**An Acad Bras Cienc (2022) 94(2): e20201020 DOI 10.1590/0001-3765202220201020**
Anais da Academia Brasileira de Ciências | *Annals of the Brazilian Academy of Sciences*
Printed ISSN 0001-3765 I Online ISSN 1678-2690
www.scielo.br/aabc | www.fb.com/aabcjournal

MICROBIOLOGY

# Filamentous fungi from textile effluent and their potential application for bioremediation process

MILENE M.A. LIRA, SUZAN P.F. BERNAL, CAMILA C.J. CASTRO, PRISCILA M.M. RAMOS, MICHAEL J.S. LIRA, JÚLIA R. OTTONI, MARCELA BOROSKI & MICHEL R.Z. PASSARINI

**Abstract:** The inappropriate disposal of toxic compounds generated by industrial activity has considerably impacted the environment. Microbial communities inhabiting contaminated sites may represent ecological alternatives for the decontamination of environments. The present work aimed to search the potential of fungi isolated from wastewater treatment plant of a textile industry for bioremediation processes. Twenty-three fungi previously isolated from textile effluent were evaluated for their abilities to degrade pollutants using heavy metal and hydrocarbon tolerance assays. One isolate was subjected to pyrene degradation due its ability to tolerate hydrocarbon. The majority of isolates were resistant to at least two metals tested, i.e. chrome, copper, lead and aluminum. Isolates *Penicillium* sp. ITF 2, *Penicillium rubens* ITF 4, *Penicillium* sp. ITF 12 and ITF 20 (not identified) showed tolerance to tested heavy metals in all concentrations. ITF 12 and ITF 20 were able to tolerate benzene, toluene and hexane, separately. ITF 12 was able to degrade 24.9% of pyrene after 5 days of cultivation. The results encourage future studies to optimize the tolerance and degradation assay using the isolates that showed the best results, as well as studies on the treatment of environments contaminated with heavy metals and hydrocarbons, including industrial textile effluents.

**Key words:** heavy metal, hydrocarbon, tolerance, PAHs, pyrene.

## INTRODUCTION

The aqueous ecosystem represents an unexplored niche for new *taxa* that could potentially be used in biotechnological processes (Menezes et al. 2010, Passarini et al. 2011a). The toxicity of the compounds present in the treatment wastewater selects microbial communities capable of thriving in this environment that can be considered extreme. These microorganisms adapt to conditions and develop an enzymatic arsenal with potential use in bioremediation processes.

Wastewater from the textile industry contains high amount of hazardous toxic chemical pollutants such as synthetic dyes, hydrocarbon and heavy metals like copper, lead, mercury, nickel and cobalt, making the effluent highly toxic (Kant 2012, Ghaly et al. 2014). Synthetic dyes are widely used in textile industry. Remazol dyes are most commonly used in painting silk, cellulose fibers and wool (Ghaly et al. 2014). These compounds are derived from anthraquinone and represent an important class of toxic and recalcitrant organopolutants (Ergene et al. 2009).

Aromatic hydrocarbons such as benzene, toluene, ethylbenzene and xylenes (BTEX) are lipophilic and toxic compounds that act as central nervous system depressants and exhibit toxicity even at low concentrations (Amaral et al. 2017). On the other hand, polycyclic aromatic

hydrocarbons (PAHs) and their metabolites represent a potential health risk due to their biological effects in promoting acute and chronic toxicity, mutagenicity and carcinogenicity (Diggs et al. 2011, Passarini et al. 2011a, Yebra-Pimentel et al. 2015). Pyrene is a recalcitrant molecule that has a half-life in soil ranging from 270 days to 5.2 years (Passarini et al. 2011a).

Heavy metal contamination caused by human activities is an important environmental problem (Hefnawy et al. 2009). Heavy metals used in industry often come to the environment by industrial wastewater without proper treatment, generating a high degree of pollution and the resulting environmental impact may reach food chain at various trophic levels (Hahn et al. 2015). Interactions between heavy metals and microbial communities can be classified into different categories such as extracellular, exocellular and intracellular, in which microbial cells can mobilize, immobilize, transform, precipitate, accumulate, coordinate, exchange and absorb metal, as well as form complexes (Joshi 2014). Melanins (a dark pigment) located in the fungal cell wall and also an extracellular polymer, have the ability to reduce heavy metals toxic effects. Due to this property, fungi may grow in contaminated environments, binding with heavy metals and accumulating these substances in their cells, representing an efficient alternative in the removal of these toxic compounds in waste waters and other terrestrial and aquatic sites in natural environments. These characteristics demonstrate that heavy metal resistant microorganisms may also be applied as biological monitors or bioindicators of environmental contamination (Iram et al. 2012).

