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Tolerance to Polycyclic Aromatic Hydrocarbons (PAHs) by filamentous fungi isolated from contaminated sediment in the Amazon region

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ABSTRACT. Tolerance to Polycyclic Hydrocarbons Aromatic (PAHs) is considered an important characteristic when assessing the bioremediation potential of microorganisms. Given this, the objective of this research was to assay filamentous fungi from the Amazon region, isolated from sediments with differents levels of contamination by PAHs, for tolerance to phenanthrene and pyrene. To achieve this, fungal cultures plugs (5 mm), obtained after 7 days growth, were transferred to petri dishes containing 20% Sabouraud dextrose agar medium, after surface innoculation with phenanthrene and pyrene crystals, separately. Radial mycelial growth was evaluated after 10 days at five different concentration levels for each contaminant and control group, all in triplicate for each treatment. Fungal growth and growth inhibition rates were calculated. The average growth of the colonies in each treatment was compared with one-way ANOVA, followed by a Tukey Test ($p < 0,05$). All fungi showed tolerant to phenanthrene and pyrene. However, *Hypoxylon* sp. showed the lowest growth inhibition rate and average growth rates significantly different of the other six tested species. *Hypoxylon* sp. has been shown to be a promising genetic resource for use in new studies of PAHs degradation.

Keywords: phenanthrene, pyrene, fungal growth inhibition, Amazon.

Tolerância a Hidrocarbonetos Policíclicos Aromáticos (HPAs) por fungos filamentosos isolados de sedimentos contaminados da região Amazônica

RESUMO. A tolerância a Hidrocarbonetos Policíclicos Aromática (HPAs) é considerada como uma característica importante na avaliação do potencial de micro-organismos para biorremediação. Diante disso, o objetivo desta pesquisa foi avaliar fungos filamentosos da região amazônica, isolados de sedimentos com diferentes níveis de contaminação por HPAs, quanto à tolerância ao fenantreno e pireno. Para tanto, discos das culturas fúngicas (5 mm), obtidas após 7 dias de crescimento, foram transferidas para placas de Petri contendo meio Agar Sabouraud Dextrose a 20%, após inoculação superficial com cristais de fenantreno e pireno, separadamente. O crescimento micelial radial foi avaliado após 10 dias em cinco concentrações diferentes para cada contaminante e grupo controle, ambos em triplicata para cada tratamento. As taxas de crescimento fúngico e de inibição de crescimento foram calculadas. O crescimento médio das colônias em cada tratamento foi comparado com ANOVA *one way*, seguido pelo teste de Tukey ($p < 0,05$). Todos os fungos mostraram tolerância ao fenantreno e ao pireno. No entanto, *Hypoxylon* sp. apresentou menor taxa de inibição de crescimento e taxas médias de crescimento significativamente diferentes das outras seis espécies testadas. *Hypoxylon* sp. tem se mostrado um recurso genético promissor para uso em novos estudos sobre degradação de HPAs.

Palavras-chaves: fenantreno, pireno, inibição do crescimento fúngico, Amazônia.

Introduction

Polycyclic Aromatic Hydrocarbons (PAHs) are important environmental organic pollutants, consisting of two to eight benzene rings fused together in linear, angular or grouped forms. Due to their hydrophobic character, they are stored in soils and sediments for long periods, and are thus a target of environmental concern as they may impact the health of both aquatic biotas and humans (Jaques, Bento, & Camargo, 2007). Among the main sources of contamination, are oil spills and their derivatives, the combustion of fossil fuels, incineration plants and agro-industrial waste (Almeida, Centeno,

Bisinoti, & Jardim, 2007; Haritash & Kaushik, 2009).

Globally, the existing environmental legislation on PAHs has been determined by the US Environmental Protection Agency (United States Environmental Protection Agency [US-EPA], 1983), which ranked a group of 16 individual PAHs as priority pollutants. Among these compounds, phenanthrene, consisting of three aromatic rings is known as a major constituent of oil and its derivatives. Pyrene, composed of four aromatic rings, originates primarily from the thermal decomposition of organic matter and its subsequent recombination (Cerniglia 1993; Samanta, Sing, & Jain, 2002; Haritash & Kaushik 2009). Both of these PAHs are known for their toxicity and have been recommended as models for biodegradation studies because they possess physical characteristics similar to those other highly carcinogenic PAHs (US-EPA, 1983; Samanta et al., 2002).

