, apart from model ligninolytic fungi, only 7 species of filamentous fungi isolated from PCB polluted substrates and able to degrade PCBs at rates > 70% have been described in the literature (SM 1), highlighting the interest of searching new native strains in the biodegradation of PCBs.

To identify which ones, among the four efficient strains, were able to detoxify PCBs and/or produce less toxic metabolites and to characterize the potential underlying enzymatic activities, we studied the growth of *G. lucidum* in the presence of products resulting from the biodegradation of these strains and characterized the extracellular enzymes induced by PCBs. We included *Ac. sclerotigenum* and *T. versicolor*, which admittedly showed lower rates of biodegradation, but presented the interesting features described above.

Only *P. canescens* was able to reduce the toxicity related to PCBs, confirming its biodegradation capacities and suggesting that the metabolites generated were less toxic than the corresponding parent PCBs. These results are comparable to those obtained with the white-rot fungal Basidiomycetes *Pleurotus ostreatus* and *Pleurotus sajor-caju* for example, efficient strains in PCB removal, which showed a reduction in the toxicity of PCBs and their metabolites in their culture (Čvančarová et al., 2012; Sadañoski et al., 2019). The opposite results were observed in *P. chrysogenum* demonstrating that the generated metabolites were different from those obtained in *P. canescens* and were more toxic than the corresponding PCBs (Bhalla et al., 2016; Blasco et al., 1997). The toxicity of certain PCB metabolites to several organisms such as hydroxyl-PCBs has been reported (Carlson and Williams, 2001; Fängström et al., 2002; Mortensen et al., 2007). As far as the other strains were concerned, no change of toxicity was observed in their culture suggesting that the metabolites they produced in 7 days of incubation

were as toxic as the PCBs whose potential degradation had been observed. Consequently, our results also suggest that the biodegradation pathways of PCBs by the studied strains are different and involve various enzymatic activities as reported by numerous studies (Čvančarová et al., 2012; Harms et al., 2011; Pérignon et al., 2019). Interestingly, laccase activity, was detected only in *T. versicolor* and was relatively strongly induced by these pollutants suggesting that this enzyme could be involved in the biodegradation of PCBs and their metabolites by this strain as described in literature (Keum and Li, 2004; Takagi et al., 2007). This induction rate (10% compared to the control) was intermediate when comparing to those described by other studies (Supplementary material 2) and seemed to depend on the studied strains. Likewise, the peroxidase activity potentially involved in the biodegradation of PCBs was observed in *Ac. sclerotigenum* and seemed different from that observed in *P. canescens* possibly not involved in the biodegradation of PCBs in this species. For the other strains, we were unable to detect these extracellular enzyme activities and the biodegradation of PCBs by these strains could involve other enzyme systems, especially intracellular ones (Harms et al., 2011). The presence of toxic metabolites in *P. chrysogenum* that could correspond to hydroxyl-PCB supports this hypothesis as these metabolites seem to be produced by cytochrome P₄₅₀ enzymes (Kamei et al., 2006; Matsuzaki and Wariishi, 2005). Nevertheless, despite these different activities according to the strains, they all share a common property, the potential to degrade PCBs independently of their degree of chlorination. This property is an important advantage of the fungal system in the biodegradation of PCBs compared to *e.g.* bacterial systems described as ineffective on highly chlorinated PCBs (Ohtsubo et al., 2000; Passatore et al., 2014).

Conclusion

Our study shows that the native filamentous strains present interesting potentials for the depollution of sites polluted by PCBs, a major deleterious pollutant for environment and very dangerous for human kind. They are insensitive to the PCB-linked toxicity as described by Federici et al., (2012) and naturally have the advantage to develop easily on PCB-polluted substrates conversely to white-rot basidiomycetes fungi (Baldrian, 2008). They also have the ability to degrade PCBs through their enzymatic systems specific to each strain. *P. canescens* is additionally able to detoxify PCBs and their metabolites, making it a choice candidate for the PCB contaminated soil depollution. It would be interesting to characterize the metabolic pathways of PCB degradation by this strain and to use it alone or in consortium with other effective strains for the bioremediation of PCB polluted sites.