The constant presence of toxic compounds including heavy metals and hydrocarbons as environment pollutants have promoted changes within microorganisms metabolisms, since they developed resistance mechanisms to these compounds (Hahn et al. 2015, Souza et al. 2017). Metal tolerance by filamentous fungi is usually associated with their isolation sites. Contaminated environments are known as main sources of hydrocarbon and metal-resistant species, and filamentous fungi strains isolated from contaminated areas containing these compounds may exhibit tolerance to high concentrations of heavy metals and hydrocarbons (Oladipo et al. 2018, Souza et al. 2017). Filamentous fungi recovered from this environment have developed the ability to process these compounds making the environment viable for related organisms development (Prabha et al. 2017).

There is a clear need to treat industrial effluents in order to minimize their impacts and, in this sense, applying sustainable technologies is a promising strategy. The use of filamentous fungi species in ecosystems presenting accumulation of toxic chemical have shown not only the capability to tolerate the presence of these compounds, as well as the aptitude to assimilate and/or degrade heavy metals and hydrocarbons (Acosta-Rodríguez et al. 2018, Barnes et al. 2018, Prenafeta-Boldú et al. 2019). The use of filamentous fungi recovered from textile industrial effluents offers a prosperous strategy with potential application as xenobiotic compounds bioremediators. In this sense, the present work evaluated the ability of filamentous fungi isolated from textile wastewater treatment plant to tolerate heavy metal and hydrocarbon presence as well as to degrade pyrene hydrocarbon, aiming future bioremediation studies.

## MATERIALS AND METHODS

### Filamentous fungi

The isolates used in this study were recovered according to Bernal et al. (2021a), from a liquid

sample withdrawal from a wastewater treatment plant of a textile industry, situated in the city of São Miguel do Iguaçu, Paraná. The strains were isolated in Petri dishes and identified by morphological Bernal et al. (2021a), and molecular Bernal et al. (2021b), approaches (isolates ITF10, 27, 29, 30, 47). The fungal isolates were cultivated in Potato Dextrose Agar (PDA -10 g $L^{-1}$ of glucose, 15 g $L^{-1}$ agar in 1000 ml $L^{-1}$ potato infusion) culture medium for 7 days at 28 °C.

## Heavy metal tolerance assay

### *Screening for tolerant strain in liquid medium*

Firstly, twenty three isolates were subjected to an initial tolerance to heavy metals test. Isolates were cultivated in 3 ml of PDB medium (10 g $L^{-1}$ of glucose in 1000 ml $L^{-1}$ potato infusion) added of the following heavy metals, separately: $Al(NO_3)_3.9H_2O$ (500 mg $L^{-1}$), $Pb(C_2H_3O_2)_2.3H_2O$ (100 mg $L^{-1}$), $Cr(NO_3)_3$ (125 mg $L^{-1}$) and $Cu(CH_3COO)_2$ (125 mg $L^{-1}$). Fungi were grown for 13 days at 28°C and 150 rpm and assays were performed in triplicate. Isolates that presented higher mycelial growth compared to the control (isolate grown in PDB medium without the addition of metals) were selected for heavy metal tolerance assay, as describe below.

### *Heavy metal tolerance in solid medium*

The method used to evaluate heavy metal tolerance in solid medium was established by Oladipo et al. (2018) with modifications. Isolates were tested for heavy metal tolerance through PDA medium cultivation, with the addition of the following metals, separately: chrome (125, 250 mg $L^{-1}$), copper (125, 250 and 500 mg $L^{-1}$), lead (100, 200 and 300 mg $L^{-1}$), and $Al(NO_3)_3.9H_2O$ (200 and 500 mg $L^{-1}$). Experiment was conducted in triplicates and control assay was not added of heavy metal. Discs of 5 mm diameter of PDA containing the isolates previously cultivated for 7 day were individually inoculated into a PDA plate containing the respective metal. All plates were incubated at 28 °C for 13 days. Mycelia radial growth was monitored every three days. Fungi heavy metal tolerance was rated as follow: 0.00–0.59 (low tolerance), 0.60–0.79 (moderate tolerance), 0.80–0.99 (high tolerance) and 1.00–>1.00 (very high tolerance). The higher the values the higher the fungal tolerance to the heavy metal. The isolates heavy metal tolerance potential was calculated in relation to the control radial growths as follow:

Tolerance index = Radial growth (cm) of test fungus in heavy metal incorporated medium

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Radial growth (cm) of fungus in non-incorporated medium

## Hydrocarbon tolerance assay

Screening assays to detect hydrocarbon tolerance was performed with all isolates. The method used was proposed by Tiso et al. (2016), with modifications. Filamentous fungi were tested for their tolerance to four hydrocarbon including benzene, toluene, hexane and xylene (all in analytical grade). Tolerance tests were carried out in liquid minimal media (MM) (0.2 g $L^{-1}$ magnesium sulfate, 0.02 g $L^{-1}$ calcium chloride, 0.7 g $L^{-1}$ monobasic potassium phosphate, 0.7 g $L^{-1}$ dibasic potassium phosphate, 0.5 g $L^{-1}$ ammonium sulfate, 0.5 g $L^{-1}$ sodium nitrate). The four hydrocarbons, separately, were used as the only carbon source. Isolates were transferred to test tubes containing 1.5 ml of MM in separate test tubes containing 0.5 mL of each organic solvent (1 tube for each fungus). Control was prepared with 1.5 ml MM in each test tube containing 0.5 mL of PDB medium (one control for each fungus). Experiments were performed in triplicate.