PAH degradation may occur naturally in the environment, mostly as a result of bacteria and fungal action. Filamentous fungi stand out because they have the ability to form extensive mycelial networks, have catabolic enzymes with low specificity and abilities to use pollutants as substrates. In addition, they tolerate stressful environmental conditions, acidic pH and nutrient poor environments (Harms, Schlosser, & Wick, 2011; Lemos, Barros, Oliveira, & Reichi, 2008). Although they may potentially be important biotechnological components for the remediation of contaminated environments, either alone or in combination with bacteria and plants, the potential use of fungi in this area has not received the attention they deserve (Harms et al., 2011).

The evaluation of fungal tolerance in the presence of different concentrations of environmental pollutants have been one among a number of strategies used by researchers to select promising species for bioremediation. Several compounds, including crude oil, naphthalene, phenanthrene, anthracene, fluoranthene, pyrene, benzo(a)pyrene (Da Silva, Umbuzeiro, Pfenning, Canhos, & Esposito, 2003; Argumedo-Delira, Alarcon, Ferreira-Cerrato, Almaraz, & Peña Cabriales, 2012; Lee et al., 2014; Zafra, Absálon, Cuevas, & Cortes-Espinosa, 2014), triazine herbicides (Colla, Primaz, Lima, Bertolini, & Costa, 2008) and heavy metals (Vale, Abreu, Gouveia, Leitão, & Santaella, 2011), has been used in these types of tests. The literature reports that high growth rates and tolerance to certain levels of contaminants are considered desirable characteristics in microorganisms, as are indicators of an ability to degrade such xenobiotics as PAHs (Lamar, Larsen, & Kirk, 1990; Matsubara, Lynch, & De Leij, 2006). The isolation of microorganisms from contaminated environments is a strategy that has also been successfully applied in bioremediation, since polluted environments act as a selective culture medium for microorganisms to adapt to the pollutant (Dritsa, Rigas, Natsis, & Marchant, 2007; Colla et al., 2008; Lima et al., 2017). Thus, obtaining filamentous fungi from contaminated environments and evaluating their tolerances to PAHs can provide information that can be usefully applied in environmental biotechnology.

Recently, a study of samples of sediments from the Rio Negro, Amazonas, found high levels of contamination with PAHs (Souza et al., 2015). Another study showed the importance of screening microorganisms collected from PAHs-contaminated areas to guarantee that species with promise for bioremediation programs are obtained (Souza et al., 2016).

Although it is a simple and effective tool, there had been little research on PAHs tolerance in Brazil. Furthermore, knowledge of the potential for use in bioremediation of filamentous fungi from the Amazon region with is still incipient. Therefore, in this context, and seeking promising species for use in future studies about biodegradation and bioremediation of PAHs, this research aimed to evaluate the tolerance to phenantherene and pyrene by filamentous fungi isolated from contaminated sediments of the Amazon region.

Material and methods

Isolation and Identification of filamentous fungi from contaminated sediments

The fungal isolates were obtained from surface sediment samples from the Negro River, Amazonas State, Brazil, in six different locations: close to the Tupé Sustainable Development Reserve (TR); the mouth of the São Raimundo basin (SR), Modern Manaus Port (MM), Panair Port (PA), Iranduba (IR) on the right bank of the river and Ceasa Port region (CE). All locations were characterized by having different levels of PAHs contamination, as described by Souza et al. (2015) (Table 1). Fungal isolation was performed by serial suspension technique, based on Gomes, Cavalcanti, and Passavante (2011) in which 25 g of sediment samples were diluted in 225 mL of sterile distilled water and further dilutions (1:1000) were performed seeding 1 mL in petri plates containing Sabouraud Dextrose Agar (SDA; 40 g L^{-1} dextrose, 10 g L^{-1} mycological peptone, and 15 g L^{-1} agar)

supplemented with chloramphenicol (100 mg L^{-1}) in quadruplicate. The inoculated plates were incubated at 28°C for 5 days and purified isolates were preserved in vials containing sterile distilled water and cryopreservation tubes at -20°C.