Acknowledgments

We gratefully acknowledge financial supports of CNRS projet Prematuration and the Agence de l'Environnement et de la Maîtrise de l'Energie (ADEME). We also thank the SERPOL company and in particular Antoine Joubert and Pascal Pomarede for the supply and sampling of PCB polluted soil. We also thank the Mass Spectrometry Platform of the Institut de Chimie Moléculaire de Grenoble for the PCB analyses and the UMR 1163 Biodiversité et Biotechnologie Fongiques (Marseille) for providing us the *Ganoderma lucidum* strain. We are deeply grateful to Viviane Barbreau for her close reading of the manuscript, her analysis and critical remarks. We address our special thanks to Nael, Ilian and Lila for their help and unconditional support.

References

- Abd El Monssef, R.A., Hassan, E.A., Ramadan, E.M., 2016. Production of laccase enzyme for their potential application to decolorize fungal pigments on aging paper and parchment. *Ann. Agric. Sci.* 61, 145–154. <https://doi.org/10.1016/j.aoas.2015.11.007>
- Altschul, S.F., Gish, W., Miller, W., Myers, E.W., Lipman, D.J., 1990. Basic local alignment search tool. *J. Mol. Biol.* 215, 403–410. [https://doi.org/10.1016/S0022-2836\(05\)80360-2](https://doi.org/10.1016/S0022-2836(05)80360-2)
- ATSDR, 2000. Toxicological Profile for Polychlorinated Biphenyls.
- Baldrian, P., 2008. Wood-inhabiting ligninolytic basidiomycetes in soils: Ecology and constraints for applicability in bioremediation. *Fungal Ecol.* 1, 4–12. <https://doi.org/10.1016/j.funeco.2008.02.001>
- Bhalla, R., Tehrani, R., Van Aken, B., 2016. Toxicity of hydroxylated polychlorinated biphenyls (HO-PCBs) using the bioluminescent assay Microtox®. *Ecotoxicology* 25, 1438–1444. <https://doi.org/10.1007/s10646-016-1693-z>
- Blasco, R., Mallavarapu, M., Wittich, R., Timmis, K.N., Pieper, D.H., 1997. Evidence that Formation of Protoanemonin from Metabolites of 4-Chlorobiphenyl Degradation Negatively Affects the Survival of 4-Chlorobiphenyl-Cometabolizing Microorganisms. *Appl. Environ. Microbiol.* 63, 427–434. <https://doi.org/10.1128/AEM.63.2.427-434.1997>
- Booth, C., 1977a. *Fusarium: Laboratory Guide to the Identification of the Major Species*. Commonwealth Mycological Institute, Kew.
- Booth, C., 1977b. *The Genus Fusarium*. Commonwealth Mycological Institute, Kew.
- Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72, 248–254. [https://doi.org/10.1016/0003-2697\(76\)90527-3](https://doi.org/10.1016/0003-2697(76)90527-3)
- Cajthaml, T., Křesinová, Z., Svobodová, K., Möder, M., 2009. Biodegradation of endocrine-disrupting compounds and suppression of estrogenic activity by ligninolytic fungi. *Chemosphere* 75, 745–750. <https://doi.org/10.1016/j.chemosphere.2009.01.034>
- Carlson, D.B., Williams, D.E., 2001. 4-hydroxy-2',4',6'-trichlorobiphenyl and 4-hydroxy-2',3',4',5'-tetrachlorobiphenyl are estrogenic in rainbow trout. *Environ. Toxicol. Chem.* 20, 351–358. <https://doi.org/10.1002/etc.5620200216>
- Chun, S.C., Muthu, M., Hasan, N., Tasneem, S., Gopal, J., 2019. Mycoremediation of PCBs by *Pleurotus ostreatus*: Possibilities and Prospects. *Appl. Sci.* 9, 4185. <https://doi.org/10.3390/app9194185>
- Čvančarová, M., Křesinová, Z., Filipová, A., Covino, S., Cajthaml, T., 2012. Biodegradation of PCBs by ligninolytic fungi and characterization of the degradation products. *Chemosphere* 88, 1317–1323. <https://doi.org/10.1016/j.chemosphere.2012.03.107>
- D'Annibale, A., Rosetto, F., Leonardì, V., Federici, F., Petruccioli, M., 2006. Role of Autochthonous Filamentous Fungi in Bioremediation of a Soil Historically Contaminated with Aromatic Hydrocarbons. *Appl. Environ. Microbiol.* 72, 28–36. <https://doi.org/10.1128/AEM.72.1.28-36.2006>
- De, S., Perkins, M., Dutta, S., 2006. Nitrate reductase gene involvement in hexachlorobiphenyl dechlorination by *Phanerochaete chrysosporium*. *J. Hazard. Mater.* 135, 350–354. <https://doi.org/10.1016/j.jhazmat.2005.11.073>
- Di Toro, S., Zanaroli, G., Fava, F., 2006. Intensification of the aerobic bioremediation of an actual site soil historically contaminated by polychlorinated biphenyls (PCBs) through bioaugmentation with a non acclimated, complex source of microorganisms. *Microb. Cell Factories* 5, 11. <https://doi.org/10.1186/1475-2859-5-11>
- Domsch, K.H., Gams, W., Anderson, T.H., 1980. *Compendium of Soil Fungi*. Academic Press, London.
- Elangovan, S., Pandian, S.B.S., S. J., G., Joshi, S.J., 2019. Polychlorinated Biphenyls (PCBs): Environmental Fate, Challenges and Bioremediation, in: Arora, P.K. (Ed.), *Microbial Metabolism of Xenobiotic Compounds*. Springer Singapore, Singapore, pp. 165–188. https://doi.org/10.1007/978-981-13-7462-3_8
- Ellis, M.B., 1971. *Dematiaceous Hyphomycetes*. Commonwealth Mycological Institute, Kew.