### Pyrene degradation analysis

#### *Microbial culture*

All experiments to evaluate pyrene degradation were performed as described by Passarini et al. (2011a, b), with adaptations. Each isolate was cultured in 3% malt extract agar for 7 days at 28 °C. Three fungal culture discs (0.5 cm diameter) taken from the edge of a pure PDA medium colony, were transferred to a 125 mL Erlenmeyer flasks containing 30 mL of Sabouraud Dextrose Broth (SDB) (10 g $L^{-1}$ of peptone and 20 g $L^{-1}$ of dextrose), in triplicate. Samples were incubated for 48h at 30 °C and 150 rpm. After incubation, 2 mg of pyrene (dissolved in 0.5 mL of dimethylsulfoxide), were added separately to each fungi culture. The samples were incubated in the dark for 5 days. The controls containing only the culture medium without the addition of pyrene and microbial strain were subjected to the same incubation conditions.

#### *Sample preparation*

Samples cultured with the presence of pyrene, as described above, were submitted to pyrene extraction from fermentative medium. For each sample, 50 mL of ethyl acetate were added followed by disintegration by Ultra-Turrax system T18 (Ika Werke, DE) (14.000 rpm for 1 minute). The samples were filtered, transferred to a separating funnel and shaken vigorously for 1 min. Anhydrous sodium was used to filter the organic phase and the aqueous phase, was re-extracted with 50 mL of ethyl acetate. The organic phases were evaporated and the residue was resuspended with 1 mL of ethyl acetate.

#### *GC–MS analysis*

Chromatographic analyses were performed using a GC-MS system TRACE 1300 equipped with an automatic sampler system TriPlus RSH and mass analyzer ISQ Single Quadrupole MS, all from ThermoScientific. Compounds were separated on a capillary column TR-5MS (5% diphenyl – 95% dimethylpolysiloxane, 30 m x 0.25 mm I.D. x 0.25 µm), ThermoScientific. Injector temperature was set at 290 °C and samples were injected (2 µL) in split mode. The oven column temperature ramped from 180 °C (1 min) to 310 °C at 5 °C $min^{-1}$, held for 10 min. Helium was used as carrier gas with a constant flow of 1 mL $min^{-1}$. Ions source was kept at 300 °C and MS transfer line temperature was set at 280°C, while a full scan monitoring mode (m/z 30–320) was set. Data processing was performed in ThermoXcalibur software, version 2.2 (Thermo Scientific). Identification was based on retention time comparison with the pyrene standard and with Nist Library.

## RESULTS AND DISCUSSION

A total of 23 filamentous fungi recovered from samples of a textile effluent treatment plant were evaluated for tolerance to heavy metals and hydrocarbons. Results derived from the screening for tolerant strain in liquid medium, showed that all twenty-three isolates were able to grow in media containing chromium and lead. Only one isolate (ITF 47) did not grow in the presence of copper. Six isolates (ITF 2, ITF 20, ITF 21, ITF 29, ITF31 and ITF 43) showed tolerance to all tested metals. Aluminum was the most toxic for the vast majority of isolates inhibiting about 65.3% of fungi tested (Table I).

Some metals are essential for the metabolism of fungi however they are toxic when present in excess. The levels of toxicity to each metal vary according to microorganism species, metal speciation and pH. These levels can inhibit fungal growth, in addition to causing morphological and physiological changes and affect reproduction. Therefore, they can reduce the number, diversity and select resistant or

