Ecotoxicology and Environmental Safety 156 (2018) 87–96

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Degradation of bisphenol A and acute toxicity reduction by different thermo-tolerant ascomycete strains isolated from arid soils

Rim Mtibaà[a,*], Dario Rafael Olicón-Hernández[b], Clementina Pozo[b,c], Moncef Nasri[a], Tahar Mechichi[a], Jesus González[b,c], Elisabet Aranda[b,c]

[a] Laboratory of Enzyme Engineering and Microbiology, Ecole Nationale d'Ingenieurs de Sfax, University of Sfax, Route de Soukra Km 4.5, BP 1173, 3038 Sfax, Tunisia
[b] Department of Microbiology, University of Granada, Campus Cartuja E-18071, Granada, Spain
[c] Institute of Water Research, University of Granada, Ramón y Cajal 4, E-18071 Granada, Spain

ARTICLE INFO

Keywords:
Bisphenol A
Ascomycete fungi
Cytochrome P450s
Laccases
LC-MS analysis
Degradation pathway

ABSTRACT

Four different laccase-producing strains were isolated from arid soils and used for bisphenol A (BPA) degradation. These strains were identified as *Chaetomium strumarium* G5I, *Thielavia arenaria* CH9, *Thielavia arenaria* HJ22 and *Thielavia arenaria* SM1(III) by internal transcribed spacer 5.8 S rDNA analysis. Residual BPA was evaluated by HPLC analysis during 48 h of incubation. A complete removal of BPA was observed by the whole cell fungal cultures within different times, depending on each strain. *C. strumarium* G5I was the most efficient degrader, showing 100% of removal within 8 h of incubation. The degradation of BPA was accompanied by the production of laccase and dye decolorizing peroxidase (DyP) under degradation conditions. The presence of aminobenzotriazole (ABT) as an inhibitor of cytochrome P450s monooxygenases (CYP) demonstrated a slight decrease in BPA removal rate, suggesting the effective contribution of CYP in the conversion. The great involvement of laccase in BPA transformation together with cell-associated enzymes, such as CYP, was supported by the identification of hydroxylated metabolites by ultra-high performance liquid chromatography-mass spectroscopy (UHPLC-MS). The metabolic pathway of BPA transformation was proposed based on the detected metabolites. The acute toxicity of BPA and its products was investigated and showed a significant reduction, except for *T. arenaria* SM1(III) that did not caused reduction of toxicity (IC_{50} < 8%), possibly due to the presence of toxic metabolites. The results of the present study point out the potential application of the isolated ascomycetes in pollutant removal processes, especially *C. strumarium* G5I as an efficient degrader of BPA.

1. Introduction

Endocrine disrupting chemicals (EDCs) are a wide group of synthetic compounds of anthropogenic origin (Erkurt, 2015). Some of them are known for their capacity to mimic and interfere with the action of endogenous hormones and thus adversely affect wildlife and human health by disrupting development, reproduction, growth, and neurological and immune systems (Daâssi et al., 2016; de Freitas et al., 2017). Bisphenol A (BPA; 2,2-bis (4-hydroxyphenyl) propane) is an organic compound with two phenolic rings connected by a bridge with two methyl functional groups (Kamaraj et al., 2014). This compound is widely used as a monomer in the production of a great variety of consumer products, including food plastic packing materials, adhesives, powder paints, thermal paper, polycarbonate plastics, epoxy resins and polyesters (Telke et al., 2009; Zhang et al., 2013; Kamaraj et al., 2014). This has led to the widespread and continuous increase in the environmental contamination by BPA detected in soils, surface water, sediments (fresh and marine) groundwater, drinking water and wastewater (Eio et al., 2014). The United States Environmental Protection Agency (USEPA) has identified bisphenol A as an environmental endocrine disruptor due to its estrogenic and genotoxic activities and continues to assess the safety of BPA in cooperation with international regulatory and public health counterparts (Zhang et al., 2013).

Due to the bioactivity, toxicity and stability structure of BPA even at low but environmentally high concentrations, the conventional physicochemical technologies, such as Fenton reaction (Mohapatra et al., 2011), ozonation (Wang et al., 2014), photodegradation (Zhang et al., 2013) and ultrasonic oxidation (Yu et al., 2014), are not effective to remove these compounds, they only transfer the pollutant from one phase to another. For this reason, biodegradation involving microorganisms has been proven to be an advanced biological approach to remove the toxic BPA compounds from the environment in a safe and economic way (Zhang et al., 2013; Eio et al., 2014; Agrawal and Shahi, 2017). Ligninolytic fungi, belonging mostly to basidiomycetes, have

* Corresponding author.
E-mail address: rim.mtibaa@enis.tn (R. Mtibaà).

https://doi.org/10.1016/j.ecoenv.2018.02.077
Received 15 December 2017; Received in revised form 28 February 2018; Accepted 28 February 2018

received considerable attention for their biodegradation potential (Lee et al., 2005; Harms et al., 2011; Fouda et al., 2015). The ability of these fungi to transform BPA has been related to the production of non-specific extracellular oxidative enzymes, i.e. lignin peroxidase (LiP, EC 1.11.1.14), manganese peroxidase (MnP, EC 1.11.1.13), versatile peroxidase (VP, EC 1.11.1.16) and laccase (or polyphenol oxidase, Lac EC 1.10.3.2) (Daâssi et al., 2016; Kabiersch et al., 2011; Taboada-Puig et al., 2011). Their non-specific nature facilitates the oxidation and mineralization of numerous substrates (Marco-Urrea and Reddy, 2012). Furthermore, ascomycete fungi represent an extremely diverse phylum (Whiteway et al., 2015) and they have demonstrated an effective role in the removal of organic pollutants (Reyes-Cesar et al., 2014; Zafra et al., 2014; Marco-Urrea et al., 2015; Yao et al., 2015). In fact, it has been demonstrated that a broad range of xenobiotics can be more effectively removed by whole-cell fungal treatment compared to enzymatic treatment, due to the combined effects of intracellular, extracellular and mycelium-bound enzymes and of sorption to the fungal biomass (Nguyen et al., 2014a; Nguyen et al., 2014b; Hofmann and Schlosser, 2016). Additionally, some Ascomycota fungi have oxidative pathways catalyzed by extracellular oxidative enzymes, including laccases and peroxidases (such as dye-decolorizing peroxidases (DyPs) and heme-containing peroxidases), which have been identified in the last decade and are widespread among several ascomycete fungi, according to the genome database (Büttner et al., 2017).

Most reports addressing the ascomycete fungal biotransformation and degradation of xenobiotics showed that the detoxification metabolism is catalyzed by phase I and phase II enzymes, mainly cytochrome P450 (CYP) proteins (Morel et al., 2013). This intracellular detoxification system constitutes the predominant pathway in the initial oxidation step of micropollutants (Cerniglia, 1997). Cytochrome P450 monooxygenases (CYP) and epoxide hydrolases (EHs) catalyze phase I oxidation reactions. The phase II conjugation reactions are mediated by transferases, including glutathione S-transferase, UDP-glucuronosyltransferase and NAD(P)H quinone oxidoreductase. This is followed by the phase III reactions which involve the transportation of the conjugated metabolites to vacuoles for the excretion of their respective derivatives, catalyzed by membrane transporters (Cerniglia, 1997). Cerniglia (1997) reported that conjugation pathways lead to detoxification whereas the oxidation products, like dihydrodiol epoxides, may be bioactive and toxic. Despite the attractive role of these intra- and extracellular enzymes in the detoxification pathways, their metabolisms have not been investigated in depth due mainly to the various enzymatic profiles. These profiles differ widely among different strains even of the same family. To our knowledge, the degradation of BPA and the enzyme mediated systems of *Chaetomium* and *Thielavia* species, isolated from soils, have not previously been described. In the present study, we investigated the removal of BPA by novel laccase-producing ascomycete strains. The degradation of BPA was monitored by high performance liquid chromatography (HPLC) and the resulting metabolites were studied by ultra high performance liquid chromatography-mass spectroscopy (UHPLC-MS). A metabolic pathway of BPA degradation was then suggested. Finally, Microtox® bioassays were performed to evaluate the acute toxicity of the fungal culture media at different assay times.

2. Material and methods

2.1. Chemicals

Bisphenol A (BPA: 2,2-bis (4-hydroxyphenyl) propane) (99% purity)), 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) (96% purity) and 1-aminobenzotriazole (98% purity) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Solvents, HPLC gradient grade acetonitrile and water were purchased from VWR (Barcelona, Spain). All other chemicals were of analytical grade.