- Fagervold, S.K., May, H.D., Sowers, K.R., 2007. Microbial Reductive Dechlorination of Aroclor 1260 in Baltimore Harbor Sediment Microcosms Is Catalyzed by Three Phylotypes within the Phylum Chloroflexi. *Appl. Environ. Microbiol.* 73, 3009–3018. <https://doi.org/10.1128/AEM.02958-06>
- Fängström, B., Athanasiadou, M., Grandjean, P., Weihe, P., Bergman, A., 2002. Hydroxylated PCB metabolites and PCBs in serum from pregnant Faroese women. *Environ. Health Perspect.* 110, 895–899. <https://doi.org/10.1289/ehp.110-1240989>
- Federici, E., Giubilei, M., Santi, G., Zanaroli, G., Negroni, A., Fava, F., Petruccioli, M., D'Annibale, A., 2012. Bioaugmentation of a historically contaminated soil by polychlorinated biphenyls with *Lentinus tigrinus*. *Microb. Cell Factories* 11, 35. <https://doi.org/10.1186/1475-2859-11-35>
- Fujihiro, S., Higuchi, R., Hisamatsu, S., Sonoki, S., 2009. Metabolism of hydroxylated PCB congeners by cloned laccase isoforms. *Appl. Microbiol. Biotechnol.* 82, 853–860. <https://doi.org/10.1007/s00253-008-1798-2>
- Galzy, P., Slominski, P., 1957. Variations physiologiques de la levure au cours de la croissance sur l'acide lactique comme seule source de carbone. *Comptes Rendus Académie Sci.* 245, 2423–2426.
- Gayosso-Canales, M., Rodríguez-Vázquez, R., Esparza-García, F.J., Bermúdez-Cruz, R.M., 2012. PCBs stimulate laccase production and activity in *Pleurotus ostreatus* thus promoting their removal. *Folia Microbiol. (Praha)* 57, 149–158. <https://doi.org/10.1007/s12223-012-0106-9>
- Harms, H., Schlosser, D., Wick, L.Y., 2011. Untapped potential: exploiting fungi in bioremediation of hazardous chemicals. *Nat. Rev. Microbiol.* 9, 177–192. <https://doi.org/10.1038/nrmicro2519>
- Kamei, I., Sonoki, S., Haraguchi, K., Kondo, R., 2006. Fungal bioconversion of toxic polychlorinated biphenyls by white-rot fungus, *Phlebia brevispora*. *Appl. Microbiol. Biotechnol.* 73, 932–940. <https://doi.org/10.1007/s00253-006-0529-9>
- Karp, S.G., Faraco, V., Amore, A., Birolo, L., Giangrande, C., Soccol, V.T., Pandey, A., Soccol, C.R., 2012. Characterization of laccase isoforms produced by *Pleurotus ostreatus* in solid state fermentation of sugarcane bagasse. *Bioresour. Technol.* 114, 735–739. <https://doi.org/10.1016/j.biortech.2012.03.058>
- Keum, Y.S., Li, Q.X., 2004. Fungal laccase-catalyzed degradation of hydroxy polychlorinated biphenyls. *Chemosphere* 56, 23–30. <https://doi.org/10.1016/j.chemosphere.2004.02.028>
- Liu, J., Qi, Z., Zhao, Z., Li, X., Buekens, A., Yan, J., Ni, M., 2015. Thermal desorption of PCB-contaminated soil with sodium hydroxide. *Environ. Sci. Pollut. Res.* 22, 19538–19545. <https://doi.org/10.1007/s11356-015-5136-9>
- Lo Piccolo, S., Alfonzo, A., Giambra, S., Conigliaro, G., Lopez-Llorca, L.V., Burrano, S., 2015. Identification of *Acremonium* isolates from grapevines and evaluation of their antagonism towards *Plasmopara viticola*. *Ann. Microbiol.* 65, 2393–2403. <https://doi.org/10.1007/s13213-015-1082-5>
- Matsuzaki, F., Wariishi, H., 2005. Molecular characterization of cytochrome P450 catalyzing hydroxylation of benzoates from the white-rot fungus *Phanerochaete chrysosporium*. *Biochem. Biophys. Res. Commun.* 334, 1184–1190. <https://doi.org/10.1016/j.bbrc.2005.07.013>
- Mortensen, A.S., Braathen, M., Sandvik, M., Arukwe, A., 2007. Effects of hydroxy-polychlorinated biphenyl (OH-PCB) congeners on the xenobiotic biotransformation gene expression patterns in primary culture of Atlantic salmon (*Salmo salar*) hepatocytes. *Ecotoxicol. Environ. Saf.* 68, 351–360. <https://doi.org/10.1016/j.ecoenv.2006.11.019>
- Mouhamadou, B., Faure, M., Sage, L., Marçais, J., Souard, F., Geremia, R.A., 2013. Potential of autochthonous fungal strains isolated from contaminated soils for degradation of polychlorinated biphenyls. *Fungal Biol.* 117, 268–274. <https://doi.org/10.1016/j.funbio.2013.02.004>
- Ohtsubo, Y., Nagata, Y., Kimbara, K., Takagi, M., Ohta, A., 2000. Expression of the bph genes involved in biphenyl/PCB degradation in *Pseudomonas* sp. KKS102 induced by the biphenyl degradation intermediate, 2-hydroxy-6-oxo-6-phenylhexa-2,4-dienoic acid. *Gene* 256, 223–228. [https://doi.org/10.1016/S0378-1119\(00\)00349-8](https://doi.org/10.1016/S0378-1119(00)00349-8)