**Table I. Tolerance of fungal species to different concentrations of heavy metal.**

| Isolates | Tolerance index levels of fungal strains | | | | | | | | Identification |
|---|---|---|---|---|---|---|---|---|---|
| | Chrome (mg $L^{-1}$) | | Copper (mg $L^{-1}$) | | Lead (mg $L^{-1}$) | | Aluminum (mg $L^{-1}$) | | |
| | 250 | 500 | 250 | 500 | 200 | 300 | 200 | 300 | |
| ITF 2 | 0,95 | 0,86 | 0.93 | 0.90 | 0.76 | 0.83 | 0.86 | 0,83 | *Penicillium* sp. |
| ITF 3 | 0,70 | 0,76 | 0.85 | 0.61 | 0.70 | 0.79 | ----- | ----- | *Penicillium* sp. |
| ITF 4 | 0,74 | 0,81 | 0.9 | 0.45 | 0.81 | 0.81 | 0.97 | 0,87 | *Penicillium rubens* |
| ITF 8 | 0.66 | 0,78 | ----- | ----- | 0.75 | 0.84 | ----- | ----- | *Penicillium* sp. |
| ITF 9 | 0,74 | 0,96 | 1.09 | 0.52 | 1.0 | 0.96 | ----- | ----- | *Aspergillus* sp. |
| ITF 10 | 0.67 | 1,24 | 0.95 | 0.62 | 0.9 | 0.95 | ----- | ----- | *Aspergillus sydowii* |
| ITF 11 | 0.74 | 0,96 | 1.07 | 0.67 | 0.93 | 0.96 | ----- | ----- | *Penicillium* sp. |
| ITF 12 | 0.88 | 0,94 | 1.06 | 1.06 | 0.88 | 1.29 | 1.82 | 1,59 | *Penicillium* sp. |
| ITF 14 | 0.60 | 0,87 | 0.87 | 0.63 | ----- | ----- | ----- | ----- | *Penicillium* sp. |
| ITF 20 | 0.76 | 0,68 | 0.84 | 0.76 | 0.74 | 0.72 | 0.76 | 0,72 | NI |
| ITF 21 | 0.87 | 0,78 | 0.96 | 0.71 | ----- | ----- | 0.84 | 0,76 | *Penicillium* sp. |
| ITF 22 | 0.95 | 1,33 | 0.95 | 1.19 | ----- | ----- | ----- | ----- | *Penicillium citrinum* |
| ITF 27 | 0.81 | 1,12 | 0.92 | 1.04 | 0.92 | 1.0 | ----- | ----- | *Aspergillus sydowii* |
| ITF 29 | 1.00 | 0,20 | 1.05 | ----- | 1.1 | ----- | ----- | ----- | *Trichoderma harzianum* |
| ITF 30 | ----- | ----- | 1.25 | 1.56 | ----- | ----- | ----- | ----- | *Aspergillus sydowii* |
| ITF 31 | 0.82 | 0,67 | ----- | ----- | 1.1 | 0.71 | ----- | ----- | NI |
| ITF 34 | 1.0 | 0,87 | ----- | ----- | 0.97 | 1.0 | 1.07 | 0,53 | *Penicillium* sp. |
| ITF 39 | 0.65 | 0,84 | 0.87 | 0.71 | 0.81 | 0.9 | ----- | ----- | *Penicillium* sp. |
| ITF 40 | 0.79 | 0,86 | 1.0 | 0.75 | 0.93 | 1.0 | ----- | ----- | *Penicillium* sp. |
| ITF 43 | 0.83 | 0,80 | 1.0 | 0.83 | ----- | ----- | 0.88 | 0,76 | *Aspergillus* sp. |
| ITF 44 | 0.74 | 0,84 | 0.9 | 0.58 | 0.81 | 0.84 | ----- | ----- | NI |
| ITF 47 | 0.67 | 1,47 | ----- | ----- | ----- | ----- | 1.33 | 1,60 | *Aspergillus amoenus* |
| ITF 60 | ----- | ----- | 0.82 | 0.56 | 0.76 | 0.91 | ----- | ----- | NI |

**-----: there was no growth on screening for tolerant strain in liquid medium assay. Macroscopic, microscopic and molecular analyses were performed by Bernal et al. (2021a). ITF = textile industry fungi. NI = not identified. ITF 4, 10, 27, 29, 30 and 47 = isolates were identified by molecular approaches Bernal et al. (2021b).**

tolerant microbial populations. As microorganism growth reflects cellular metabolism, it has been used as a key indicator of heavy metal toxicity to microorganisms (Vale et al. 2011). The isolates considered tolerant (which grew in the presence of metals), were evaluated in radial growth tests of the mycelium in solid culture medium, containing the same heavy metals in higher concentrations (except for aluminum, which showed a higher toxicity to the tested isolates, thus its concentration was reduced). Through this assay, it was possible to evaluate the Metal Tolerance Index (Table I).