The fungi obtained were identified by classical taxonomy and molecular techniques. Genomic DNA from seven-day growth cultures was extracted by physical lysis with glass beads (425-600 µm diameter) following a combined protocol from Moller, Bahnweg, Sandermann & Geiger, (1992). After DNA extraction, the ITS region was amplified with the primer pair ITS1 (5'-TCCTCCGCTTATTGATATGC-3') and ITS4 (5'-CGTAACAAGGTTTCCGTAGG-3') (White & Lee 1990). Amplification reactions consisted of 0.2 mM of each dNTP, 5x KCl buffer, 1.5 mM $MgCl_2$, 0.5 µM of each primer and 1U of Taq polymerase (Promega) in a final volume of 25 µL. Amplicon purification was performed using the Wizard® SV Gel and PCR Clean-up System kit (Promega) and quantified in the NanoDrop® (Thermo Scientific). DNA sequencing was performed using the BigDye® Terminator Cycle Sequencing kit v.3.1 (Life Technologies) according to the manufacturer's protocol. Forward and reverse sequences were compiled in contigs in BioEdit v7.1.3. The contigs compared with were queried in the NCBI-GenBank (www.ncbi.nlm.nih.gov) of the Fungal Biodiversity Centre (CBS, www.cbs.knaw.nl) databases for homologous sequences of closely related species (Table 1).

Evaluation of tolerance to polycyclic aromatic hydrocarbons

Crystals of phenanthrene and pyrene (Sigma-Aldrich 98%) were weighed, solubilized in acetone and used to make-up a 3000 µg mL^{-1} stock solution. Aliquots of this solution, at concentrations: 240, 540, 780, 1020 and 2040 µg mL^{-1}, were withdrawn and applied directly to culture medium containing Sabouraud Dextrose Agar to 20% (ASD 20%) (8 g L^{-1} of dextrose, 2 g L^{-1} mycological peptone, 15 g L^{-1} agar), which was spread superficially in the culture media with the aid of a drigalski handle. Plates were incubated for 12 hours to volatilize the solvent and fix each aliquot on the surface of the culture medium (adapted from Argumedo-Delira et al., 2012).

Filamentous fungi were cultured in a Sabouraud Dextrose Agar culture medium (ASD) (40 g L^{-1} of dextrose, 10 g L^{-1} mycological peptone, 15 g L^{-1} agar) and incubated for 7 days at 28°C. Fungal culture plugs (5 mm diameter) were then removed from the edge of the colonies and transferred to the center of petri dishes containing ASD 20%, once these had been separately surface contaminated with phenanthrene and pyrene as mentioned above. The plates were then incubated at 28°C for 10 days (240h). The experiments were performed in triplicate. Plates lacking added phenanthrene, and pyrene were used as experimental controls. To evaluate the radial growth of the mycelium, the culture plates were marked with three lines and the colony diameter measured with calipers (method adapted from Colla et al., 2008).

Data analysis

Fungal growth rate (FG) was calculated from the radial mycelial growth record, using Equation 1:

$$FG\ (\%) = D_{HPA} / D_C \text{ x } 100$$, where:

D_{HPA} = diameter of fungal colony exposed to HPA, D_C = diameter of the control fungal colony.

Fungal growth inhibition (FI) of the colonies, due to exposure to different concentrations of phenanthrene and pyrene were calculated using Equation 2:

$$FI\ (\%) = 100 - FG$$, where:

FI = fungal growth inhibition, and FG = fungal growth rate obtained from Equation 1 (Argumedo-Delira et al., 2012).

Table 1. Filamentous fungi isolated from surface sediments with different levels of contamination by Polycyclic Aromatic Hydrocarbons (PAHs) at different locations on the Negro River, Amazon, Brazil (available in Souza et al., 2015).

