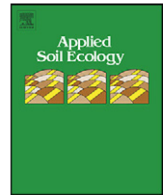
Applied Soil Ecology 51 (2011) 102–110

Contents lists available at SciVerse ScienceDirect

Applied Soil Ecology

journal homepage: www.elsevier.com/locate/apsoil



Biodegradation of 4-hydroxybenzoic acid by *Phomopsis liquidambari*

Yan Chen[a], Yao Peng[a], Chuan-Chao Dai[a,*], Qun Ju[b]

[a] Jiangsu Key Laboratory for Microbes and Functional Genomics, Jiangsu Engineering and Technology Research Center for Industrialization of Microbial Resources, College of Life Science, Nanjing Normal University, Jiangsu Province, 210046, China
[b] Jurong Medical Limited Company, Jurong, Jiangsu Province, 212441, China

ARTICLE INFO

Article history:
Received 18 March 2011
Received in revised form 3 September 2011
Accepted 7 September 2011

Keywords:
Endophytic fungi
4-Hydroxybenzoic acid
Phenolic acid
Soil degradation
HPLC–MS
GC–MS

ABSTRACT

Phenolic acid allelochemicals released by decomposing foliage can have negative impacts on growth of plants and microbes in soil. However, these chemicals can potentially be degraded by broad-spectrum endophytic fungi such as, *Phomopsis liquidambari* (strain B3), which is capable of growing on phenolic 4-hydroxybenzoic acid (4-HBA) as the sole carbon and energy source. In this study, the optimum concentration of 4-HBA supplied in culture as the carbon source was determined to be 600 mg L^{-1}, with maximum biomass of fungal growth and, 94% and 99% degraded by *P. liquidambari* in liquid culture and soil conditions, respectively, within 48 h. The fungal metabolic pathway of 4-HBA was investigated using high performance liquid chromatography–mass spectrometry (HPLC–MS) and gas chromatography–mass spectrometry (GC–MS). The results showed that 4-HBA was first hydroxylated to 3,4-dihydroxybenzoic acid, converted to catechol and then oxidated to *cis*,*cis*-muconic acid in the tricarboxylic acid (TCA) cycle. The benzene ring was opened through *ortho* catechol cleavage. During the course of fungal incubation, activities of three main 4-HBA degradation pathway enzymes exhibited a continuous cascading pattern of induction. 4-HBA hydroxylase showed the highest activity after 8 h of incubation, with 3,4-dihydroxybenzoic acid decarboxylase reaching a peak at 24 h and catechol 1,2-dioxygenase peaking at 32 h, which corresponded with changes in corresponding substrate concentrations. These results indicated the bioremediation potential of this endophytic fungus to degrade accumulated phenolic acid allelochemicals in soil to alleviate the effects of their ecological suppression.

© 2011 Elsevier B.V. All rights reserved.

1. Introduction

In China, the effect of phenolic allelochemicals in soil is one of the most important factors for crop decline in terms of growth, yield and crop quality, especially in many continuous cropping systems which have increasingly significant agricultural losses every year (Seal et al., 2004; Ma et al., 2005; Li et al., 2010a). 4-HBA is a common and typical phenolic allelochemical that is released into the environment by plants through foliar leachate and residue decomposition (Inderjit and Nilsen, 2003; Jilani et al., 2008). Direct phytotoxicity of 4-HBA includes the inhibition of seed germination and root growth through inhibition of metabolic enzymatic activities involved in glycolysis and the oxidative pentose phosphate pathway and through disturbance of auxin synthesis (Muscolo et al., 2001; Lim et al., 2002). Zhang et al. (2010a) and Weir et al. (2004) also demonstrated that 4-HBA along with other phenolic acids not only cause damage to DNA and proteins but also hinder carbon and nitrogen metabolic processes in plants. An indirect negative effect of 4-HBA is that it disrupts the soil microbial community balance by modifying the soil population and community structure and regulation of pathogen growth. Ye et al. (2006) and Li et al. (2010a,b) demonstrated that 4-HBA and several other phenolic acids can promote the growth and infection rate of *Fusarium* sp. in cucumber and peanuts.

Endophytic fungi are microorganisms that can be detected within the tissues of apparently healthy host plants (Wilson, 1995). In recent years, an increasing number of studies have shifted from examining the ecological functions of endophytic fungi *in vivo* toward *in vitro* experiments. Their function in rapidly degrading plant leaf litter has been repeated confirmed (Muller et al., 2001; Fukasawa et al., 2009; Dai et al., 2010a; Sun et al., 2011). As the surrounding endophytic environment is damaged (leaf senescence, abscission, or damage), endophytic fungi are triggered to grow and perhaps sporulate like saprobes by decomposing recalcitrant compounds in litter (Muller et al., 2001; Promputtha et al., 2010). However, no information is available on the endophytic fungal metabolic pathway of natural recalcitrant compounds.

Phomopsis spp. are common endophytic fungi that exist in a wide variety of herbaceous and woody plants and establish symbiotic relations with these hosts (Huang et al., 2008, 2009; Chaeprasert et al., 2010; Maehara et al., 2010; Rocha et al., 2011). Chen et al.

* Corresponding author. Tel.: +86 25 85891382; fax: +86 25 85891526.
E-mail address: daichuanchao@njnu.edu.cn (C.-C. Dai).

