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Research article

Simple screening protocol for identification of potential mycoremediation tools for the elimination of polycyclic aromatic hydrocarbons and phenols from hyperalkalophile industrial effluents

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ABSTRACT

A number of fungal strains belonging to the ascomycota, basidiomycota and zygomycota genera were subjected to an *in vitro* screening regime to assess their ligninolytic activity potential, with a view to their potential use in mycoremediation-based strategies to remove phenolic compounds and polycyclic aromatic hydrocarbons (PAHs) from industrial wastewaters. All six basidiomycetes completely decolorized remazol brilliant blue R (RBBR), while also testing positive in both the guaiacol and gallic acid tests indicating good levels of lignolytic activity. All the fungi were capable of tolerating phenanthrene, benzo-α- pyrene, phenol and *p*-chlorophenol in agar medium at levels of 10 ppm. Six of the fungal strains, *Pseudogymnoascus* sp., *Aspergillus caesiellus*, *Trametes hirsuta* IBB 450, *Phanerochate chrysosporium* ATCC 787, *Pleurotus ostreatus* MTCC 1804 and *Cadophora* sp. produced both laccase and Mn peroxidase activity in the ranges of 200–560 U/L and 6–152 U/L, respectively, in liquid media under nitrogen limiting conditions. The levels of adsorption of the phenolic and PAHs were negligible with 99% biodegradation being observed in the case of benzo-α-pyrene, phenol and *p*-chlorophenol. The aforementioned six fungal strains were also found to be able to effectively treat highly alkaline industrial wastewater (pH 12.4). When this wastewater was supplemented with 0.1 mM glucose, all of the tested fungi, apart from *A. caesiellus*, displayed the capacity to remove both the phenolic and PAH compounds. Based on their biodegradative capacity we found *T. hirsuta* IBB 450 and *Pseudogymnoascus* sp., to have the greatest potential for further use in mycoremediation based strategies to treat wastestreams containing phenolics and PAHs.

1. Introduction

Wastes generated by industrial processes are mainly composed of ubiquitous organic compounds. In general, aromatic compounds are extensively distributed in nature, showing a rich structural diversity and are considered as strong pollutants (Fuchs et al., 2011). Polycyclic aromatic hydrocarbons (PAHs) and phenolic compounds are well established as being among the most toxic environmental

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priority pollutants known (Gupta et al., 2015; Tisa et al., 2015). Since aromatic compounds have a poor reactivity, they can stably remain in the environment for long periods of time (Fuchs et al., 2011). However, when activated, they are extremely dangerous because they can react with biological molecules, such as DNA, as well as lipids present in cellular membranes and with proteins (Bragin et al., 2016; Long et al., 2016).

PAHs are a wide and heterogeneous group of compounds (more than 100 compounds) with two or more condensed benzene rings in linear, angular or cluster arrangements (Scott, 2015). They can be either naturally produced or derived from anthropogenic sources. PAHs can be found in oil and carbon deposits, and their adverse effects which include toxicity, teratogenicity, mutagenicity and carcinogenicity have been extensively demonstrated (Bragin et al., 2016; Long et al., 2016). PAHs are semi-volatile, thermodynamically stable, and show low aqueous solubility (Keyte et al., 2013). Consequently, they are highly recalcitrant compounds that persist over long periods of time in ecosystems (Kuśmierz et al., 2016). PAHs immobilization in nature mainly occurs through adsorption into soil or sediment particles due to their hydrophobic characteristics and by bioaccumulation processes in trophic chains (Petit et al., 2013; Shuttleworth and Cerniglia, 1995).

Phenols are widely used in numerous industrial processes. Consequently, several toxic phenols are generated from industrial operations (Pontes et al., 2010). They are highly toxic even at low concentrations; are one of the most prevalent forms of chemical contaminants, and the major pollutants found in industrial wastewaters (Gayathri and Vasudevan, 2010). Since 1989, phenolic compounds have been considered as the second major class of pollutants in the environment (Guang, 1998). Particularly, phenols can be found in liquid effluents such as petrochemical, papermaking, oil refining, resin manufacturing, coking, iron-smelting, pharmaceutical industries, among others (Tisa et al., 2015). Phenolic compounds present in wastewaters represent a potential risk for the aquatic biota (Gayathri and Vasudevan, 2010).

