ELSEVIER

Soil Biology & Biochemistry 39 (2007) 1040–1046

Soil Biology & Biochemistry

www.elsevier.com/locate/soilbio

Humic acid bleaching by white-rot fungi isolated from biosolids compost

Tzafrir Granit[a], Yona Chen[a,*], Yitzhak Hadar[b]

[a]*Department of Soil and Water Sciences, Faculty of Agricultural, Food and Environmental Quality Sciences, Rehovot, The Hebrew University of Jerusalem, P.O. Box 12, Rehovot 76100, Israel*

[b]*Department of Plant Pathology and Microbiology, Faculty of Agricultural, Food and Environmental Quality Sciences, Rehovot, The Hebrew University of Jerusalem, Israel*

Received 27 July 2006; received in revised form 15 November 2006; accepted 20 November 2006
Available online 17 January 2007

Abstract

A screening assay for isolating humic-substances degrading fungi from biosolids compost at the thermophillic phase employed using plates containing 2,2′-azino-bis(3-ethylbenzothiazoline-sulfonic acid) (ABTS), $MnCl_2$ or the monoazo dye Acid Red 183. Two of the most active fungi out of 70 fungal strains isolated were identified based on rDNA sequences and designated *Trametes* sp. M23 and *Phanerochaete* sp. Y6 (accessions no. DQ408582 and DQ438910). These isolates, when compared to a model white-rot fungi *T. versicolor* and *P. chrysosporium*, showed the ability to bleach humic acids extracted from biosolids compost while growing under solid-state conditions using perlite as a solid support. *T. versicolor* and *Trametes* sp. M23 also exhibited the ability to bleach humic acids from a peat source. Interestingly, only *Trametes* sp. M23 bleached leonardite humic acid, which is considered to be a highly aromatic and stable type of natural organic matter. To the best of our knowledge, this is the first report of white-rot fungi being isolated and identified from thermophilic composts. Since these fungi are capable of degrading lignin and humic acid and were found active in organic-matter-degradation processes, we suggest that they may play a significant role in the degradation and transformation of these refractory substances during composting.

Keywords: Humic acid; White-rot fungi; *Trametes*; *Phanerochaete*; Bleaching; Compost; Biosolids

1. Introduction

Humic substances (HS) are the most widespread and ubiquitous natural nonliving organic materials in terrestrial and aquatic environments and they represent the major fraction of soil organic matter (OM). Based on their solubility in acids and alkalis, they can be divided into three main fractions: humic acid (HA), which is soluble in alkali and insoluble in acid; fulvic acid (FA), which is soluble in alkali and acid, and humin, which is insoluble in both alkali and acid (Stevenson, 1994).

White-rot fungi (WRF) are the most efficient lignin degraders, due to their ligninolytic system which is comprised of manganese peroxidase (MnP), lignin peroxidase (LiP) and laccase (Hatakka, 1994; Higuchi, 2004). Moreover, WRF play a crucial role in the transformation and degradation of relatively recalcitrant OM (Haider and Martin, 1988; Dehorter and Blondeau, 1992; Gramss et al., 1999; Steffen et al., 2002; Yanagi et al., 2003). To the best of our knowledge, Burges and Latter (1960) were the first to show that WRF are able to degrade HAs from podzol soil. Numerous studies on HS degradation by WRF focused on coal-HS (Catcheside and Ralph, 1999; Fakoussa and Hofrichter, 1999). Haider and Martin (1988) showed that *Phanerochaete chrysosporium* is able to mineralize ^{14}C-labeled HA (originating from ^{14}C-wheat straw) and ^{14}C-xenobiotics bound to HAs. Steffen et al. (2002) showed that the litter decomposing fungus *Collybia dryophila* can degrade both soil-HAs and synthetic ^{14}C-HAs prepared from catechol and suggested that MnP is involved in the mineralization process. Nevertheless, only a

*Corresponding author. Tel.: +972 8 9489234; fax: +972 8 9468565.
E-mail address: yonachen@agri.huji.ac.il (Y. Chen).

0038-0717/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.soilbio.2006.11.021

few reports have been published on the isolation and characterization of fungi from compost with respect to their oxidizing enzymatic activity (Chefetz et al., 1998; Kluczek-Turpeinen et al., 2003, 2005). Composting is a sustainable way of harnessing the microbial population to the rhythm of the modern world. Moreover, intermediates and final products formed during composting resemble those of OM decomposed in natural aerobic environments such as litter layers and surfaces horizons of soils (Inbar et al., 1990).

In addition to their role in composting and the global carbon cycle, ligninolytic fungi, WRF in particular, are of great interest due to the potential use of their enzymes for bioremediation, industrial and biotechnological applications (Jordaan et al., 2004; Novotny et al., 2004).

To the best of our knowledge, the isolation and identification of WRF from a compost environment at its thermophillic stage has not been reported. Thus, the aim of this study was to elucidate the role of ligninolytic fungal populations in the composting of biosolids (BS) in relation to their ability to degrade and transform HS.