2.2. Isolation, screening and fungal identification

Fungal strains were isolated from soil samples collected from arid regions in the south of Tunisia using the method of Waksman (1992). They were screened for laccase production on Malt Extract Agar (MEA) plates amended by 2,6-dimethoxyphenol (2,6-DMP) as described by Mtibaà et al. (2017). Among the isolated laccase-producing fungi, a total of four strains were selected for the BPA degradation study on the basis of their notable laccase production. They were genetically identified by amplifying, cloning and further sequencing fragments corresponding to the internal transcribed spacer (ITS) region in their total genomic DNA. The PCR amplification was performed using universal primers ITS1 (5′–TCCGTAGGTGAACCTGCGG) and ITS4 (5′-TCCTCCG CTTATTGATATGC) (White et al., 1990). PCR reaction mixtures, amplification conditions, electrophoresis and products purification were performed as described by Godoy et al. (2016). The resulted PCR products were analyzed using an ABI 3130xl automatic capillary sequencer (Applied Biosystems). A homology search was performed by using the BLAST software tool (http://blast.ncbi.nlm.nih.gov/Blast.cgi) to compare nucleotide acid sequence similarities against the GenBank database. The obtained homologous sequences were used for ClustalW multiple sequence alignments (Thompson et al., 1994) using BioEdit software (Hall, 1999). The phylogenetic tree was inferred by using the Maximum Likelihood estimation method based on the Tamura-Nei model (Tamura et al., 2004). The evolutionary analyses were conducted in MEGA7 (Kumar et al., 2016). Neighbor-Joining and BIONJ algorithms were applied to a matrix to generate the phylogenetic tree and the one showing the highest log likelihood was selected.

2.3. Fungal culture conditions

Cultures were carried out in 250 mL Erlenmeyer flasks containing 50 mL of synthetic medium composed of 2.5% glucose, 0.35% soya peptone and 0.4% yeast extract at pH 6.0. In order to induce extracellular laccase, fungi were cultured in an orbital shaking incubator at 120 rpm and 35 °C for 4 days in presence of 0.25 mM $CuSO_4$ as inducer (Farnet et al. (1999) according to a previous optimization process (data not shown). Inoculation was carried out using 0.5 mL of mycelial suspension prepared as described by Aranda et al. (2009). BPA diluted in acetonitrile was aseptically added to the cultures at a final concentration of 0.1 mM after 2 days of incubation. Heat-inactivated mycelium and non-inoculated cultures were used as adsorption and abiotic controls, respectively, as reported by Aranda et al. (2017). In order to study the role of CYP on BPA degradation, additional cultures included 1 mM of the CYP inhibitor 1-aminobenzotriazole (ABT). Experiments were performed in triplicate for 2 days. Samples of each individual flask were taken every 2 h to analyze the extracellular enzymatic activities (laccase and dye decolorizing peroxidase (DyP)), sugar content and pH. BPA concentrations in the remaining liquid culture were determined by HPLC as described previously by Aranda et al. (2010). The reported data represent the mean values with standard deviation of triplicate measurements.

2.4. Enzyme assays

Laccase activity was determined spectrophotometrically by monitoring the oxidation of 0.3 mM ABTS at 420 nm ($\varepsilon = 36{,}000\ M^{-1}\ cm^{-1}$) (Eggert et al., 1995) in 50 mM sodium citrate buffer (pH 4.5). Dye decolorizing peroxidase activity (DyP) was measured as previously described by Liers et al. (2010). One unit of enzyme activity was defined as the amount of enzyme that catalyzed the oxidation of one micromole of substrate per minute and per milliliter (Telke et al., 2009).

2.5. Characterization of the fungal growth medium

During the BPA degradation, the variation in pH and sugar content were determined. The total sugar content of fungal culture media amended with BPA was evaluated using the dinitrosalicylic acid reagent (DNS colorimetric method) previously described by Miller (1959), by measuring the absorbance at 540 nm. A standard curve of glucose was used to calculate the total sugar content.

2.6. Chromatographic analyses of BPA degradation

2.6.1. Analysis of BPA concentration by HPLC

The residual BPA concentration in fungal culture supernatants was analyzed by HPLC (HP 1050, Agilent®, Germany) as described previously by Aranda et al. (2010). Detection wavelength was set at 278 nm. Remaining concentrations of BPA were calculated using a calibration curve of BPA, by comparing the retention times and the chromatogram of BPA.

2.6.2. Analysis of BPA degradation metabolites by LC–MS

For the identification of BPA degradation metabolites, an UHPLC Acquity I-Class System (Waters, Milford, USA) and a SYNAPT G2S QToF mass spectrometer (Waters, Milford, USA) were used. Chromatographic separation was carried out on a CORTECS-UHPLC® HILIC® C18 1.6 µm column (2.1 × 50 mm; Waters, Milford, USA) with mobile phases A (H_2O with 0.01% ammonium acetate) and B (methanol with 0.01% ammonium acetate) in a gradient flow rate of 0.4 L min^{-1} (initial 5% B; 5 min, 100% B; 6 min, 100% B; 6.10 min, 5% B; 7 min, 5% B). Column temperature was set at 40 °C and 10 µL of each sample was injected. Z-Spray electrospray ionization (ESI) interface working in negative ion mode and the detection mass range (m/z) was 50–1200. The reference capillary voltage was 2.5 kV and reference scan frequency was 20 s. The source temperature was set to 100 °C and the desolvation temperature to 500 °C. The sampling cone voltage was set to 35 V and the source offset voltage to 40 V. The data were acquired in resolution mode with a cone gas flow and desolvation flow of 50 L h^{-1} and 800 L h^{-1}, respectively. The system control and computer metabolite analyses were made by the MassLynx software (version 4.1, Waters Milford, USA).

2.7. Toxicity analyses

The Microtox® bioassay was used to measure acute toxicity after fungal conversion based on changes on the luminescence of the bacteria *Vibrio fischeri* (Qureshi et al., 1982). A Microtox Model 500 toxicity analyzer (Instrumentación Analítica S.A. Madrid, Spain) was used. The remaining liquid culture was analyzed at the beginning and the end of the experiment according with the degradation time of each strain. Additionally, an initial heat-killed inactivated control with BPA (0.1 mM) was measured for comparison. Acute toxicity was expressed as EC_{50} after 15 min of exposure (Qureshi et al., 1982).

3. Results and discussion

3.1. Isolation, screening and fungal identification

Four fungal strains (G5I, CH9, HJ22, and SM1(III)) isolated from arid soils were selected, based on their capability to produce laccase activity, for BPA degradation experiments and further identification using biology molecular tools was investigated. Three of them (CH9, HJ22, and SM1(III)) were identified as *Thielavia arenaria* (99% homology) and the other one (G5I) as *Chaetomium strumarium* (99% homology). All identified fungal sequences have been deposited in the NCBI GenBank database and have been assigned accession numbers as summarized in Supplementary Table 1. Fig. 1 shows the phylogenetic tree inferred with 32 different representatives belonging to the orders Sordariales, Xilariales, Boliniales, Coniochaetales, and Diaporthales. GenBank accession numbers are listed in Supplementary Table 1. Based on the phylogenetic analysis, strains CH9, SM1(III) and HJ22 exhibited more than 99% homology to several *Thielavia arenaria* strains with accession numbers JN709489.1, KP101209.1 and KU872811.1. The strain G5I showed 99% of identity to the strain *Chaetomium strumarium* TC22 with accession number JQ796877.1 and other 99% similar strains, which are *Achaetomium strumarium* AY681201.1 and *Achaetomium strumarium* SGLAf19. It is noteworthy that the strain *T. arenaria* HJ22 is distant from the rest of *Thielavia* strains.