Locations	Sampling sites	Geographical position	ΣPAH (ng g^{-1})⋆	Code	Molecular identification	GenBank Accession number	Identification Confidence
São Raimundo	SR3	S 03°08'01.55" W 060°01'58.30"	2460.5	S08	*Penicillium* sp.	GU981570.1	99%
Panair Port	PA1	S 03°08'49.2" W 060°00'42.4"	892.7	S05	*Talaromyces* sp.	JX677937.1	100%
	PA2	S 03°08'46.64" W 060°00'40.50"	995.5	S61	*Penicillium* sp.	JQ796872.1	99%
Iranduba	IR1	S 03°09'36.2" W 060°02'10.8"	22.8	S13	*Talaromyces* sp.	KF917583.1	99%
				S41	*Penicillium* sp.	HQ343437.1	98%
Ceasa Port	CE1	S 03°08'06.7" W 059°56'17.4"	2375.3	S29	*Hypoxylon* sp.	AJ390406.1	100%
	CE2	S 03°08'08.15" W 059°56'16.04"	437.6	S66	*Penicillium* sp.	JX500716.1	99%

⋆Concentration of Total Polycyclic Aromatic Hydrocarbons from Souza et al. (2015).

Based on data for average fungal growth after 240 hours (10 days) Analysis of Variance (ANOVA) *one way*, followed by the Tukey test ($p < 0.05$), using the Statistical Program "R" were used to compare growth responses of the strains of filamentous fungi to the five concentrations for each of the two PAHs.

Results

The study results indicated that the seven tested fungi had different responses to the presence of the assayed PAHs. All fungi grew in the presence of phenanthrene. However, fungal growth rates were lower, indicating greater growth inhibition capacity. Unlike the control group, where PAHs were present we observed fungal growth only after 48 hours. There was total inhibition of growth (100%) in assays with concentration of 2040 μg mL^{-1}. Mycelial growth of *Talaromyces* sp. (S05) was not inhibited by phenanthrene concentrations of 240 μg mL^{-1} and showed low inhibition at a concentration of 540 μg mL^{-1} (29.2%). However, *Hypoxylon* sp. (S29) showed the lowest growth inhibition rates at concentrations of 240 μg mL^{-1} (20.5%), 540 μg mL^{-1} (22.2%) 780 μg mL^{-1} (43%) and 1020 μg mL^{-1} (42.9%) (Figure 1 A). Analysis of mean mycelial growth rates shows those of *Hypoxylon* sp. (S29) to be significantly different ($p < 0,05$) to those of the other the six tested species, confirming a greater phenanthrene tolerance for this fungus (Table 2).

All fungi grew in the presence of pyrene, and fungal growth rates were much higher when compared with tests using phenanthrene. Consequently, fungal growth inhibition rates were lower (below 23%). Shortly after 24 hours, growth was observed for *Talaromyces* sp. (S05), *Talaromyces* sp. (S13), *Hypoxylon* sp. (S29) and *Penicillium* sp. (S61) in the presence of pyrene. Among the tested strains *Hypoxylon* sp. (S29) showed the lowest growth inhibition in all treatments, followed by *Penicillium* sp. (S66) at concentrations of 540 and 780 μg mL^{-1} (<1%) (Figure 1 B). For average fungal growth in the presence of pyrene, *Hypoxylon* sp. (S29) growth rates were significantly different from the other fungi ($p < 0,05$), as it grew well in all tested concentrations (Table 3). For trials of both PAHs, changes in the patterns of sporulation and pigmentation formation characteristic of each fungus were noted during the daily recording of mycelial growth (Figure 2).

