Mycoremediation of PAHs mixture by filamentous fungi.

G. Greco[1,2,*], G M. Capello[1], Cecchi[1,2], L. Cutroneo[1], S. Di Piazza[1,2], G. Vagge[1], M. Zotti[1,2].
[1]DISTAV - Department of Environmental, Earth and Life Sciences, Laboratory of Mycology[2], University of Genoa, Genoa, I-16132, Italy
Keywords: Marine sediment, autochthonous fungi, organic polluted substances, biodegradability.
* Presenting Author email giuseppe.greco@edu.unige.it

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a large group of widespread environmental pollutants (Clemente et al. 2001; Jiang et al. 2009) distributed in aquatic environments (Shi et al., 2005), sediments, and soils (Lima et al. 2005). These polluting substances end up in the atmosphere and then fall back on specific environmental compartments, such as soils, compromising their protective but also productive and ecological functions (Samanta et al. 2002). Furthermore, these compounds have been widely studied because PAHs are known to be both persistent in the environments and potential carcinogenic (Wang et al 2013; Farrington et al. 2014). Some organisms, such as fungi, utilizing a wide range of different carbon sources, are capable to transform PAHs into non-toxic compounds (Verdin et al 2004; Cerniglia et al. 2010). Many fungal species are adaptable to adverse environmental conditions and they result particularly able to metabolize polycyclic aromatic hydrocarbons (Reyes-César et al. 2014; Marco-Urrea et al. 2015). In particular, some mycelia would be capable to degrade and absorb PAHs through specific enzymes, such as laccase, lignin peroxidase, and manganese peroxidase (Baborová et al. 2006). Some fungal species play a significant role in the PAHs degradation processes, such as *Aureobasidium* (Sihag et al. 2014), *Rhodotorula* (Cerniglia et al. 2010, Sihag et al. 2014), and *Sporobolomyces* spp. (Sihag et al. 2014). Biodegrading capability was also proven for *Geotrichum* (Giraud et al. 2001; Cerniglia et al 2010) and *Rhizopus* (Cerniglia et al. 2010; Fernández-Luqueño et al. 2011) isolated from soil and water samples. Garon et al. (2004) found that *Absidia cylindrospora* Hagem, in a hydrocarbon-contaminated soil, about 90% fluorene removed in 288 h, whereas in the absence of the fungus the process spends 576 h. Ye et al. (2011) showed that *Aspergillus fumigatus* Fresen is able to break up anthracene: the molecular structure was modified into a series of compounds with lower toxicity levels as phthalic anhydride, anthrone, and anthraquinone. This study is devoted to demonstrate the potential degrading role of PAHs by many filamentous fungal species and to select a pool of fungal strains in order to optimize the real feasibility of large-scale biodegradation processes.

Materials and methods

Samples of bilge water affected by PAHs were collected from December 2015 to February 2017. All the samples were stored in sterile plastic jars and conserved at 5 ± 1 °C to preserve their chemical and physical *in situ* characteristics. A specific method was employed to isolate vital fungal strains biologically adapted to live in contaminated substrate (Greco et al. 2017). The goal is to select a pool of fungal strains usable in future mycoremediation protocols. The strains were identified by a polybasic approach (morphological, physiological and molecular) and maintained, in axenic cultures, in the collection of the Laboratory of Mycology, for bioremediation purposes. Five isolates were selected to test their ability to degrade sludge. PAHs mixture were added in sterile 9-mm Petri dishes to MEA medium, at three different concentrations (25%, 50%, and 75%). All the Petri dishes were incubated at 24±1°C, in the dark, and the colonies were checked daily for 28 days to monitor fungal growth.

Results

Among the isolated species, *Fusarium solani* (Mart.) Sacc., *Pseudalleschiera boydii* (Shear) McGinnis, A.A. Padhye & Ajello, *Sordaria fimicola* (Roberge ex Desm.) Ces. & De Not., *Talaromyces amestolkiae* N. Yilmaz, Houbraken, Frisvad & Samson were selected to test their ability to utilize sludge and MEA as source of carbon and energy for growth. These species demonstrated the ability to degrade a large set of PAHs at different rates. In particular, at 25% of concentration, *F. solani* showed the capability of degrading acenaphthene, anthracene, benzo(a)anthracene, fluorene and pyrene of up 95% of the initial added amount.

Discussion

Our research was carried out on complex mixtures of PAHs. Among the isolated fungi, *F. solani* has degraded/broken up more PAHs because it produces specific lignin-useful enzymes for the degradation/break up process. Some works report its ability to degrade/break up benzo[a]pyrene (Verdin et al.,2004), pyrene (Ravelet et al. 2000; Romero et al.,2002), and anthracene (Wu et al.2010).

Conclusions

This study has been proved the capability of filamentous fungi isolated from polluted bilge waters to biodegrade PAHs compounds. This appears strictly connected to the capability of this kind of fungi bio-mineralize and/or biotransform PAHs in less hazardous compounds.

The work represents a first step in the research on sustainable remediation techniques of polluted marine sediments.

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