- Passatore, L., Rossetti, S., Juwarkar, A.A., Massacci, A., 2014. Phytoremediation and bioremediation of polychlorinated biphenyls (PCBs): State of knowledge and research perspectives. *J. Hazard. Mater.* 278, 189–202. <https://doi.org/10.1016/j.jhazmat.2014.05.051>
- Plačková, M., Svobodová, K., Cajthaml, T., 2012. Laccase activity profiling and gene expression in PCB-degrading cultures of *Trametes versicolor*. *Int. Biodeterior. Biodegrad.* 71, 22–28. <https://doi.org/10.1016/j.ibiod.2012.03.005>
- Pérignon, S., Massier, M., Germain, J., Binet, M.-N., Legay, N., Mouhamadou, B., 2019. Metabolic adaptation of fungal strains in response to contamination by polychlorinated biphenyls. *Environ. Sci. Pollut. Res.* 26, 14943–14950. <https://doi.org/10.1007/s11356-019-04701-5>
- Prathyusha, P., Rajitha Sri, A.B., Ashokvardhan, T., Satya Prasad, K., 2015. Antimicrobial and Siderophore Activity of the Endophytic Fungus *Acremonium sclerotigenum* Inhabiting *Terminalia bellerica* Roxb. *Int J Pharm Sci Rev Res* 30, 84–87.
- Ross, G., 2004. The public health implications of polychlorinated biphenyls (PCBs) in the environment. *Ecotoxicol. Environ. Saf.* 59, 275–291. <https://doi.org/10.1016/j.ecoenv.2004.06.003>
- Ruiz-Aguilar, G.M.L., Fernández-Sánchez, J.M., Rodríguez-Vázquez, R., Poggi-Varaldo, H., 2002. Degradation by white-rot fungi of high concentrations of PCB extracted from a contaminated soil. *Adv. Environ. Res.* 6, 559–568. [https://doi.org/10.1016/S1093-0191\(01\)00102-2](https://doi.org/10.1016/S1093-0191(01)00102-2)
- Sadañoski, M.A., Benítez, S.F., Fonseca, M.I., Velázquez, J.E., Zapata, P.D., Levin, L.N., Villalba, L.L., 2019. Mycoremediation of high concentrations of polychlorinated biphenyls with *Pleurotus sajor-caju* LBM 105 as an effective and cheap treatment. *J. Environ. Chem. Eng.* 7, 103453. <https://doi.org/10.1016/j.jece.2019.103453>
- Sadañoski, M.A., Velázquez, J.E., Fonseca, M.I., Zapata, P.D., Levin, L.N., Villalba, L.L., 2018. Assessing the ability of white-rot fungi to tolerate polychlorinated biphenyls using predictive mycology. *Mycology* 9, 239–249. <https://doi.org/10.1080/21501203.2018.1481152>
- Saitou, N., Nei, M., 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* <https://doi.org/10.1093/oxfordjournals.molbev.a040454>