3.2. Whole-cell strain BPA degradation

The results of BPA transformation of all the strains are represented in Fig. 2. In the heat-inactivated fungal cultures no significant reduction of the BPA concentration was detected. It remained constant for all cultures, except for *Thielavia arenaria* HJ22 (Fig. 2c), which decreased slightly to 70 µM, suggesting the negligible contribution of the sorption process on the fungal biomass. In active fungal cultures, the BPA had completely removed by all the tested strains within different times. A total BPA depletion was achieved rapidly by the strain *C. strumarium* G5I, within only 8 h (Fig. 2a). This result was similar to that obtained in a previous study (Hofmann et al., 2016) by the ascomycete fungus *Phoma* sp. showing about 77% of BPA degradation rate within 4 h of incubation, but it was slightly higher than that achieved by the white rot fungi *Irpex lacteus* and *Trametes versicolor* which were able to remove 100% of BPA within 12 h of incubation (Shin et al., 2007). A complete removal was observed after 12, 16, and more than 30 h of treatment by the *T. arenaria* strains CH9, HJ22, and SM1(III), respectively (Fig. 2b, c and d). The low degradation rate of BPA by the strain *T. arenaria* SM1(III) (> 30 h) could be likely due to the toxic effect of BPA on the mechanism of this fungus (Shin et al., 2007). The degradation half-life of BPA by this fungus was observed at 16 h, similar to that showed by the bacterial consortium reported by Eio et al. (2014) which was at 17 h for a concentration of 20 mg L^{-1} (~100 µM). In contrast, some other reports showed transformation times of several days to achieve similar removal rates (Chai et al., 2005; Zhang et al., 2015). These findings demonstrate that all the tested fungi, particularly *C. strumarium* and *T. arenaria* CH9 were efficient degraders and could be able to remove BPA in thermal conditions. This is in agreement with different studies showing the capability and the tolerance of ascomycetes in the transformation of EDCs and xenobiotic compounds, such as BPA (Shin et al., 2007; Jové et al., 2016; Kamaraj et al., 2017). Experiments supplemented with ABT as a non-selective inhibitor of CYP, showed a slight reduction in the BPA degradation efficiency of all the tested strains. At initial time, the transformation of BPA was slightly decreased by 25% for the strains *C. strumarium* G5I and *T. arenaria* CH9, and by 19% and 16% for *T. arenaria* HJ22 and SM1(III), respectively, compared to 100% in cultures lacking ABT (Fig. 2). This decrease was observed during the incubation time of all cultures. These findings suggest that the intracellular mechanism had contributed to BPA removal, which is in agreement with several reported studies showing the involvement of CYP in EDC transformation (Morel et al., 2013; Zhang et al., 2015; Olicón-Hernández et al., 2017). Fig. 2 shows the pH and sugar content during the bioconversion of BPA by the selected strains. The pH of the *C. strumarium* G5I culture medium changed slightly during the culture period from 6.0 to 7.0, suggesting the production of alkaline metabolites. For the other strains, the pH remained constant. Miao et al. (2006) reported that pH variation was due mainly to the type of metabolites produced by the cultivated strain. The sugar content decreased gradually until the end time of the cultures and achieved approximately the same amount around 10 g L^{-1} for *T. arenaria* strains and only 2 g L^{-1} for *C. strumarium* G5I (Fig. 2a). This depletion in sugar concentration when evaluated in parallel with the increase in enzyme production could indicate the co-metabolic nature of the process.

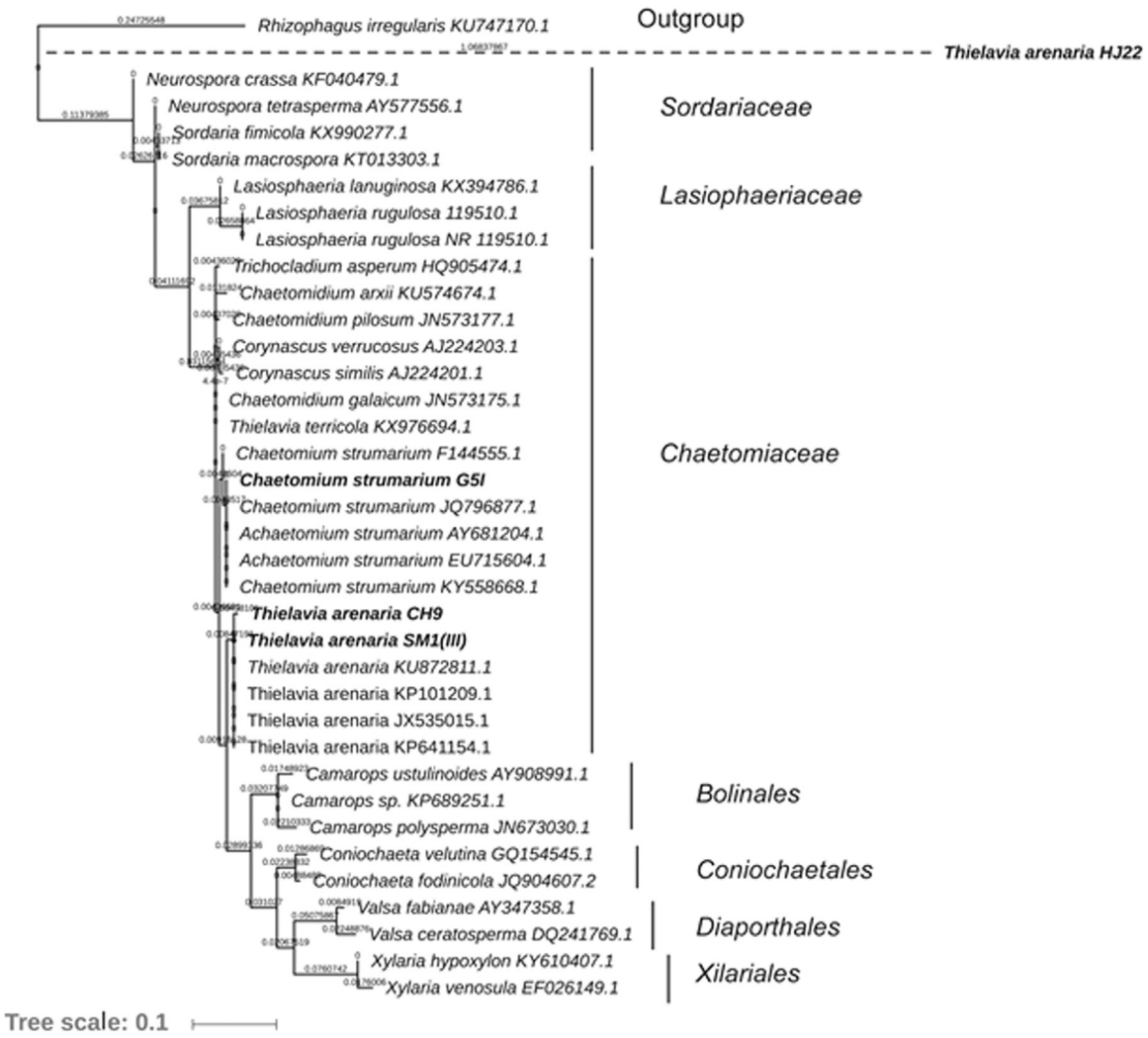


Fig. 1. Phylogenetic tree constructed based on the 5.8S rDNA gene of four isolated fungi and relative organisms using the Neighbor-Joining method. Evolutionary analyses were conducted in MEGA7. Numbers at branches represent the bootstrap values of 1000 replications.

3.3. Enzymatic activities study during BPA removal

Laccase and DyP activities were measured during the BPA degradation assay. As shown in Fig. 2, both extracellular enzymes showed a continuous increase in activity, in active fungal cultures until the end of the treatment, concomitantly with the removal of BPA. Therefore, those two enzyme based degradation mechanisms might be contributing to BPA conversion under the tested conditions. In fact, *C. strumarium* G5I (Fig. 2a) and *T. arenaria* CH9 (Fig. 2b) showed a substantial decrease of BPA (70% and 50% of removal) immediately after BPA addition (at time 0) corresponded with 0.3 and 0.8 U mL^{-1} of laccase activity, respectively. For *T. arenaria* HJ22 a slight decrease of BPA was detected at the starting sampling point (2 days) (18% decrease) which probably corresponded to the 0.2 U mL^{-1} of laccase and no removal of BPA was detected in *T. arenaria* SM1(III) (Fig. 2d). In the presence of inhibitors, results showed a sharp decrease in the conversion of BPA at the beginning of the experiment by *C. strumarium* G5I and *T. arenaria* CH9 by about 35% and 40%, respectively, indicating the involvement of intracellular and extracellular enzymatic system in BPA conversion. However, no initial removal was detected by *T. arenaria* HJ22 and SM1(III) in the presence of inhibitors, which suggest that the CYP system was markedly involved in BPA transformation by these strains at the initial times. Furthermore, in ABT-containing cultures, although the laccase and DyP activities remained at a low level, they showed a similar pattern of activity compared to those of the active fungal cultures. It is important to note that a close correlation was found between the extracellular enzymatic activities, the intracellular system and the decrease of BPA during the period of incubation. *Chaetomium* and *Thielavia* sp. have previously been shown to have the ability to secrete laccases (Mtibaà et al., 2017). A recent study reported by Zámocký et al. (2016) showed an abundance of genes encoding putative enzymes from all four known heme peroxidase superfamilies, including, cytochrome c peroxidase (CcP), manganese peroxidase, cyclooxygenase-like enzymes and DyPs, in the genome of *Chaetomium cochliodes* CCM F-232. However, the detection of the recently described DyP-type peroxidase activity in *Thielavia* sp. is described here for the first time. Further confirmation with purification techniques will be necessary to validate this statement. According to the genome database, the distribution of DyP enzymes seems to be widespread in other ascomycete fungi, such as *Kretzschmaria deusta* (Büttner et al., 2017).