The results found indicated a high tolerance (with tolerance index between 0.80 to 0.99) to the metals by most of the tested fungi (60.8%). The isolates ITF 2, ITF 4, ITF 12, and ITF 20 presented the tolerance index in all concentrations of

metals evaluated in the solid culture medium. We can highlight the isolate ITF 20 (not identified), with tolerance to all metals in all concentrations tested in liquid and solid medium (Table I), followed by the isolate ITF 12 *Penicillium* sp., which presented high levels of tolerance (between 0.88 to 1.82) for the concentrations of heavy metals evaluated. Only isolate ITF 30 (*Aspergillus sydowii*) showed tolerance only to copper in the two concentrations assayed. Chromium was the heavy metal that least affected microbial growth, while aluminum was the heavy metal with the greatest negative effect on fungal mycelial growth. The isolate ITF 2 (*Penicillium* sp.) showed exceptional capacity to tolerate copper (1.06 for 250 and 500 mg $L^{-1}$), lead (1.29 for 300 mg $L^{-1}$) and aluminum (1.82 and 1.59 for 200 and 300 mg $L^{-1}$) (Table I).

Most isolates able to tolerate almost all the heavy metals analyzed was represented by the genus *Penicillium* (n = 13), thus demonstrating its potential use as a bioremediation agent for heavy metals. Reports in the literature describe the investigation of fungal resistance from the same genus to heavy metals from industrial discharge effluent and their adaptation to these toxic substances (Iskandar et al. 2011, Mohammadian et al. 2017). Muñoz-Silva et al. (2019) studied the degree of tolerance to heavy metals from fungi isolated from the soil with mining, in order to know their potential for applications in bioremediation works. Of the 23 isolated fungi, the genus *Penicillium* had the best tolerance rates for the metals lead (Pb), zinc (Zn), nickel (Ni), and chromium (Cr). The authors concluded that the isolates may be useful for bioremediation processes development. *Penicillium* sp. and *Penicillium janthinellum* isolated from tea fields showed aluminum tolerance when cultivated in medium supplemented with 10, 50, 100, 150 and 200 mM of this metal (Kawai et al. 2000). However, few studies have been carried out focusing on heavy metal tolerance by filamentous fungi isolated from textile industrial effluents for application in bioremediation processes of heavy metal contaminated environments. Presence of species such as *Penicillium* and *Aspergillus,* in sites contaminated with heavy metals, occurs due to their ability to remove heavy metals from the environment (Iram et al. 2012). According to Kant (2012), wastewater from the textile industry contains large amounts of heavy metals which may explain the presence of a large number of microbial isolates resistant to these metals.

Concerning hydrocarbon tolerance, from 23 isolates tested, seven isolates (30.4%) were able to tolerate one or more hydrocarbon including benzene, toluene, xylene and hexane. The isolates ITF 12 (*Penicillium* sp.) and ITF 20 (not identified) were the fungi that showed greater tolerance to hydrocarbons, and were able to thrive in culture medium supplemented with benzene, toluene and hexane, separately. In comparison to the respective controls, the isolate ITF 12 showed mycelial growth greater than the isolate ITF 20, being then selected to undergo pyrene hydrocarbon degradation tests. The isolate ITF 34 (*Penicillium* sp.) was able to grow in medium supplied with toluene and hexane, separately. No isolate was able to grow in medium supplied with xylene (Table II).

It is possible to verify that all isolates tolerant to hydrocarbons are representatives of the genus *Penicillium*. The genus *Penicillium* can be present in different habitats with unfavorable conditions for microbial development such as high concentrations of pH, salinity, temperature, and low availability of nutrients (Naraian & Gautam 2018). The fact that it is an organism that produces many spores may be the reason for the high tolerance of this genus demonstrated in the present study. *Penicillium* species isolated from extreme environments can be used to

**Table II. Tolerance capability of fungal species to hydrocarbons.**

| Isolates | Hydrocarbon tolerance | | | |
|---|---|---|---|---|
| | **Benzene** | **Toluene** | **Xylene** | **Hexane** |
| *Penicillium* sp. ITF 2 | ----- | ----- | ----- | ----- |
| *Penicillium* sp. ITF 3 | ----- | ----- | ----- | ----- |
| *Penicillium rubens* ITF 4 | ----- | ----- | ----- | ----- |
| *Penicillium* sp. ITF 8 | x | ----- | ----- | ----- |
| *Aspergillus* sp. ITF 9 | ----- | ----- | ----- | ----- |
| *Aspergillus sydowii* ITF 10 | ----- | ----- | ----- | ----- |
| *Penicillium* sp. ITF 11 | ----- | ----- | ----- | ----- |
| *Penicillium* sp. ITF 12 | x | x | ----- | x |
| *Penicillium* sp. ITF 14 | ----- | ----- | ----- | ----- |
| NI ITF 20 | x | x | ----- | x |
| *Penicillium* sp. ITF 21 | ----- | x | ----- | ----- |
| *Penicillium citrinum* ITF 22 | ----- | ----- | ----- | ----- |
| *Aspergillus sydowii* ITF 27 | ----- | ----- | ----- | ----- |
| *Trichoderma harzianum* ITF 29 | ----- | ----- | ----- | ----- |
| *Aspergillus sydowii* ITF 30 | ----- | ----- | ----- | ----- |
| NI ITF 31 | ----- | ----- | ----- | ----- |
| *Penicillium* sp. ITF 34 | ----- | x | ----- | x |
| *Penicillium* sp. ITF 39 | ----- | ----- | ----- | ----- |
| *Penicillium* sp. ITF 40 | ----- | ----- | ----- | ----- |
| *Aspergillus* sp. ITF 43 | ----- | ----- | ----- | x |
| NI ITF 44 | ----- | ----- | ----- | ----- |
| *Aspergillus amoenus* ITF 47 | ----- | ----- | ----- | ----- |
| NI ITF 60 | ----- | ----- | ----- | x |