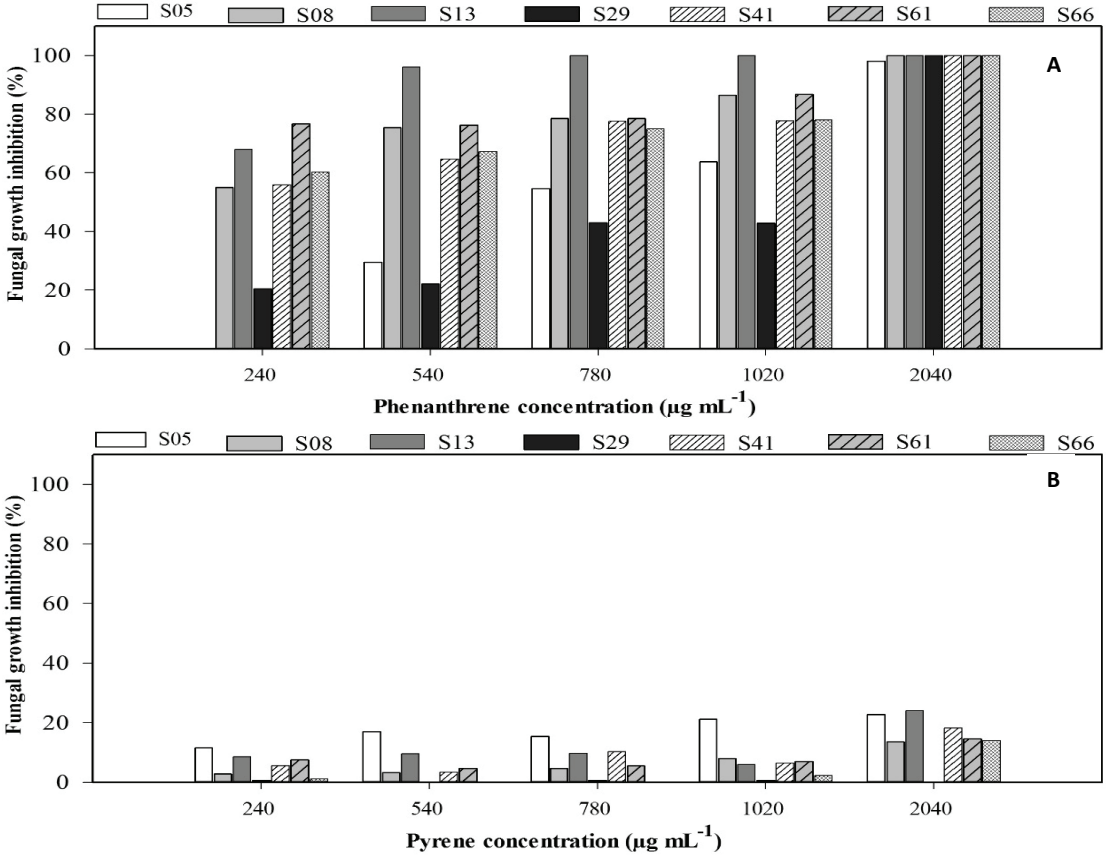

Figure 1. Fungal growth inhibition after 10 days of contact with different concentrations of phenanthrene (A) and pyrene (B). *Talaromyces* sp. (S05); *Penicillium* sp. (S08); *Talaromyces* sp. (S13); *Hypoxylon* sp. (S29); *Penicillium* sp. (S41); *Penicillium* sp. (S61); *Penicillium* sp. (S66).

Table 2. Fungal growth average (mm) after 10 days of contact with different concentrations of phenanthrene. *Talaromyces* sp. (S05); *Penicillium* sp. (S08); *Talaromyces* sp. (S13); *Hypoxylon* sp. (S29); *Penicillium* sp. (S41); *Penicillium* sp. (S61); *Penicillium* sp. (S66).

	Filamentous fungi						
	S05	S08	S13	S29	S41	S61	S66
240 μg mL^{-1}	61.37±3.87cd	80.33±1.18ab	71.33±2.02bc	90.00±0.00a	65.71±6.75cd	71.08±4.96bc	60.58±1.01d
540 μg mL^{-1}	58.19±1.59e	80.00±1.32b	70.50±3.90cd	90.50±0.86a	67.16±4.90cd	73.33±4.40bc	64.58±1.70
780 μg mL^{-1}	58.66±2.93e	78.75±0.50b	70.42±2.10bcd	90.00±0.00a	62.33±6.02de	72.67±4.04bc	63.92±4.68cde
1020 μg mL^{-1}	54.75±1.39e	76.08±1.13b	75.00±1.50b	90.00±0.00a	65.50±2.78c	71.58±2.50b	59.92±1.51d
2040 μg mL^{-1}	53.58±2.90d	71.50±3.07b	59.17±2.60cd	92.17±2.02a	56.83±4.26d	65.67±3.41bc	52.00±1.00d

Table 3. Fungal growth average (mm) after 10 days of contact with different concentrations of pyrene. *Talaromyces* sp. (S05); *Penicillium* sp. (S08); *Talaromyces* sp. (S13); *Hypoxylon* sp. (S29); *Penicillium* sp. (S41); *Penicillium* sp. (S61); *Penicillium* sp. (S66).