Numerous studies have documented the ability of laccases to catalyze the degradation of BPA (Shin et al., 2007; Wang et al., 2013; Cajthaml, 2015; Marco-Urrea et al., 2015; Daâssi et al., 2016; Shin et al., 2007). The production of extracellular ligninolytic enzymes, including laccase and DyP-type peroxidase, by Ascomycota fungi growing in non-ligninolytic conditions has been previously described (Kiiskinen et al., 2002) and these enzymes have received a great deal of attention during the last decade for their possible application in real scenarios (Marco-Urrea et al., 2015; Aranda, 2016). Attention has also been paid to the transformation of various EDCs by the fungal CYP system, which

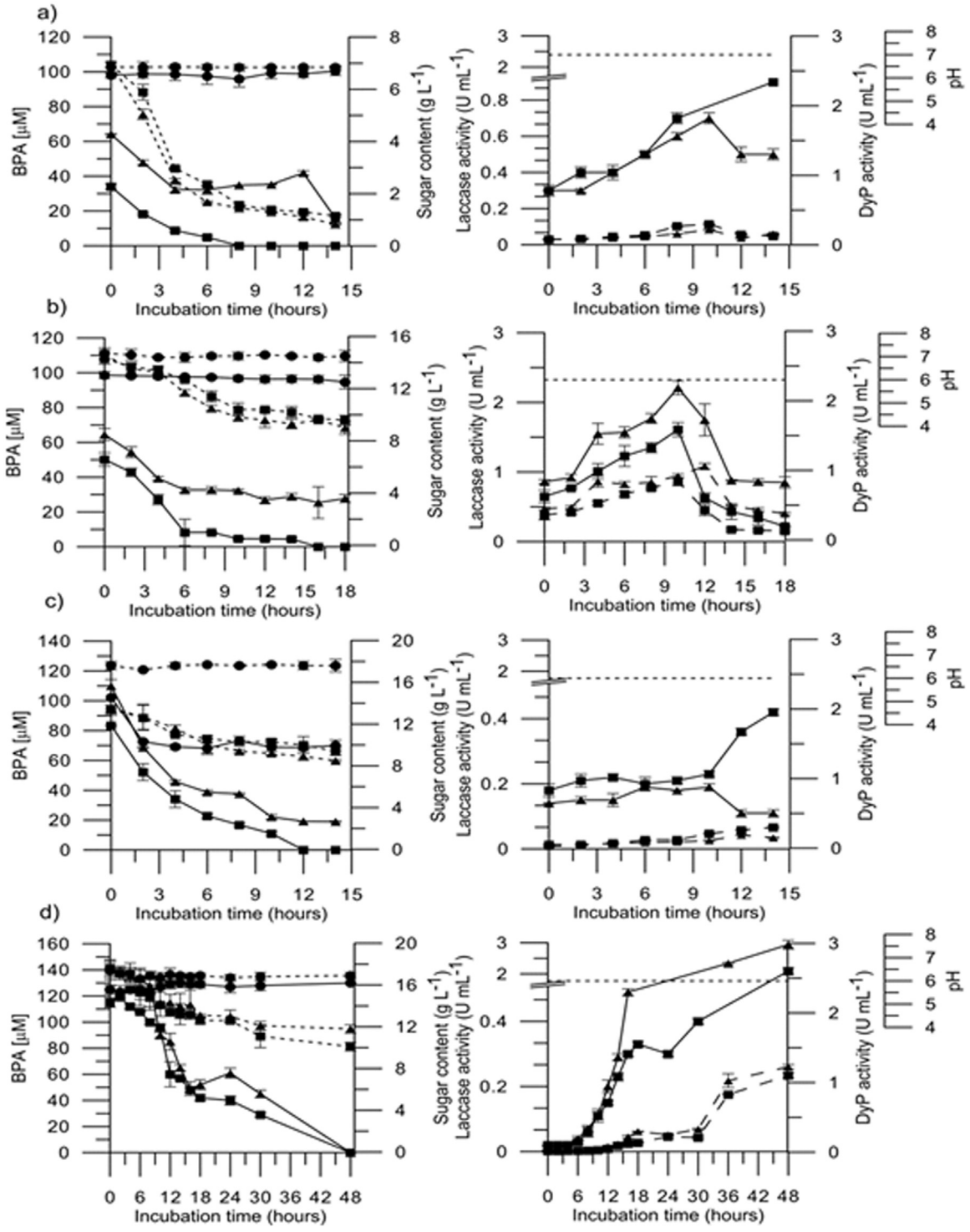


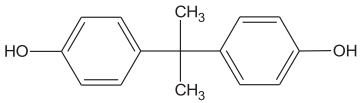
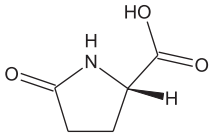
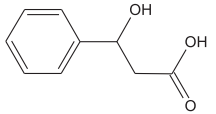
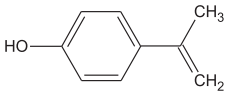
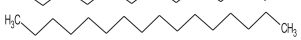
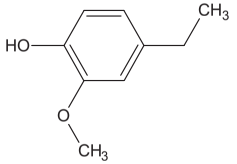
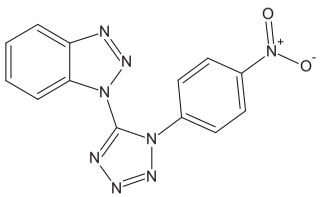
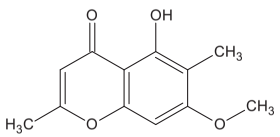
Fig. 2. Time course of BPA degradation (full line) and sugar content (dotted line) (left), and enzymatic activities of laccase and dye decolorizing peroxidase (right) in (a) *Chaetomium strumarium* G5I, (b) *Thielavia arenaria* CH9, (c) *Thielavia arenaria* HJ22 and (d) *Thielavia arenaria* SM1(III) in liquid fermentation at 35 °C. Full line: BPA degradation, dotted line: sugar content. Key: control (•), fungal strain (■), and fungal strain + ABT (▲). Full line: laccase activity, and dotted line DyP activity. Values represent averages and error bars represent standard deviation of tree replicates.

is the main enzymatic system involved in the degradation of xenobiotics in non-ligninolytic fungi (Cajthaml, 2015; Marco-Urrea et al., 2015). The important role occupied by CYP in the transformation and detoxification of a wide variety of pollutants, such as polycyclic aromatic hydrocarbons (PAHs), has been described in detail (Hata et al., 2010; Wang et al., 2012; Morel et al., 2013; Wang et al., 2013) and has an important role in the metabolism of xenobiotics.

3.4. Metabolite identification and putative pathway

Metabolites originating from BPA degradation by the different strains were identified by UHPLC-MS analysis and the results are shown in Table 1. No differences were found in the samples supplemented with ABT with the methodology used for metabolite analysis in this study, thus, more detailed and sensitive tests must be performed to corroborate the presence of other biotransformation products. However, the

Table 1
Metabolites of bisphenol A biotransformation in the different strains. The symbol (+) indicate the presence of the compound in the sample. No differences were found between the samples with ABT as CYP inhibitor.