PAHs and phenolic compounds in the environment can be transformed by different mechanisms such as photolysis and chemical or biological oxidation (Biache et al., 2015; Riva et al., 2015). Microbial transformation is recognized as the main process in their mineralization in nature (Haritash and Kaushik, 2009; Tisa et al., 2015). Different microbial catabolic pathways are involved in the PAHs and phenolic biodegradation or mineralization. PAHs and phenol catabolic genes have been studied in both bacteria and fungi, and several microbial treatment strategies have been described in response to these pollutants (Aranda, 2016; Haritash and Kaushik, 2009). The resonance energy that stabilizes the aromatic ring systems is the main base of the aromatic substance recalcitrance, which confers a high redox potential for electron transfer reactions. For instance, only a small group of enzymes are able to cleave the aromatic rings of PAHs or phenol-based compounds (Fuchs et al., 2011). While dioxygenases, dihydroxygenases, monooxygenases, phenol hydroxylases, serine hydrolases and aldolases are involved in the bacterial degradation of PAHs and phenols via either metabolism or cometabolism (Moody et al., 2004; Rentz et al., 2008), only two major fungal mechanisms have been described. They are *(i)* the cytochrome P-450 monooxygenase pathway and *(ii)* extracellular enzymes of lignin catabolism such as lignin peroxidases (LiP), manganese peroxidase (MnP) and laccases (Lac) (Aranda, 2016; Cerniglia, 1997; Krastanov et al., 2013; Mhuantong et al., 2015). These three ligninolytic enzymes oxidize a wide range of organic compounds (including several oil fractions, phenols and PAHs) by virtue of their low specificity (Andriani et al., 2016; Pang et al., 2015; Zafra and Cortes-Espinosa, 2015).

The bacterial metabolism of PAHs and phenolic compounds have been extensively studied and their molecular mechanisms extensively elucidated. However, some fungi have shown the ability to remove and mineralize PAHs and phenolic compounds in a more competent way than bacteria (Fernández-Luqueño et al., 2010; Fuentes et al., 2014; Juhasz and Naidu, 2000). Moreover, microbial-based approaches to remove phenols or PAHs under extreme conditions (e.g. high pH and salinity) provide an opportunity for the screening of microorganisms with extremophile characteristics. The use of fungi to remove aromatic compounds from industrial wastewaters enriched with phenols or PAHs derived from chemical industry, provides an opportunity for downstream biotechnological applications on hypersaline or alkaline liquid wastes (Acikgoz and Ozcan, 2016; Christen et al., 2011). Fungi growing under extremophile conditions (e.g. alkaline pH) in real wastewaters provide an attractive resource for the development of bioprocesses and ecological restoration.

There is an increasing interest in exploring as yet unstudied or understudied fungal species/strains in order to degrade PAHs and phenol-based compounds, and to select ideal candidates for bioremediation of industrial wastewaters contaminated with organic pollutants. The environmental problems caused by the anthropogenic activities demand new screening regimes to uncover fungal phylotypes with robust potencies for downstream biotechnological applications.

This work aimed to study the removal of phenols and PAHs *in vitro* by the screening of a fungal collection and to identify relevant strains capable of eliminating phenolic compounds and PAHs from biorefinery industrial wastewaters. To this end, twelve fungal strains were screened based on their ability to secrete ligninolytic enzymes to degrade PAHs and phenols. Additionally, we tested the tolerance of these strains at a variety of different PAHs and phenol concentrations, and their efficiency in the removal of these compounds from wastewaters.

2. Materials and methods

2.1. Microorganisms and chemicals

Zygomycetes, ascomycetes and basidiomycetes species isolated from different environments or deposited in different microbial collections were analyzed in this work (Table 1). Spores and mycelia

Table 1
Zygomycetes, ascomycetes and basidiomycetes utilised in this study.

Species	Remarks
Zygomycete	
Cunninghamella elegans ATCC 36112	
Ascomycetes	
Cadophora sp. TS2	Isolated from the deep sea
Emericellopsis sp. TS11	sponge *Stelleta normani*
Pseudogymnoascus sp. TS12	
Aspergillus caesiellus H1	Halophilic fungus
Trichoderma atroviride CEIB 206040	
Trichoderma atroviride + Lac of	Recombinant clone
Trametes sanguineus CeIB MD01	of *T. atroviride*
Basidiomycetes	
Pleurotus dryinus IBB 903	
Trametes hirsuta IBB 450	
Phanerochaete chrysosporium ATCC 787	
Trametes hirsuta MTCC 1171	
Pleurotus ostreatus MTCC 1804	

ATCC: American Type Culture Collection.
TS: Reference in School of Microbiology, University College Cork. Cork, Ireland.
CEIB: Biotechnology Research Center of the Autonomous University of the Morelos State.
IBB: Institute of Biochemistry and Biotechnology, Tbilisi, Georgia.
MTCC: Indian Microbial Type Culture Collection.