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Short Communication

Bioremediation potential of basidiomycetes isolated from compost

Antonella Anastasi [a], Giovanna C. Varese [a,*], Francesca Bosco [b], Fabiana Chimirri [b], Valeria Filipello Marchisio [a]

[a] *Dipartimento di Biologia Vegetale, Università degli Studi di Torino, Viale Mattioli 25, 10125 Torino, Italy*
[b] *Dipartimento di Scienza dei Materiali ed Ingegneria Chimica, Politecnico di Torino, Corso Duca degli Abruzzi 24, 10129 Torino, Italy*

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Abstract

The potential of a consortium of three basidiomycete mycelia isolated from compost to degrade polycyclic aromatic hydrocarbons (PAH) was first evaluated using a test based on decolorization of Poly R-478 dye. When pre-grown on straw, the consortium decolorized the dye by 83% in 7 days and generated a laccase activity of 663 IU l^{-1}. Its ability to degrade naphthalene was investigated in soil microcosms specially suited for this volatile PAH. The kinetic study was conducted at a maximal naphthalene concentration of 500 mg kg^{-1} of soil. Naphthalene concentration, CO_2 evolution and phytotoxicity (germination index, GI%) on *Lepidium sativum* seeds were monitored. The naphthalene concentration decreased by about 70% in three weeks in the presence of metabolic activity, while the GI% increased indicating reduced phytotoxicity.

Keywords: Fungi; Naphthalene; PAH degradation; Phytotoxicity tests; Poly R-478

1. Introduction

Polycyclic aromatic hydrocarbons (PAH) are common, persistent and recalcitrant contaminants in soil. Their distribution and fate within the environment are a matter of growing concern, especially since many are cytotoxic, mutagenic and carcinogenic and have been classified as priority pollutants by the EPA (Aitken and Long, 2004). PAH can be eliminated from polluted soil by incineration, thermal desorption, soil washing etc. These methods, however, are expensive and have an adverse impact on the environment (Canet et al., 2001). Bioremediation is a competitive alternative.

Composts have an enormous potential for bioremediation through their support of mesophilic and thermophilic bacteria and ligninolytic fungi endowed with the ability to degrade aromatic pollutants (Semple et al., 2001). However, a better understanding of the compost microflora involved in PAH degradation is still needed, with particular emphasis on the impact of ligninolytic fungi (Antizar-Ladislao et al., 2004). Lignin degrading basidiomycetes normally do not assimilate PAH as the sole carbon source, and hence require cometabolites (e.g. glucose) to degrade them (Singh, 2006). Their degradation results in the formation of PAH-quinones catalysed by extracellular ligninolytic enzymes such as lignin peroxidase (LiP), manganese peroxidase (MnP), laccases (Lcc) and H_2O_2-generating enzymes (Singh, 2006). These enzymes are non-specific, non-stereoselective and effective against a broad spectrum of aromatic compounds including anthracene, benzo[*a*]pyrene, naphthalene and pyrene (Muncnerovà and Augustin, 1994; Novotny et al., 1999).

However, basidiomycetes are rarely isolated from compost, both because many of them cannot withstand the temperatures of more than 50 °C generated during the thermophilic stage (Ryckeboer et al., 2003), and because their isolation and morphological identification are impeded by the fact that they are often found as sterile mycelia, while their molecular detection is hampered by the

* Corresponding author. Tel.: +39 0116705984; fax: +39 0116705962.
E-mail address: cristina.varese@unito.it (G.C. Varese).

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incompleteness of gene databases and the low taxonomic resolution of DNA sequences. The genera observed in compost include *Armillaria*, *Clitopilus*, *Coprinus*, *Fomes*, *Lentinus*, *Lenzites*, *Pleurotus* and *Trametes*. The degradative capability of basidiomycetes from compost, however, has not been investigated.

We have previously used a test based on the decolorization of Poly R-478 dye in solid culture to assess the ligninolytic activity of 266 fungal isolates (33 were basidiomycetes) from different kinds of composts (Anastasi et al., 2005). Decolorization ability and ligninolytic enzymes production of the three most promising basidiomycetes were then investigated in greater detail in liquid culture (Anastasi et al., 2006) containing Poly R-478, since the ability to decolorize this dye is also regarded as indicative of a PAH degradation potential (Alcade et al., 2002; Canet et al., 2001). The aim of the present work was first to investigate the ability to decolorize Poly R-478 of the same three basidiomycetes in a consortium and determine its inhibitory/synergic effects on decolorization and enzyme activities, and then to evaluate its PAH degradation capability in soil microcosms polluted with naphthalene. Phytotoxicity due to naphthalene and/or any of its metabolites was monitored by calculating the germination index (GI%) of *Lepidium sativum* seeds during the incubation period.