Compound	Structure	Elemental composition	*m/z*	Average Retention Time (s)	Error (ppm)	G5I			CH9			HJ22			SM1(III)		
						T0	T6	T12	T0	T8	T16	T0	T6	T12	T0	T24	T48
Bisphenol A[a]		$C_{15}H_{16}O_2$	227.1072	3.63	−2.2	+	+		+	+		+	+		+	+	
Pyroglutamic acid[b]		$C_5H_7NO_3$	128.0348	0.59	−17.22	+	+	+	+	+	+	+	+	+	+	+	+
3-hidroxy − 3- phenyl propionic acid		$C_9H_{10}O_3$	165.0552	1.08	−9.1	+	+	+	+	+	+	+	+	+	+	+	+
p- Isopropenylphenol		$C_9H_{10}O$	133.0653	1.73	−7.5	+	+	+	+	+	+	+	+	+	+	+	+
Nonadecane		$C_{19}H_{40}$	267.3052	4.71	−10.1	+	+	+	+	+	+			+	+	+	+
Hexadecane		$C_{16}H_{34}$	225.2582	2.15	−19.5									+			
4-ethyl − 2-methoxyphenol		$C_9H_{12}O_2$	151.0759	1.31	−13.9												+
1-[1-(4-Nitrophenyl) − 1H-tetrazol − 5-yl] − 1H-benzotriazole[b]		$C_{13}H_8N_8O_2$	307.0665	2.56	−16.9				+	+	+					+	+
Eugenitin[c]		$C_{12}H_{12}O_4$	219.0644	2.40	−6.4				+	+						+	+

[a] The original molecule was present in the initial time of the degradation until its completely elimination depending on each strain.
[b] Is not clear if they are ABT or ABTS metabolites.
[C] Is not clear if its a sub-product generated under experimental conditions.

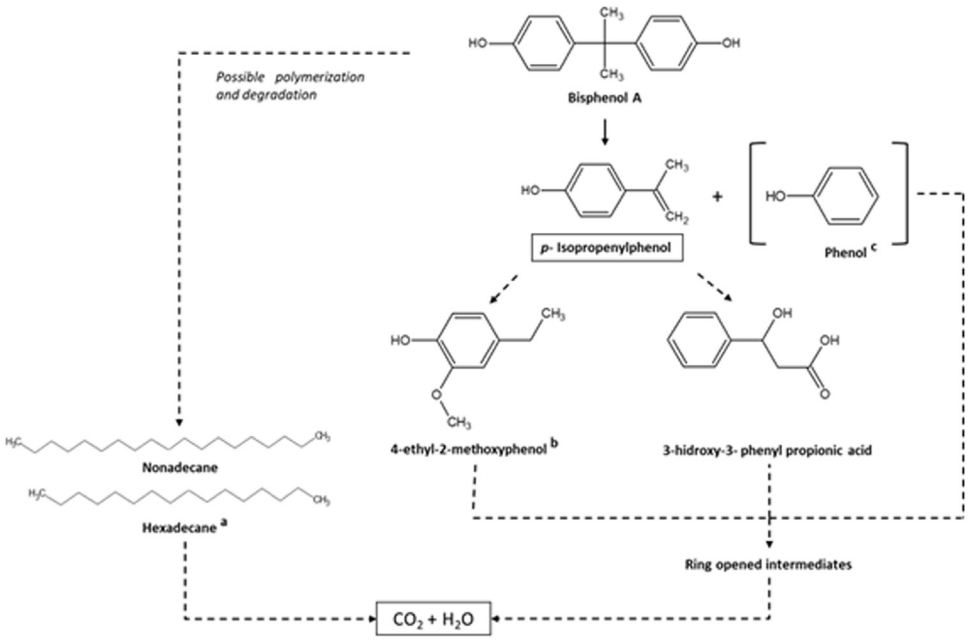


Fig. 3. Partial metabolic pathway for the degradation of bisphenol A by different strains. *p*-Isopropenylphenol is the central metabolite in the degradation of BPA. (a) Compounds found only in *Thielavia arenaria* HJ22; (b) compounds found only in *Thielavia arenaria* SM1(III); and (c) phenol was not detected in this analysis.

participation of CYP in the biodegradation of BPA cannot be ruled out due to the existence of a difference in the biodegradation of BPA with or without CYP inhibitor (Fig. 2). Moreover, these enzymes can participate in detoxification processes of metabolites or sub-products, as well as trigger protective processes against external stress in the strains evaluated. The distinctive chemical structure of BPA facilitates the generation of a series of aromatic compounds as a consequence of the breakdown of the main aromatic rings (Gassara et al., 2013). Fig. 3 represents the partial pathway for the degradation of BPA. As shown in this figure, the main mechanism for the biotransformation of BPA is the oxidation of the aromatic substrate resulting in acidic (3-hydroxy-3-phenyl propionic acid) and hydroxylated (4-ethyl-2-methoxyphenol, *p*-isopropenylphenol) metabolites. It has been reported that in the microbiological and/or enzymatic treatment for the removal of BPA, these structures are representatives of the biodegradation products of this endocrine disruptor and they are present in different concentrations (Chai et al., 2005; Husain and Qayyum, 2013; Daâssi et al., 2016). The metabolites found during the biodegradation of BPA by the different strains (Fig. S1) are consistent with the results reported previously. These metabolites are related to the enzymatic actions of a variety of enzymatic systems, including mainly extracellular enzymes such as laccases, and peroxidases, as well as in some cases, the combined effect of these enzymes with CYP enzymes (Chairin et al., 2013; Chhaya and Gupte, 2013; Wang et al., 2013; Daâssi et al., 2016; de Freitas et al., 2017). de Freitas et al. (2017) reported that *p*-isopropenylphenol was formed after laccase-catalyzed degradation of BPA via the oxidative condensation pathway, with a C-C and/or C-O coupling between phenolic groups followed by its cleavage. Similar structures were reported by Erkurt (2015) following laccase-catalyzed treatment of *Funalia trogii*, such as 4-ethyl-2-methoxyphenol and the aliphatic molecule nonadecane. The formation of pyroglutamic acid has been associated with ABT/ABTS degradation, which is added at the beginning of the fungal inoculation, for this reason, the presence of this byproduct without BPA degradation in some strains has been observed (Daâssi et al., 2016). In particular, in the strain SM1(III), the presence of metabolites resulting from the degradation of BPA as well as ABT/ABTS at the beginning of the experiment was detected in absence of enzymatic activity (Fig. S2). These molecules could be the result of the action of other enzymatic or non-enzymatic systems that are involved in the degradation of xenobiotic compounds as mechanisms of protection for the fungus, or as a result of the basal metabolism of aromatic compounds such as amino acids. Additional experiments in this strain are necessary to elucidate the origin of these metabolites, i.e. the use of isotopic labeling to corroborate the mechanisms of BPA removal. An interesting result is the presence of long chain hydrocarbons as a part of the metabolites observed in BPA transformation in all strains (nonadecane), and in particular the strain *Thielavia arenaria* HJ22 (nonadecane and hexadecane). The presence of these long chain metabolites suggests a possible polymerization of the aromatic rings of bisphenol A to produce oligomers of different sizes, which are then reduced to smaller hydrocarbons to be integrated into the cellular metabolism of the fungi. Similar results have been previously described in white rot fungi for the elimination of this emerging contaminant (Hou et al., 2014; de Freitas et al., 2017). However, in our results, the presence of bisphenol polymers was not detected. Also, the presence of eugenitin in the strains *T. arenaria* CH9 and HJ22, a fungal chromone derivative during BPA transformation has not been previously reported. This molecule is a benzopyran derivate and is present as a secondary metabolite, it has been described in fungi, such as *Tolypocladium extinguens*, *Paraphaeosphaeria quadriseptata*, *Chaetomium chiversii* and *Chaetomium brasiliense*, but its function remains unclear (Andrioli et al., 2012). The experimental conditions could trigger the formation of this secondary metabolite in the strains tested, although it cannot be confirmed whether its role is in the degradation of BPA.