- Steffen, K.T., Schubert, S., Tuomela, M., Hatakka, A., Hofrichter, M., 2007. Enhancement of bioconversion of high-molecular mass polycyclic aromatic hydrocarbons in contaminated non-sterile soil by litter-decomposing fungi. *Biodegradation* 18, 359–369. <https://doi.org/10.1007/s10532-006-9070-x>
- Stella, T., Covino, S., Čvančarová, M., Filipová, A., Petruccioli, M., D'Annibale, A., Cajthaml, T., 2017. Bioremediation of long-term PCB-contaminated soil by white-rot fungi. *J. Hazard. Mater.* 324, 701–710. <https://doi.org/10.1016/j.jhazmat.2016.11.044>
- Svobodová, K., Plačková, M., Novotná, V., Cajthaml, T., 2009. Estrogenic and androgenic activity of PCBs, their chlorinated metabolites and other endocrine disruptors estimated with two in vitro yeast assays. *Sci. Total Environ.* 407, 5921–5925. <https://doi.org/10.1016/j.scitotenv.2009.08.011>
- Takagi, S., Shirota, C., Sakaguchi, K., Suzuki, J., Sue, T., Nagasaka, H., Hisamatsu, S., Sonoki, S., 2007. Exoenzymes of *Trametes versicolor* can metabolize coplanar PCB congeners and hydroxy PCB. *Chemosphere* 67, S54–S57. <https://doi.org/10.1016/j.chemosphere.2006.05.090>
- Tamura, K., Dudley, J., Nei, M., Kumar, S., 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) Software Version 4.0. *Mol. Biol. Evol.* 24, 1596–1599. <https://doi.org/10.1093/molbev/msm092>
- Tigini, V., Prigione, V., Di Toro, S., Fava, F., Varese, G.C., 2009. Isolation and characterisation of polychlorinated biphenyl (PCB) degrading fungi from a historically contaminated soil. *Microb. Cell Factories* 8, 5. <https://doi.org/10.1186/1475-2859-8-5>
- Tomasini, A., Hugo León-Santiesteban, H., 2019. The Role of the Filamentous Fungi in Bioremediation, in: *Fungal Bioremediation*. CRC Press, pp. 3–21. <https://doi.org/10.1201/9781315205984-1>
- UNEP, 2017. Stockholm Convention on persistent organic pollutants (POPs).
- White, T.J., Bruns, T., Lee, S., Taylor, J., 1990. AMPLIFICATION AND DIRECT SEQUENCING OF FUNGAL RIBOSOMAL RNA GENES FOR PHYLOGENETICS, in: *PCR Protocols*. Academic Press, Inc, San Diego, pp. 315–322. <https://doi.org/10.1016/B978-0-12-372180-8.50042-1>

Zeddel, A., Majcherczyk, A., Hüttermann, A., 1993. Degradation of polychlorinated biphenyls by white-rot fungi *pleurotus ostreatus* and *trametes versicolor* in a solid state system. *Toxicol. Environ. Chem.* 40, 255–266. <https://doi.org/10.1080/02772249309357947>