2. Methods

2.1. Microorganisms and inoculum preparation

The three basidiomycetes were patented and deposited in the DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH) collection (DSM 15214, DSM 15215, DSM 15216); they were isolated from compost as sterile mycelia and identified as basidiomycetes morpho-physiologically (Anastasi, 2003); rDNA sequencing (Internal Transcribed Spacer – ITS 1, 2 – and 18S) and comparison with sequences in gene databases showed that strains DSM 15214 and DSM 15215 had 100% similarity to *Trametes versicolor* (L.) Pilát, while strain DSM 15216 had 99% similarity to different species of basidiomycetes [*Bjerkandera adusta* (Willd.) P. Karst., *Bjerkandera fumosa* (Pers.) P. Karst., *Lopharia spadicea* (Pers.) Boidin] (GenBank, NCBI). All three strains had been shown to display Lcc and/or MnP activity during their decolorization of Poly R-478 in single cultures (Anastasi et al., 2006).

Equal portions of straw cultures of each strain, prepared as previously described (Anastasi et al., 2006), were used to inoculate flasks for Poly R-478 decolorization and naphthalene degradation studies.

2.2. Decolorization tests and enzyme assays in liquid cultures

Decolorization tests were performed in 500 ml, hermetically sealed Erlenmeyer flasks containing 30 ml of a synthetic glucose medium (Gold et al., 1988) supplemented with an aqueous Poly R-478 solution (Sigma Aldrich Co., Ltd.) at a final concentration of 0.02%. Inoculum consisted of 0.2 g straw cultures for each strain. A heat-killed control (straw cultures autoclaved at 121 °C for 20 min) and an abiotic (consortium-free) control were set up to check for absorption of the dye on the mycelium or a decrease in absorbance not due to fungal presence. Triplicate cultures were incubated statically at 27 °C and flushed about every 2 days with O_2 in sterile conditions. Decolorization was monitored daily for 7 days on 0.3 ml cell free culture medium diluted 10-fold in water and expressed as the percentage decrease of absorbance at 514 nm, i.e. the maximum visible wavelength of absorbance of Poly R-478, with a UV–visible spectrophotometer (Ultrospec 3300 pro; Amersham).

LiP activity was evaluated spectrophotometrically *via* the oxidation of veratryl alcohol at the wavelength of 310 nm in 250 mM sodium tartrate buffer, pH 5, at 39 °C (Tien and Kirk, 1988). MnP activity was evaluated spectrophotometrically *via* the oxidation of curcumin at the wavelength of 430 nm in 0.5 M sodium tartrate buffer, pH 5, at 22 °C (Paszczynski et al., 1988). Lcc activity was evaluated spectrophotometrically by following the oxidation of 2,2′-azino-bis-(3-ethylbenzothiazoline-6 sulphonic acid) (ABTS) at the wavelength of 420 nm in 100 mM sodium acetate buffer, pH 5, at 22 °C (Johannes and Majcherczyk, 2000). Activities were expressed as international units per litre (IU l^{-1}).

The Spearman correlation test was used to evaluate correlations between decolorization and enzyme activity.

2.3. Soil microcosms

The static microcosms were prepared, as previously described by Mollea et al. (2005), by placing in hermetically sealed, 500 ml Erlenmeyer flasks 1 g of straw cultures of each strain, 20 ml of a 20 g/l glucose:water solution and 10 g of an uncontaminated, natural soil (pH 6.63, total moisture 12%) then artificially spiked with naphthalene (500 mg kg^{-1}) dissolved in ethyl acetate (EA) (Fluka). Heat-killed controls were obtained by preparing microcosms as described above, but with straw cultures pre-autoclaved at 121 °C for 20 min. Microcosms were incubated statically at 27 °C and periodically flushed with wetted pure oxygen at a constant 27 °C, since the results of preliminary experiments (not shown) had made it clear that temperature control during oxygenation was essential to limit the increase in the volatility of the pollutant. However, checking PAH losses during oxygenation was necessary; for this purpose the flowing gaseous phase was absorbed in 40 ml *N*,*N*-dimethylformamide (DMF) (Fluka) at the end of the oxygenation system. Ten milliliters of the 20 g/l glucose:water solution were added on days 14 and 28 to re-establish the system's C source and humidity.

2.4. Respirometric analysis

To determine metabolic activity in each microcosm we periodically performed a respirometric analysis by moni-