3.5. Microtox© bioassay

Fig. 4 illustrates the acute toxicity values (expressed as EC_{50} after 15 min exposure) of fungal liquid culture media amended with BPA after different incubation times. The negative controls (heat inactivated controls) showed the highest toxicity. At the beginning of the experiment (initial degradation time), we detected a significant decrease in the toxicity in *C. strumarium* G5I medium, this depletion was less patent in the case of *T. arenaria* CH9. For *T. arenaria* HJ22 and *T. arenaria* SM1(III) media, the decrease was not so evident at the beginning of the experiment, a fact that demonstrates the differences among the fungal strains. However, in both cases the toxicity values gradually decreased, a fact that coincides with the total removal of BPA (Fig. 2). The strain

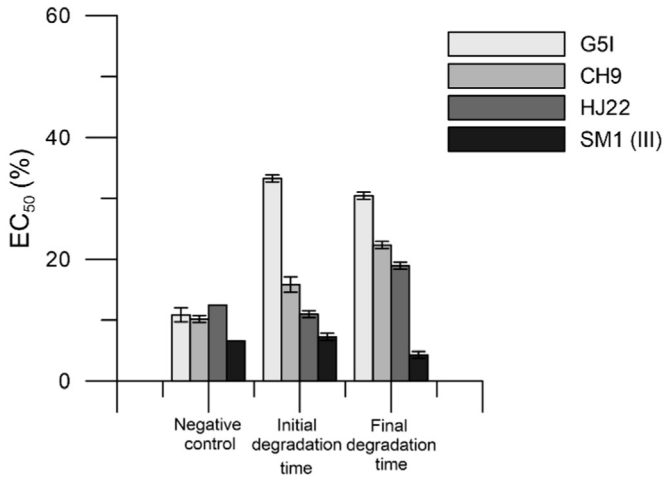


Fig. 4. Acute toxicity of the remaining liquid culture of BPA degradation by the different strains expressed as EC_{50} (%) at 15 min. Negative control represents the heat inactivated control, initial degradation time represents T0 after BPA addition, and final time of degradation was selected according to the removal efficiency of each strain: *Chaetomium strumarium* G5I = after 12 h, *Thielavia arenaria* CH9 = after 16 h, *Thielavia arenaria* HJ22 = after 12 h and *Thielavia arenaria* SM1(III) = after 48 h of incubation. Error bars represent standard deviation of tree replicates.

SM1(III) was the only fungus unable to reduce the toxicity of BPA throughout the incubation time, despite the observation of the total BPA depletion after 48 h of incubation. From this observation, it is clear that the measured toxicity before and after treatment does not only originate from BPA. In this case, the appearance of one or more metabolites, as toxic as BPA, contributed to the continued acute toxicity despite the complete removal of the target pollutant BPA. A similar result was found by Olmez-Hanci et al. (2013) who showed that some intermediates formed during the BPA biotransformation were more toxic than BPA itself. Several studies investigated similar toxicity tests of BPA and their degradation products (Nguyen et al., 2014a; Nguyen et al., 2014b; Erkurt, 2015; de Freitas et al., 2017). It was reported that the presence of some products could be associated with the generation of more toxic effects than that of BPA (de Freitas et al., 2017), especially those with aromatic rings, such as: *p*-hydroxybenzaldehyde, 2-phenylpropenal, phenol, *p*-hydroxybenzoic acid, hydroquinone, *p*-isopropylphenol and 4-ethyl-2-methoxyphenol. The latter metabolite was the only one among the cited compounds detected after BPA degradation by SM1(III), which may explain the remaining toxicity observed by this strain after the total removal of BPA following the 48 h incubation period as shown in Table 1. Similarly, de Freitas et al. (2017) reported a decrease in BPA toxicity using *Pleurotus ostreatus* laccase but not with *Pleurotus pulmonarius* laccase. Moreover, Erkurt (2015) reported the reduction in the toxicity of the products of BPA in parallel to its disappearance.

4. Conclusion

The present study is the first report describing the biodegradation of BPA by ascomycetes fungi belonging to the *Chaetomiaece* family isolated from arid soil. The isolates were identified as *Chaetomium strumarium* G5I, *Thielavia arenaria* CH9, *Thielavia arenaria* HJ22 and *Thielavia arenaria* SM1(III). All tested fungi were found able to totally remove BPA using the whole fungal cell cultures within 48 h. The strain *C. strumarium* G5I showed the highest degree of BPA removal. Research on enzymatic system demonstrated that the transformation of BPA was mainly contributed by two different mechanisms simultaneously: extracellular conversion by laccase and DyP and intracellular hydroxylation by CYP monooxygenases. The BPA breakdown was catalyzed by the isolated fungi into different metabolites. After identification of the resulted metabolites through LC-MS, hydroxylated and acidic products were detected, showing different toxicity levels. However, the removal of BPA by the *C. strumarium* G5I produced less toxic metabolites compared with the other tested fungi, evidenced by the low acute toxicity found. A possible degradation pathway was proposed. The results presented indicates that *Chaetomiaece* ascomycetes could be a potential new tool for bioremediation in xeric and thermophilic conditions. However, further research is needed for practical applications.

Acknowledgments

This study was supported by the Ramón y Cajal Program (project P09-CVI-4778). RM thanks Erasmus + program KA1 with the University of Granada (Spain) for providing the financial support. EA would like to thank the Ministry of Economy and Competitiveness (MINECO) and FEDER funds for co-funding the Ramón y Cajal contract (RYC-2013–12481) and DRO thanks Conacyt Mexico for the post-doctoral fellowship.

Conflict of interest statement

The authors declare that there is no conflict of interest.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.ecoenv.2018.02.077.

References

Agrawal, N., Shahi, S.K., 2017. Degradation of polycyclic aromatic hydrocarbon (pyrene) using novel fungal strain *Coriolopsis byrsina* strain APC5. Int. Biodeterior. Biodegrad. 122, 69–81. http://dx.doi.org/10.1016/j.ibiod.2017.04.024.

Andrioli, W.J., Silva, T.M., da Silva, V.B., Damásio, A.R.L., Maller, A., Conti, R., Jorge, J.A., Araújo, J.M., Silva, C.H.T.P., Pupo, M.T., Polizeli, M.L.T.M., Bastos, J.K., 2012. The fungal metabolite eugenitin as additive for *Aspergillus niveus* glucoamylase activation. J. Mol. Catal. B Enzym. 74, 156–161. http://dx.doi.org/10.1016/j.molcatb.2011.08.003.

Aranda, E., Kinne, M., Kluge, M., Ullrich, R., Hofrichter, M., 2009. Conversion of dibenzothiophene by the mushrooms *Agrocybe aegerita* and *Coprinellus radians* and their extracellular peroxygenases. Appl. Microbiol. Biotechnol. 82, 1057. http://dx.doi.org/10.1007/s00253-008-1778-6.

Aranda, E., Ullrich, R., Hofrichter, M., 2010. Conversion of polycyclic aromatic hydrocarbons, methyl naphthalenes and dibenzofuran by two fungal peroxygenases. Biodegradation 21, 267–281. http://dx.doi.org/10.1007/s10532-009-9299-2.

Aranda, E., 2016. Promising approaches towards biotransformation of polycyclic aromatic hydrocarbons with Ascomycota fungi. Curr. Opin. Biotechnol. 38, 1–8. http://dx.doi.org/10.1016/j.copbio.2015.12.002.

Aranda, E., Godoy, P., Reina, R., Badia-Fabregat, M., Rosell, M., Marco-Urrea, E., García-Romera, I., 2017. Isolation of Ascomycota fungi with capability to transform PAHs: insights into the biodegradation mechanisms of *Penicillium oxalicum*. Int. Biodeterior. Biodegrad. 122, 141–150. http://dx.doi.org/10.1016/j.ibiod.2017.05.015.

Büttner, E., Gebauer, A.M., Hofrichter, M., Liers, C., Kellner, H., 2017. Draft Genome Sequence of the Wood-Degrading Ascomycete *Kretzschmaria deusta* DSM 104547. Genome Announc. 5, e01076–17. http://dx.doi.org/10.1128/genomeA.01076-17.

Cajthaml, T., 2015. Biodegradation of endocrine disrupting compounds by ligninolytic fungi: mechanisms involved in the degradation. Environ. Microbiol. 17, 4822–4834. http://dx.doi.org/10.1111/1462-2920.12460.

Cerniglia, C.E., 1997. Fungal metabolism of polycyclic aromatic hydrocarbons: past, present and future applications in bioremediation. J. Ind. Microbiol. Biotechnol. 19, 324–333. http://dx.doi.org/10.1038/sj.jim.2900459.

Chai, W., Handa, Y., Suzuki, M., Saito, M., Kato, N., Horiuchi, C.A., 2005. Biodegradation of bisphenol a by fungi. Appl. Biochem. Biotechnol. 120, 175–182. http://dx.doi.org/10.1385/abab:120:3:175.

Chairin, T., Nitheranont, T., Watanabe, A., Asada, Y., Khanongnuch, C., Lumyong, S., 2013. Biodegradation of Bisphenol A and Decolorization of Synthetic Dyes by Laccase from White-Rot Fungus *Trametes polyzona*. Appl. Biochem. Biotechnol. 169, 539–545. http://dx.doi.org/10.1007/s12010-012-9990-3.