**-----: there was no growth. NI: not identified. ITF: textile industry – fungus.**

understand adaptive processes that allow life in these types of environments (Yadav et al. 2018).

The results showed clearly that most isolates (n=16) were not able to tolerate hydrocarbons at concentrations of 25% because the hydrocarbons assayed were toxic for microbial growth, mainly xylene. The xylene hydrocarbon (MM 106.16 g $moL^{-1}$) has a higher molecular weight than the other hydrocarbons, which may have restricted the growth of microorganisms in the culture medium. According to Baldantoni et al. (2017), resistance of PAHs to microbial degradation increases with increasing molecular weight. The PAHs biodegradation depends on the stability of the aromatic rings and the solubility and bioavailability of the PAHs, as well as hydrocarbon molecule concentration and size (Wetler-Tonini et al. 2010).

Literature reports describe the investigation of hydrocarbon tolerance by filamentous fungi from contaminated samples. According to Lima et al. (2011) species from genera *Penicillium* and *Aspergillus*, including *A. niger,* are the main degrading fungi found in the literature. Santos et al. (2008) performed an isolation of four *Aspergillus* spp. from contaminated

soil samples from Rio Grande, Brazil, using a method involving the determination of colony growth rates on medium containing specific hydrocarbons including phenol, hexane, chlorobenzene, benzene, toluene and xylene. The LEBM1 isolate was the only strain capable of growing in all hydrocarbon supplemented medium, showing a high tolerance to benzene, toluene and xylene. Bhuvaneswari et al. (2012) reported toluene biodegradation by about 75% of strains identified as *Penicillium* sp., *Aspergillus niger* and *Trichoderma viride*. The fungi were isolated from municipal sewage water from Koyambedu, India. Rodrigues et al. (2017) using a continuous flow reactor, observed the ability of an *Aspergillus niger* strain to remove BTEX (benzene, toluene, ethylbenzene and xylene). The fungus was able to degrade benzene, toluene, ethylbenzene and xylene in the percentages of 88%, 90%, 90% and 91%, respectively. Studies using filamentous fungi isolated from industrial textile effluents were not found, thus demonstrating the importance in the search for microbial cells recovered from these environments capable of metabolizing hydrocarbons.

With respect to the pyrene degradation assay, the textile industry-derived fungus *Penicillium* sp. (ITF 12) was able to degrade 24.9% of pyrene after five days of cultivation (Figure 1). Comparing degradation experiments with their respective controls, it was possible to observe that pyrene had not toxic effects on the fungal strain. The dry weight of the biomass of the *Penicillium* sp. (ITF 12) was similar to that produced by the controls (without addition of PAH, data not shown). Two strategies, described by Passarini et al. (2011a) and by Passarini et al. (2011b) were applied to ensure that the reduction of pyrene was not caused by pyrene adsorption on fungal mycelium: *i)* the use of Ultra-Turrax system, which promotes greater efficiency in the extraction of pyrene by the rupture of cells under intensive shaking; *ii*) the use of two subsequent extractions to ensure efficiency greater than 99% for the recovery of pyrene.

These results demonstrated that in just five days the *Penicillium* sp. (ITF 12) strain showed aptitude in hydrocarbons degradation. More expressive results can be obtained by extending the action time, since the results were achieved in a five days assay. Works have described the biodegradation of hydrocarbon by fungal isolates from different samples. Souza et al. (2017) screened filamentous fungi isolated from sediments sampled from Rio Negro, Amazon, Brazil, contaminated with polycyclic aromatic hydrocarbons. All fungi showed tolerance to phenanthrene and pyrene, among them, four *Penicillium* spp. strains. These strains were able to tolerate 240, 540, 780, 1020 and 2040 μg $mL^{-1}$ of pyrene after 10 days of growth. According to Passarini et al. (2011a), the marine-derived fungi *Aspergillus sclerotiorum* (CBMAI 849) showed 84.9% of pyrene biodegradation after 4 days of growing in liquid medium. The same isolate was able to metabolize pyrene to the corresponding pyrenylsulfate. The authors suggested that a cytochrome P-450 monooxygenase was responsible for hydroxylation followed by conjugation with sulfate ions.