	Filamentous fungi						
	S05	S08	S13	S29	S41	S61	S66
240 μg mL^{-1}	38,75±3,25b	35,34±1,33b	22,59±1,41cd	65,75±0,75a	26,91±0,08c	19,00±0,67d	23,83±1,60cd
540 μg mL^{-1}	26,33±0,33b	18,50±0,44cd	3,057±5,29e	63,00±1,45a	17,33±1,66cd	16,083±0,41cd	19,67±2,08c
780 μg mL^{-1}	17,66±2,33b	17,66±2,92b	0,00±0,00d	52,00±0,50a	13,17±0,60c	18,00±0,33b	15,00±0,00bc
1020 μg mL^{-1}	14,59±4,41b	11,17±0,83b	0,00±0,00c	48,66±0,33a	13,06±059b	12,74±0,58b	14,16±0,66b

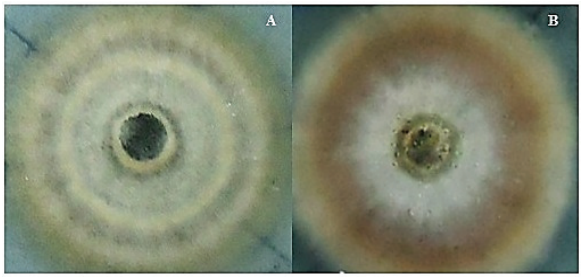

Figure 2. Morphology and coloring of the mycelium *Hypoxylon* sp. in the experimental control (A) and in the presence of phenanthrene (B).

Discussion

The highest rates of fungal growth inhibition in the phenanthrene tests can be attributed to its higher toxicity compared to pyrene. Da Silva et al. (2003) evaluated the phenanthrene and pyrene tolerance of filamentous fungi isolated from estuarine sediments contaminated with PAHs. Some 59% were tolerant of pyrene, and 30% showed phenanthrene tolerance. Moreover, they observed that in many cases the fungi did not grow at all in the presence of phenanthrene, though there was always some growth in the presence of pyrene. In the current study, the high inhibition of fungal growth by phenanthrene and the low growth inhibition by pyrene confirm the greater toxicity of phenanthrene, thus corroborating the results previously reported in the literature.

The increase in fungal growth inhibition rates occurred as the fungi were exposed to greater concentrations of both PAHs. Tests with 2040 µg mL^{-1} phenanthrene completely inhibited growth, indicating the limits of fungal tolerance. Similar results were reported by Zafra et al. (2014), who observed complete inhibition of mycelial growth and sporulation in assays containing high concentrations of a mixture of PAHs.

Several physical and chemical factors can influence the capacity of fungi to tolerate PAHs, including their vapor pressure, solubility and adsorption. Argumedo-Delira et al. (2012) attributed to the combined effects of vapor pressure and solubility of a mixture of PAHs as being the determining factors affecting the growth of *Trichoderma* strains. The vapor pressure of phenanthrene (0.02 *Pa*) is much higher than that of pyrene (0.0006 *Pa*), permitting a higher vapor concentration, so saturating the air trapped in the petri dish. This enhances the impact of phenanthrene's toxicity in the micro-environment, and interfers significantly with fungi growth capacity when compared to pyrene. The solubility of phenanthrene (1.1 mg L^{-1}) is also higher than that of pyrene (0.132 mg L^{-1}), favoring the attachment of crystals of the compound with the culture medium, which also increases the contact surface with the mycelium during growth (Latimer & Zheng 2003; Argumedo-Delira et al. 2012).

The change in patterns of sporulation and pigmentation of fungi as a result of PAHs presence was also mentioned by Zafra et al. (2014) and Reyes-César, Absálon, Fernández, González, and Cortés-Espinosa (2014). The literature mentions that phenanthrene may inhibit spore germination of fungi (Lisowska, Palecz, & Dlugonski, 2004). Morphological changes and interference with germination demonstrate the toxic effect that PAHs have on fungi. Although these tests do not allow us to state the nature of the metabolic activity of PAHs degradation, it is known that PAHs oxidation by non-ligninolytic fungi can form genotoxic compounds that combine with DNA, such as certain trans-9,10-dihydrodiols metabolites, which are soluble in water, but bind to DNA, RNA and proteins, causing direct damage to cells and further