Chhaya, U., Gupte, A., 2013. Possible role of laccase from *Fusarium incarnatum* UC-14 in bioremediation of Bisphenol A using reverse micelles system. J. Hazard. Mater. 254, 149–156. http://dx.doi.org/10.1016/j.jhazmat.2013.03.054.

Daâssi, D., Prieto, A., Zouari-Mechichi, H., Martínez, M.J., Nasri, M., Mechichi, T., 2016. Degradation of bisphenol A by different fungal laccases and identification of its degradation products. Int. Biodeterior. Biodegrad. 110, 181–188. http://dx.doi.org/10.1016/j.ibiod.2016.03.017.

de Freitas, E.N., Bubna, G.A., Brugnari, T., Kato, C.G., Nolli, M., Rauen, T.G., Moreira, R.F.P.M., Peralta, R.A., Bracht, A., de Souza, C.G.M., Peralta, R.M., 2017. Removal of bisphenol A by laccases from *Pleurotus ostreatus* and *Pleurotus pulmonarius* and

evaluation of ecotoxicity of degradation products. Chem. Eng. J. 330, 1361–1369. http://dx.doi.org/10.1016/j.cej.2017.08.051.
Eggert, C., Temp, U., Dean, J.F.D., Eriksson, K.E.L., 1995. Laccase-mediated formation of the phenoxazinone derivative, cinnabarinic acid. FEBS Lett. 376, 202–206. http://dx.doi.org/10.1016/0014-5793(95)01274-9.
Eio, E.J., Kawai, M., Tsuchiya, K., Yamamoto, S., Toda, T., 2014. Biodegradation of bisphenol A by bacterial consortia. Int. Biodeterior. Biodegrad. 96, 166–173. http://dx.doi.org/10.1016/j.ibiod.2014.09.011.
Erkurt, H.A., 2015. Biodegradation and detoxification of BPA: involving laccase and a mediator. CLEAN–Soil, Air, Water 43, 932–939. http://dx.doi.org/10.1002/clen.201400628.
Farnet, A.M., Tagger, S., Le Petit, J., 1999. Effects of copper and aromatic inducers on the laccases of the white-rot fungus *Marasmius quercophilus*. Comptes Rendus De. l'Académie Des. Sci.-Ser. III-Sci. De. la Vie 322, 499–503. http://dx.doi.org/10.1016/S0764-4469(99)80100-0.
Fouda, A., Khalil, A.M.A., El-Sheikh, H.H., Abdel-Rhaman, E.M., Hashem, A.H., 2015. Biodegradation and detoxification of bisphenol-A by filamentous fungi screened from nature. J. Adv. Biol. Biotechnol. 2, 123–132. http://dx.doi.org/10.9734/JABB/2015/13959.
Gassara, F., Brar, S.K., Verma, M., Tyagi, R.D., 2013. Bisphenol A degradation in water by ligninolytic enzymes. Chemosphere 92, 1356–1360. http://dx.doi.org/10.1016/j.chemosphere.2013.02.071.
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, 20985–20996. http://dx.doi.org/10.1007/s11356-016-7257-1.
Hall, T.A., 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic acids Symp. Ser. 41, 95-98. [London]: Information Retrieval Ltd., c1979-c2000.
Harms, H., Schlosser, D., Wick, L.Y., 2011. Untapped potential: exploiting fungi in bioremediation of hazardous chemicals. Nat. Rev. Microbiol. 9, 177–192. http://dx.doi.org/10.1038/nrmicro2519.
Hata, T., Kawai, S., Okamura, H., Nishida, T., 2010. Removal of diclofenac and mefenamic acid by the white rot fungus *Phanerochaete sordida* YK-624 and identification of their metabolites after fungal transformation. Biodegradation 21, 681–689. http://dx.doi.org/10.1007/s10532-010-9334-3.
Hofmann, U., Schlosser, D., 2016. Biochemical and physicochemical processes contributing to the removal of endocrine-disrupting chemicals and pharmaceuticals by the aquatic ascomycete *Phoma* sp. UHH 5-1-03. Appl. Microbiol. Biotechnol. 100, 2381–2399. http://dx.doi.org/10.1007/s00253-015-7113-0.
Hou, J., Dong, G., Ye, Y., Chen, V., 2014. Enzymatic degradation of bisphenol-A with immobilized laccase on TiO_2 sol–gel coated PVDF membrane. J. Membr. Sci. 469, 19–30. http://dx.doi.org/10.1016/j.memsci.2014.06.027.
Husain, Q., Qayyum, S., 2013. Biological and enzymatic treatment of bisphenol A and other endocrine disrupting compounds: a review. Crit. Rev. Biotechnol. 33, 260–292. http://dx.doi.org/10.3109/07388551.2012.694409.
Jové, P., Olivella, M.A., Camarero, S., Caixach, J., Planas, C., Cano, L., de las Heras, F.X., 2016. Fungal biodegradation of anthracene-polluted cork: a comparative study. J. *Environ. Sci. Health A.* 51, 70–77. http://dx.doi.org/10.1080/10934529.2015.1079114.
Kabiersch, G., Rajasärkkä, J., Ullrich, R., Tuomela, M., Hofrichter, M., Virta, M., Hatakka, A., Steffen, K., 2011. Fate of bisphenol A during treatment with the litter-decomposing fungi *Stropharia rugosoannulata* and *Stropharia coronilla*. Chemosphere 83, 226–232. http://dx.doi.org/10.1016/j.chemosphere.2010.12.094.
Kamaraj, M., Sivaraj, R., Venckatesh, R., 2014. Biodegradation of Bisphenol A by the tolerant bacterial species isolated from coastal regions of Chennai, Tamil Nadu, India. Int. Biodeterior. Biodegrad. 93, 216–222. http://dx.doi.org/10.1016/j.ibiod.2014.02.014.
Kamaraj, M., Rajeshwari, S., Aravind, J., 2017. Isolation of *Virgibacillus* sp. strain KU4 from agricultural soil as a potential degrader of endocrine disruptor bisphenol-A. International. J. Environ. Sci. Technol. 1–6. http://dx.doi.org/10.1007/s13762-017-1398-8.
Kiiskinen, L.L., Viikari, L., Kruus, K., 2002. Purification and characterisation of a novel laccase from the ascomycete *Melanocarpus albomyces*. Appl. Microbiol. Biotechnol. 59, 198–204. http://dx.doi.org/10.1007/s00253-002-1012-x.
Kumar, S., Stecher, G., Tamura, K., 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol. Biol. Evol. 33, 1870–1874. http://dx.doi.org/10.1093/molbev/msw054.
Lee, S.M., Koo, B.W., Choi, J.W., Choi, D.H., An, B.S., Jeung, E.B., Choi, I.G., 2005. Degradation of bisphenol A by white rot fungi, *Stereum hirsutum* and *Heterobasidium insulare*, and reduction of its estrogenic activity. Biol. Pharm. Bull. 28, 201–207. http://dx.doi.org/10.1248/bpb.28.201.
Liers, C., Arnstadt, T., Ullrich, R., Hofrichter, M., 2010. Patterns of lignin degradation and oxidative enzyme secretion by different wood-and litter-colonizing basidiomycetes and ascomycetes grown on beech-wood. FEMS Microbiol. Ecol. 78, 91–102. http://dx.doi.org/10.1111/j.1574-6941.2011.01144.x.
Marco-Urrea, E., Reddy, C.A., 2012. Degradation of Chloro-organic Pollutants by White Rot Fungi. In Microbial Degradation of Xenobiotics. Springer, Berlin Heidelberg, pp. 31–66. http://dx.doi.org/10.1007/978-3-642-23789-8-2.
Marco-Urrea, E., García-Romera, I., Aranda, E., 2015. Potential of nonligninolytic fungi in bioremediation of chlorinated and polycyclic aromatic hydrocarbons. N. Biotechnol. 32, 620–628. http://dx.doi.org/10.1016/j.nbt.2015.01.005.
Miao, L.I., Kwong, T.F., Qian, P.Y., 2006. Effect of culture conditions on mycelial growth, antibacterial activity, and metabolite profiles of the marine-derived fungus *Arthrinium* cf *saccharicola*. Appl. Microbiol. Biotechnol. 72, 1063–1073. http://dx.doi.org/10.1007/s00253-006-0376-8.
Miller, G.L., 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal. Chem. 31, 426. http://dx.doi.org/10.1021/ac60147a030.
