Isolation and characterization of a white rot fungus *Bjerkandera* sp. strain capable of oxidizing phenanthrene

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Received 25 February 2005; Revision requested 3 March 2005; Revisions received 19 April 2005; Accepted 19 April 2005

**Key words:** bioremediation, manganese peroxidase, polycyclic aromatic hydrocarbons, white rot fungi

**Abstract**

Strain BOL13 was selected from 18 fungal strains isolated from an oil-spill contaminated site in Oruro, Bolivia. It was identified as a basidiomycete with high homology to *Bjerkandera*. The fungus degraded 100 mg phenanthrene l⁻¹ at 0.17 mg l⁻¹ d⁻¹ at 30 °C at pH 7. During phenanthrene degradation, a maximum manganese peroxidase activity of 100–120 U l⁻¹ was measured after 10 days of incubation. The ability of *Bjerkandera* sp. to produce lignin-modifying enzymes and to oxidize phenanthrene under various pH and temperature conditions was confirmed.

**Introduction**

Polycyclic aromatic hydrocarbons (PAHs) are listed as priority pollutants, because of their ubiquity in the environment and their toxicity, mutagenicity, and carcinogenicity. PAHs are found in particularly high concentrations at many industrial sites, especially those associated with petroleum and gas production. Due to their hydrophobic nature, most bind to particulate matter in soil and sediments, rendering them less available for biological uptake and more likely to accumulate in food chains (Boonchan & Britz 2000). Therefore, PAH biodegradation often begins with co-metabolism by higher microorganisms, such as rot-causing fungi capable of producing extracellular enzymes that can break down poorly soluble contaminants (Meulenberg *et al.* 1997). In particular, white rot fungi secrete extracellular oxidative enzymes that are essential for lignin degradation and are therefore capable of oxidizing PAHs (Pointing 2001).

Although the biodegradation of various organic pollutants by *Bjerkandera* species has been reported (Kotterman *et al.* 1996, Soares *et al.* 2005), their ability to oxidize phenanthrene is not well understood. Phenanthrene usually cannot be degraded by lignin-modifying enzymes because of its high ionization potential (8.19 eV), and white rot fungi are not generally able to degrade this compound (Bezalel *et al.* 1996). Furthermore, in the case of lignin and PAHs, optimum levels of degradation usually occur in environments that are acidic and rich in organic carbon. It is not known if these fungi can degrade pollutants in environments which are poor in organic carbon or which have a pH close to neutral.

In this context, *Bjerkandera* sp. strain BOL13 was characterized by its morphology and by rDNA sequencing. Its capacity to biodegrade
phenanthrene was tested in liquid cultures at pH 5 and 7 at 10, 20 and 30 °C.

Materials and methods

Screening of fungi

Soil samples were collected from an oil-polluted site in Toma–Toma (Oruro, Bolivia). Eighteen fungi were isolated and identified by their colony morphology on Saboraoud agar plates (Barnett & Hunter 1972). Their ability to produce lignin-modifying enzymes was then determined using a solid medium containing poly R-478 (Table 1). The white rot fungus Trametes versicolor (obtained from the Biotechnology Department, Lund University, Sweden) was used as a positive control. The most active isolate (BOL13) was further characterized.

Microbial characterization of strain BOL13

Morphological characterization

The morphology of the strain was described after being cultured on malt extract/agar.

Table 1. Fungal strains isolated from an oil-polluted site (Toma–Toma) in Oruro, Bolivia.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genus/species</th>
<th>Poly R-478 decolorization</th>
</tr>
</thead>
<tbody>
<tr>
<td>BOL13</td>
<td>Unidentified strain</td>
<td>++ ++</td>
</tr>
<tr>
<td>BOL15</td>
<td>Botrytis</td>
<td>+</td>
</tr>
<tr>
<td>BOL22</td>
<td>Geotrichum</td>
<td>+</td>
</tr>
<tr>
<td>BOL132</td>
<td>Pythium</td>
<td>-</td>
</tr>
<tr>
<td>BOL178</td>
<td>Wardomyces</td>
<td>+</td>
</tr>
<tr>
<td>BOL182</td>
<td>Verticillium</td>
<td>+</td>
</tr>
<tr>
<td>Control</td>
<td>Trametes versicolor</td>
<td>+ ++</td>
</tr>
</tbody>
</table>

*rDNA sequencing*

A fungal mycelium was grown in a broth containing yeast extract (1 g l⁻¹) and glucose (5 g l⁻¹). After 4 days of incubation at 25 °C, 1.5 ml of mycelium was withdrawn, washed twice with ultra-pure water, and placed in sterile Eppendorf tubes containing 0.3 g 425–600 µm glass beads (Sigma). Chromosomal DNA was extracted and purified according to Sambrook et al. (1989). Partial sequences of the nuclear rDNA ribosomal genes and spacer regions 18S, ITS1, 5.8S, ITS2 and 28S were amplified using the following primers (Gardes & Bruns 1993): ITS1- F (5′-CTT GGT CAT TTA GAG GAA GTA A-3′) and ITS4-B (5′-CAG GAG ACT TGT ACA CGG TCC AG-3′) (http://plant-bio.berkeley.edu/~bruns/primers.html). For the D1/D2 domain, the primers used were F63 (5′-GCA TAT CAA TAA GCG GAG GA-3′) and LR3 (5′-TGC TGT TTC AAG ACG G-3′) (Altschul et al. 1990). PCR reactions were performed in a Gene Amp PCR System 9700 (PE Applied Biosystems). PCR products were purified after electrophoretic separation using a gel extraction kit (Qiagen). DNA sequencing of both strands was performed using the dideoxy chain termination method with an ABI Prism 3100 DNA Analyzer, and the ABI Prism Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems).

Optimization of the production of lignin-modifying enzymes

To determine the optimum conditions for the expression of lignin-modifying enzymes, a basal medium was prepared with different concentrations of glucose, manganese and yeast extract (Table 2). Serum bottles (100 ml) with rubber septa were supplied with 10 ml sterile medium and inoculated with a 10-mm-diameter piece of malt extract agar covered with fungal mycelium. The bottles were incubated in the dark at 20 °C. All tests were performed in duplicate.

Phenanthrene biodegradation tests

Phenanthrene biodegradation tests were carried out in 100 ml serum flasks closed with rubber septa. Phenanthrene was dissolved in hexane at 1 g l⁻¹ and added to the flasks to give 100 mg l⁻¹. Once the solvent had evaporated, 5 ml culture medium at pH 5 or 7 was added.