carcinogenic effects (Cavalieri et al., 2005; Cerniglia & Sutherland, 2010). Thus, the absorption of phenanthrene and pyrene by fungal hyphae may lead to possible interference in the gene expression of the individual concerned. Of all evaluated fungi, *Hypoxylon* sp. (S29) proved to be the most tolerant to both the PAHs assayed. To date, there is no record of this fungus as one tolerant to environmental contaminants, and this work the first to highlight it as one with promise for biodegradation. Currently, it is known that white rot fungi such as *Phanerochaete*, *Pleurotus* and *Trametes* have been recorded as having extracellular enzymes capable of degrading various types of PAHs. Among the Ascomycetes, non-ligninolytic fungi has also been mentioned as promising in bioremediation, due to their capacity to use the intracellular enzyme cytochrome P450 monooxygenase to perform the initial oxidation of the PAH aromatic ring. Together with oxygen originating from arene oxide, followed by epoxide hydrolases and phenol, this can be conjugated to form glucuronide, glycosides, xilosídeos and sulfates, with subsequent mineralization (Cerniglia & Sutherland, 2010). Thus, studies on production of extra and intracellular enzymes should be performed with *Hypoxylon* sp. (S29) to evaluate its feasibility in PAHs biodegradation process.

According to Ju and Rogers (1996), the genus *Hypoxylon* is cosmopolitan, with its greater diversity in the tropics and sub-tropics, and is usually found colonizing dead wood. The *Hypoxylon* sp. fungus used in this study is brownish black in color, that is, belonging to the dematiaceous group. As Conceição, Angelis, Bidoia, and Angelis, (2005) reported that the presence of melanin in dark-colored fungi is directly associated with resistance mechanisms to adverse environmental conditions, this may explain the ability of this species to tolerate high concentrations of phenanthrene and show lower grown rate inhibition in presence of pyrene, as observed in the current study. Of the other fungi tested, *Talaromyces* sp. (S05) demonstrated an ability to tolerate lower concentrations of phenanthrene (240-780 μg mL^{-1}) and growth inhibition below 54.6%. *Penicillium* sp. (S66) also showed a very low growth inhibition rate in the presence of pyrene (<1%) at concentrations of 240-780 μg mL^{-1}, indicating some ability of this fungus to tolerate PAHs. There are reports in the literature concerning species of *Talaromyces* and *Penicillium* being collected from soil contaminated with aromatic hydrocarbons and how some species show tolerance to PAHs (Da Silva et al., 2003; Chaillan et al., 2004; Reyes-Cesar et al., 2014).

Obtaining filamentous fungi from sites contaminated with PAHs has been strategic for researchers interested in bioremediation programs for impacted areas. According to Colla et al. (2008), contaminated sites act as a selective medium for the microorganisms, which become adapted to those pollutants which can be used as nutrients. In the biotechnological context, such environments provide an ample variety microorganismal gene pool pertaining to the special requirements of bioremediation (Conceição et al., 2005).

In the current study, the *Hypoxylon* sp., which proved to be more promising taxon in terms of its ability to tolerate PAHs was obtained from a sample that came from contaminated sediments of an old port area of Manaus (Porto Ceasa - CE2). Here recorded contamination levels reach 2375.3 ng g^{-1} for total PAHs (Table 1) and 1879.6 ng g^{-1} for alkylated PAH (Souza et al., 2015). This result confirms the general opinion in the literature, that fungi have great potential for use in contaminated environments, reinforces the need for studies aimed at screening to promising microorganisms to future studies on biodegradation and bioremediation of PAHs, and indicates that the best place to search in the future for fungi with bioremediation potential may well be sites already suffering high levels of pollution.

Conclusion

Filamentous fungi evaluated in this study were phenanthrene and pyrene tolerant. Some, especially *Hypoxylon* sp., showed high tolerance to phenanthrene and pyrene. New studies with selected species should be conducted to assess the potential of PAHs degradation and production of enzymes involved in the oxidation of aromatic rings. Studies like this are the basis for efficient bioremediation programs because they provide relevant information necessary for screening biota with potential to degrade xenobiotics stored in the environment.

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