2. Materials and methods

2.1. Compost samples

Composted BS were sampled (a total of 45 samples, 30 g each) from the Shaham Dlila composting facility near kibbutz Kfar Menachem, Israel. The raw material used for composting was sewage sludge from a municipal source, after a secondary purification treatment followed by anaerobic digestion, mixed with wood chips (1:1, *v*/*v*). Composting was performed using a windrow system and sampled after three months, when the average temperature at a depth of 1 m was 60.5 °C. The compost samples were stored at 4 °C.

2.2. Fungi isolation

Each sample (10 g) was placed in 100 ml of sterile water and shaken at 150 rpm for 30 min at room temperature. The suspension was then diluted twice (10^1, 10^2 for quantitative analyses) and 100-μl aliquots inoculated onto plates containing (per l) 39 g potato dextrose agar (PDA) (Difco), 5 g agar (Difco) and 250 mg chloramphenicol. The plates were incubated at 35 °C. Fungi isolated at this stage were examined further to detect ligninolytic enzyme activity. All fungi examined in the screening tests were isolated from the same BS compost. *Trametes versicolor* was used as a positive control in the screening assay. *Phanerochaete chrysosporium* (strain BKM) and *T. versicolor* were used as references in the bleaching assay.

2.3. Screening assays for ligninolytic enzyme activity

Each fungal colony emerging from the 70 isolates was transferred to plates containing (per l) 39 g PDA, 5 g agar, 50 ml of a microelement stock solution containing (per l): 40 mg $ZnNO_3 \cdot 4H_2O$, 1 g $Ca(NO_3)_2 \cdot 4H_2O$, 60 mg $CuSO_4 \cdot 5H_2O$. Each plate contained one of the following indicators which were used to detect oxidative enzyme activity: (i) 100 mg ABTS [2,2′-azino-bis(3-ethylbenzothiazoline-sulfonic acid)]; (ii) 100 mg $MnCl_2$ (Steffen et al., 2002); and (iii) 0.02% (*w*/*v*) monoazo dye Acid Red 183 (C.I. 18800, Aldrich) (Jarosz-Wilkolazka et al., 2002). The $MnCl_2$ and ABTS were filtered through a 0.45-μm filter and added after autoclaving.

2.4. Using rDNA for fungal identification

The fungi were grown on potato dextrose broth (PDB) (Difco). The mycelium was filtered, frozen in liquid nitrogen and lyophilized. The dry mycelium was pulverized. DNA was extracted in one volume of buffer containing 0.35 M sorbitol, 0.1 M Tris base and 5 mM Na_2-ethylenediaminetetraacetic acid (EDTA), and one volume of buffer containing 0.2 mM Tris base, 0.05 M Na_2-EDTA, 2 M NaCl, 2% (*w*/*v*) cetyl-trimethylammonium bromide (CTAB) and 0.4 volume of sarkosyl (5%, *w*/*v*). The suspension was treated with chloroform:octanol (24:1, *v*/*v*), precipitated in 3 M sodium acetate and isopropanol, washed with 75% EtOH and resuspended in water.

For amplification of the rDNA sequences, we used the fungal primers NS1 and FR1 (White et al., 1990; Vainio and Hantula, 2000) for the 18S region, ITS1F and ITS4 (White et al., 1990; Bruns and Gardes, 1993) for the internal transcribed spacer (ITS) region, and LR0R and LR16 (Klonowska et al., 2003) for the large subunit (LSU) region. Each PCR mixture contained 1.5 U (per 50 μl) of Taq polymerase (Red Taq, Sigma Chemical Co.) and the following reagents: 1X Sigma PCR buffer, 0.20 mM PCR nucleotide mix (Promega), 4.0 mM $MgCl_2$, 6.25 μg (per 50 μl) bovine serum albumin (BSA; Roche Diagnostics) and 25 pmol of each primer. Samples were initially PCR-amplified using the aforementioned primer sets in a reaction volume of 25 μl. These reactions were conducted as follows: the 18S rRNA samples were initially denatured for 3 min at 95 °C and then cycled 35 times through three steps: denaturation (94 °C; 30 s), annealing at 57 °C and elongation (72 °C; 105 s). A 2-min incubation at 72 °C was added to the end of each PCR program. The ITS regions samples were initially denatured for 4 min at 94 °C, and then cycled 32 times through three steps, denaturation (94 °C; 30 s), annealing (62 °C; 30 s) and elongation (72 °C; 45 s). A 7-min incubation at 72 °C was added to the end of each PCR program. For the LSU region, the PCR was conducted as described previously (Klonowska et al., 2003). Amplification-product sizes and yield were assessed by gel electrophoresis in 1% agarose gels stained with ethidium bromide.

PCR products of the ITS and LSU regions were excised from agarose gels following electrophoresis, purified using the Wizard® SV gel and PCR Clean-Up System Kit (Promega) and sequenced (forward and reverse) with the