Mtibaà, R., de Eugenio, L., Ghariani, B., Louati, I., Belbahri, L., Nasri, M., Mechichi, T., 2017. A halotolerant laccase from Chaetomium strain isolated from desert soil and its ability for dye decolourization. 3 Biotech 7 (5), 329. http://dx.doi.org/10.1007/s13205-017-0973-5.
Mohapatra, D.P., Brar, S.K., Tyagi, R.D., Surampalli, R.Y., 2011. Concomitant degradation of bisphenol A during ultrasonication and Fenton oxidation and production of biofertilizer from wastewater sludge. Ultrason. Sonochem. 18, 1018–1027. http://dx.doi.org/10.1016/j.ultsonch.2011.03.013.
Morel, M., Meux, E., Mathieu, Y., Thuillier, A., Chibani, K., Harvengt, L., Jacquot, J.P., Gelhaye, E., 2013. Xenomic networks variability and adaptation traits in wood decaying fungi. Microb. Biotechnol. 6, 248–263. http://dx.doi.org/10.1111/1751-7915.12015.
Nguyen, L.N., Hai, F.I., Yang, S., Kang, J., Leusch, F.D.L., Roddick, F., Price, W.E., Nghiem, L.D., 2014a. Removal of pharmaceuticals, steroid hormones, phytoestrogens, UV-filters, industrial chemicals and pesticides by Trametes versicolor: role of biosorption and biodegradation. Int. Biodeterior. Biodegrad. 88, 169–175. http://dx.doi.org/10.1016/j.ibiod.2013.12.017.
Nguyen, L.N., Hai, F.I., Price, W.E., Leusch, F.D., Roddick, F., McAdam, E.J., Magram, S.F., Nghiem, L.D., 2014b. Continuous biotransformation of bisphenol A and diclofenac by laccase in an enzymatic membrane reactor. Int. Biodeterior. Biodegrad. 95, 25–32. http://dx.doi.org/10.1016/j.ibiod.2014.05.017.
Olicón-Hernández, D.R., González-López, J., Aranda, E., 2017. Overview on the Biochemical Potential of Filamentous Fungi to Degrade Pharmaceutical Compounds. Front. Microbiol. 8, 1792. http://dx.doi.org/10.3389/fmicb.2017.01792.
Olmez-Hanci, T., Arslan-Alaton, I., Genc, B., 2013. Bisphenol A treatment by the hot persulfate process: oxidation products and acute toxicity. J. Hazard. Mater. 263, 283–290. http://dx.doi.org/10.1016/j.jhazmat.2013.01.032.
Qureshi, A.A., Flood, K.W., Thompson, S.R., Janhurst, S.M., Inniss, C.S., Rokosh, D.A., 1982. Comparison of a luminescent bacterial test with other bioassays for determining toxicity of pure compounds and complex effluents. In: Pearson, J.G., Foster, R.B., Bishop, W.E. (Eds.), Aquatic Toxicology and Hazard Assessment In: Proceedings of the Fifth Conference. ASTM STP 766,179–195. https://dx.doi.org/Doi:10.1520/STP36718S.
Reyes-Cesar, A., Absalon, A.E., Fernandez, F.J., Gonzalez, J.M., Cortés-Espinosa, D.V., 2014. Biodegradation of a mixture of PAHs by nonligninolytic fungal strains isolated from crude oil-contaminated soil. World J. Microbiol. Biotechnol. 30, 999–1009. http://dx.doi.org/10.1007/s11274-013-1518-7.
Shin, E., Choi, H.T., Song, H., 2007. Biodegradation of endocrine-disrupting bisphenol A by white rot fungus *Irpex lacteus*. J. Microbiol. Biotechnol. 17, 1147 (PMDI: 18051326).
Taboada-Puig, R., Junghanns, C., Demarche, P., Moreira, M.T., Feijoo, G., Lema, J.M., Agathos, S.N., 2011. Combined cross-linked enzyme aggregates from versatile peroxidase and glucose oxidase: production, partial characterization and application for the elimination of endocrine disruptors. Bioresour. Technol. 102 (11), 6593–6599. http://dx.doi.org/10.1016/j.biortech.2011.03.018.
Tamura, K., Nei, M., Kumar, S., 2004. Prospects for inferring very large phylogenies by using the neighbor-joining method. Proc. Natl. Acad. Sci. Usa. 101, 11030–11035. http://dx.doi.org/10.1073/pnas.0404206101.
Telke, A.A., Kalyani, D.C., Jadhav, U.U., Parshetti, G.K., Govindwar, S.P., 2009. Purification and characterization of an extracellular laccase from a *Pseudomonas* sp. LBC1 and its application for the removal of bisphenol A. J. Mol. Catal. B Enzym 61, 252–260. http://dx.doi.org/10.1016/j.molcatb.2009.08.001.
Thompson, J.D., Higgins, D.G., Gibson, T.J., 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. 22, 4673–4680. http://dx.doi.org/10.1093/nar/22.22.4673.
Wang, J., Hirai, H., Kawagishi, H., 2012. Biotransformation of acetamiprid by the white-rot fungus *Phanerochaete sordida* YK-624. Appl. Microbiol. Biotechnol. 93, 831–835. http://dx.doi.org/10.1007/s00253-011-3435-8.
Wang, J., Yamamoto, R., Yamamoto, Y., Tokumoto, T., Dong, J., Thomas, P., Hirai, H., Kawagishi, H., 2013. Hydroxylation of bisphenol A by hyper lignin-degrading fungus *Phanerochaete sordida* YK-624 under non-ligninolytic condition. Chemosphere 93, 1419–1423. http://dx.doi.org/10.1016/j.chemosphere.2013.07.026.
Wang, X.D., Lv, Y., Liu, H.Y., Li, M.M., 2014. Removal of bisphenol A from water by ozone. Adv. Mat. Res 864–867, 155–160. http://dx.doi.org/10.4028/www.scientific.net/AMR.864-867.155.
Waksman, S.A., 1992. A method of counting the number of fungi in the soil. J. Bacteriol. 7, 339–341 (PMID: 16558961).
White, T.J., Bruns, T., Lee, S., Taylor, J., 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, M.A., Gelfand, D.H., Sninsky, J.J., White, T.J. (Eds.), PCR protocols: a guide to methods and amplifications 18. Academic Press, Inc, New York, pp. 315–322.
Whiteway, M., Tebung, W.A., Choudhury, B.I., Rodríguez-Ortiz, R., 2015. Metabolic regulation in model ascomycetes–adjusting similar genomes to different lifestyles. Trends Genet. 31, 445–453. http://dx.doi.org/10.1016/j.tig.2015.05.002.
Yao, L., Teng, Y., Luo, Y., Christie, P., Ma, W., Liu, F., Wu, Y., Luo, Y., Li, Z., 2015. Biodegradation of polycyclic aromatic hydrocarbons (PAHs) by Trichoderma reesei FS10-C and effect of bioaugmentation on an aged PAH-contaminated soil. Bioremediat. J. 19, 9–17. http://dx.doi.org/10.1080/10889868.2014.939137.
Yu, L., Wang, C., Ren, X., Sun, H., 2014. Catalytic oxidative degradation of bisphenol A using an ultrasonic assisted tourmaline-based system: influence factors and mechanism study. Chem. Eng. J. 252, 346–354. http://dx.doi.org/10.1016/j.cej.2014.05.014.
Zafra, G., Absalón, Á.E., Cuevas, M.D.C., Cortés-Espinosa, D.V., 2014. Isolation and

selection of a highly tolerant microbial consortium with potential for PAH biodegradation from heavy crude oil-contaminated soils. Water, Air, Soil Pollut. 225, 1826. http://dx.doi.org/10.1007/s11270-013-1826-4.

Zámocký, M., Tafer, H., Chovanová, K., Lopandic, K., Kamlárová, A., Obinger, C., 2016. Genome sequence of the filamentous soil fungus *Chaetomium cochliodes* reveals abundance of genes for heme enzymes from all peroxidase and catalase superfamilies. BMC Genom. 1, 763. http://dx.doi.org/10.1186/s12864-016-3111-6.

Zhang, C., Li, M., Chen, X., Li, M., 2015. Edible fungus degrade bisphenol A with no harmful effect on its fatty acid composition. Ecotoxicol. Environ. Saf. 118, 126–132. http://dx.doi.org/10.1016/j.ecoenv.2015.04.020.

Zhang, W., Yin, K., Chen, L., 2013. Bacteria-mediated bisphenol A degradation. Appl. Microbiol. Biotechnol. 97, 5681–5689. http://dx.doi.org/10.1007/s00253-013-4949-z.