In addition to intracellular enzymes such as monooxygenase, pyrene can also be degraded by extracellular enzymes such as ligninolytics. According to Agrawal & Shahi (2017), the white rot fungi *Coriolopsis byrsina*, isolated from Surguja district of Chhattisgarh, India, was able to degrade 96.1% of pyrene with the help of their ligninolytic enzymes including laccase, lignin peroxidase and manganese peroxidase. In this work, the authors identified the intermediate metabolites pyruvic acid, benzoic acid, benzoic acid 2-hydroxy pentyl ester, phenanthrene,

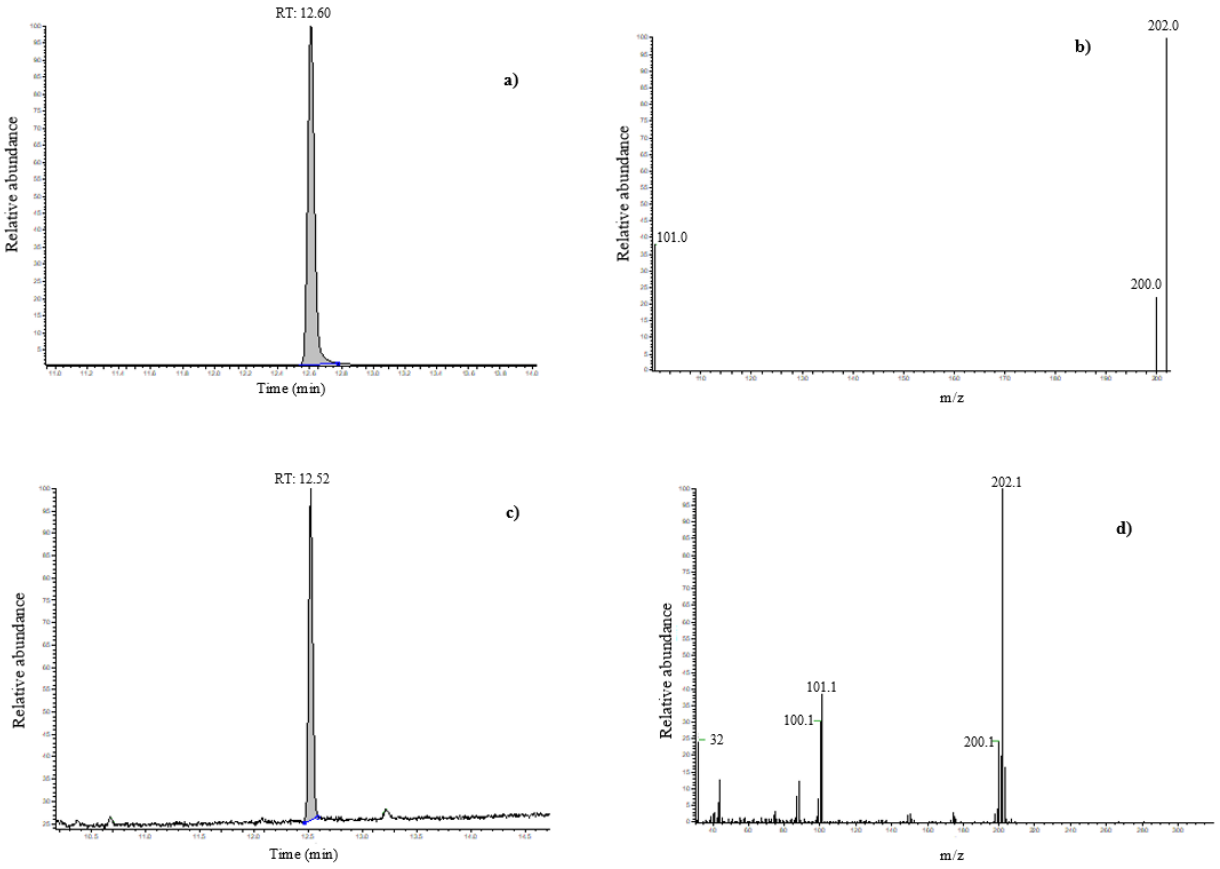


**Figure 1. Analysis of pyrene degradation after five days of microbial growth. Chromatograms and fragmentogram obtained by GC–MS after pyrene extraction approaches. a) and c) standard pyrene solution; b) and d): ITF12 (*Penicillium* sp.). RT: retention time.**

pthalic acid diisopropyl ester and 4, 5 di hydroxy pyrene by using GC-MS.

In the present study, it is possible to state that the *Penicillium* sp. isolate (ITF 12) was the fungus that presented the best results, both for tolerance to the tested heavy metals (chrome, copper, lead and aluminum) in all concentrations, as well as for the presence of benzene, toluene and hexane hydrocarbons, being in addition able to degrade 24.9% of pyrene after 5 days incubation. However, it was not possible to find the intermediate metabolites of the pyrene degradation in the first days of microbial fermentation (5 days). Perhaps, studies with longer fermentation times (10 to 14 days) could show the formation of metabolites from pyrene compound degradation. On the other hand, works using filamentous fungi isolated from industrial textile effluents, for pyrene hydrocarbon degradation were not found. Thus demonstrating the relevance in research using fungi recovered from these samples capable of degrading PAHs including pyrene. Despite the lack of visualization of intermediate metabolites during the degradation of pyrene, we can say that the *Penicillium* sp. ITF 12 isolate represents a potentially degrading agent of aromatic hydrocarbons and can be used in bioremediation processes. As industrial textile effluents present several compounds including hydrocarbon and heavy metals (Upadhyay et al. 2016, Ghaly et al. 2014), present work results corroborate with the expected profile of microorganisms which inhabit an environment with such specific

characteristics as the studied samples. Thus, the *Penicillium* sp. ITF 12 showed biotechnological potential for bioremediation process possibly due to evolutionary adaptations that could have occurred in the presence of toxic compounds including dyes, hydrocarbon and heavy metals.

Results from the present work demonstrated the biotechnological potential that microbial communities recovered from textile wastewater treatment plant samples may present. Tolerance and degradation results showed the importance and the adaptive potential of filamentous fungi recovered from textile effluents which can be used in studies of environments contaminated with heavy metals and/or hydrocarbons. Fungi that thrive in this environment can produce a range of compounds of industrial interest, as observed with the isolate *Penicillium* sp. ITS 12, which showed tolerance to heavy metals and hydrocarbons and ability to withdraw hydrocarbon from the medium. Such abilities can be harnessed in favor of bioremediation processes in contaminated environments, including industrial effluents. Further researches are needed for the characterization of the compounds or the possible enzymes involved in the pyrene degradation.

**Acknowledgments**

This work was supported by the Programa Institucional Agenda Tríplice (EDITAL PRPPG/UNILA Nº 137/2018–UNILA) and by the Programa Institucional Apoio a Grupos de Pesquisa (EDITAL PRPPG/UNILA Nº 110/2018).

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**How to cite**
LIRA MMA, BERNAL SPF, CASTRO CCJ, RAMOS PMM, LIRA MJS, OTTONI JR, BOROSKI M & PASSARINI MRZ. 2022. Filamentous fungi from textile effluent and their potential application for bioremediation process. An Acad Bras Cienc 94: e20201718. DOI 10.1590/0001-3765202220201020.

*Manuscript received on July 2, 2020;*
*accepted for publication on January 12, 2021*

**MILENE M.A. LIRA**[1]
https://orcid.org/0000-0003-1056-5825

**SUZAN P.F. BERNAL**[1]
https://orcid.org/0000-0003-0919-3761

**CAMILA C.J. CASTRO**[1]
https://orcid.org/0000-0002-6291-7434

**PRISCILA M.M. RAMOS**[2]
https://orcid.org/0000-0001-6132-4592

**MICHAEL J.S. LIRA**[3]
https://orcid.org/0000-0002-3748-901X

**JÚLIA R. OTTONI**[4]
https://orcid.org/0000-0003-1229-7676

**MARCELA BOROSKI**[2]
https://orcid.org/0000-0003-1621-5199

**MICHEL R.Z. PASSARINI**[1]
https://orcid.org/0000-0002-8614-1896

[1]Programa de Pós-Graduação em Biociências, Universidade Federal da Integração Latino-Americana (UNILA), Laboratório de Biotecnologia Ambiental, Av. Tarquínio Joslin dos Santos, 1000, Jd. Universitário, 85870-650 Foz do Iguaçu, PR, Brazil

[2]Programa de Pós-Graduação em Energia & Sustentabilidade, Universidade Federal da Integração Latino-Americana (UNILA), Av. Tancredo Neves, 6731, 85867-970 Foz do Iguaçu, PR, Brazil

[3]Coordenação Institucional de Informação e Regulação, Universidade Federal da Integração Latino-Americana (UNILA), Av. Silvio Américo Sasdelli, 1842, Itaipu A, 85866-000 Foz do Iguaçu, PR, Brazil

[4]Centro Universitário Dinâmica das Cataratas (UDC), Av. Castelo Branco, 340, Centro, 85852-010 Foz do Iguaçu, PR, Brazil

Correspondence to: **Michel Rodrigo Zambrano Passarini**
*E-mail: michel.passarini@unila.edu.br*

## Author contributions

MMAL, SPFB, CCJC, PMMR and MJSL carried out the analyses of the data; JRO, MB and MRZP wrote, reviewed, and edited the manuscript; all authors approved the final version of this manuscript.