0929-1393/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.apsoil.2011.09.004

(2010) and Promputtha et al. (2010) demonstrated the effective leaf litter decomposition of *Phomopsis* sp. through biochemical and enzymological analyses. Our former research indicated that one of these endophytic fungi exhibits efficient degradation of polycyclic aromatic hydrocarbon (PAH) phenanthrene in pure culture (Tian et al., 2007). Thus, it may be assumed that such endophytic fungi have the potential to degrade single cyclic molecules such as phenolic acid, with its simpler structures than PAH and litter recalcitrant compounds.

Studies of phenolic allelochemicals degradation have recently focused on aspects such as isolation and purification of environmental microbes and evaluation of the level of allelochemical degradation. Zhang et al. (2010b) isolated and characterized *Pseudomonus putida* from soil containing a high concentration of phenolic acid and detected the effect of microbial degradation of *p*-coumaric acid on bamboo growth. Chen et al. (2011) isolated a fungal strain of *Trichoderma harzianum* and found that, when applied to soil, it degrades compounds such as 4-HBA, vanillic acid and benzoic acid in cucumber plant soil. To study microbial catabolism of 4-HBA, Middelhoven et al. (1992) and Fairley et al. (2002) systematically analyzed aerobic metabolism of 4-HBA in ascomycetous yeast and archaea. However, previous studies on endophytic fungi degradation of 4-HBA *in vitro* are rare. In contrast to other microbes which degrade residual plant material from the outside to the inside, the endophytic fungal degradation process starts from an inner residual location to the exterior (Muller et al., 2001; Fukasawa et al., 2009), which effectively breaks down the phenolic allelochemicals in leaf litter more directly and rapidly.

In this study, the endophytic fungi *Phomopsis liquidambari* was evaluated for its ability to utilize 4-HBA as a sole carbon and energy source *in vitro*, and the 4-HBA catabolic products were investigated. The relationships between the kinetics of the 4-HBA degradation pathway enzymes and related metabolic substrates were also evaluated.

2. Materials and methods

2.1. Isolation and identification of 4-HBA degrading endophytic fungi strain B3

The endophytic fungus strain B3 was previously isolated from the inner bark of the stem of *Bischofia polycarpam* (Shi et al., 2004) and stored at 4 °C on potato dextrose agar (PDA, containing 200 g L^{-1} potato extract, 20 g L^{-1} glucose and 20 g L^{-1} agar, pH 7.0). The fungal strain was cultured in potato dextrose broth (PDB, containing 200 g L^{-1} potato extract, 20 g L^{-1} glucose, pH 7.0) medium for 3 days at 160 rpm in an orbital shaker at 28 °C.

Fungal DNA was extracted using E.Z.N.A.® Fungal DNA Midi Kit (Omega Bio-Tek, Inc., Norcross, GA, USA). The ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) primers were used for amplification of the fungal rDNA internal transcribed spacer (ITS) regions 1 and 2 (Yuan et al., 2010). The PCR reaction (50 μL total volume) contained 5 μL 10× PCR buffer, 3 μL 25 mM $MgCl_2$, 1 μL 10 mM dNTP, 1 μL of each primer (50 mM), 3 μL (5.0–10.0 ng) of total DNA, 0.5 U *Taq* polymerase and 35.5 μL dd-H_2O. Thirty five cycles were run, each consisting of a denaturation step at 94 °C (40 s), an annealing step at 54 °C (50 s) and an extension step at 72 °C (60 s). After the 35th cycle, a final 10 min extension step at 72 °C was performed. The reaction products were separated in a 1.0% (w:v) agarose gel, and bands were stained with ethidium bromide. The PCR products were then purified using a DNA Gel Extraction Kit (Axygen Bioscience, Inc., Union City, CA, USA) and sequenced by Invitrogen Corp. (Carlsbad, CA, USA).

2.2. Materials and chemicals

Soil samples for analyzing endophytic fungal 4-HBA degradation were obtained from a 5–10 cm layer below the soil surface of long-term planted traditional medicinal herb *Atractylodes lancea* (thunb) DC. in the Plant Park of Nanjing Normal University, Jiangsu Province (N32°16′, E118°79′). Abundant plant litter covered the surface of the soil. The soil was at a pH of 4.68 and had a moisture content of 16.12%. The nutrient composition of the soil was as follows: total C 14.68 g kg^{-1}, total N 0.79 g kg^{-1}, available P 334.57 mg kg^{-1} and available K 123.33 mg kg^{-1}.

4-HBA (purity ≥ 99%) and catechol (purity ≥ 99%) were purchased from Sigma–Aldrich (St. Louis, Mo, USA); 3,4-dihydroxybenzoic acid (purity ≥ 97%) was obtained from Aladdin Chemistry Co. Ltd. (Shanghai, China) and *cis*,*cis*-muconic acid (purity ≥ 98%) from Acros Organics (Morris Plains, NJ, USA). HPLC-grade acetonitrile was purchased from Honeywell Burdick & Jackson Company (Morristown, NJ, USA). HPLC-grade methanol and acetic acid were purchased from Hanbon Sci. Tech. Co. Ltd. (Jiangsu, China).

2.3. Identification of the optimal culture concentration of 4-HBA for fungal-mediated degradation

The endophytic fungal strain B3 was first activated in PDB medium in a shaker at 160 rpm, 28 °C for 48 h. Two milliliters of the active fungal suspension were then used to prepare the fungal inoculum by culturing for 1 day at 28 °C with 160 rpm shaking speed in 100 mL liquid mineral salt medium (2 g L^{-1} $NaNO_3$, 1 g L^{-1} K_2HPO_4, 0.5 g L^{-1} KCl, 0.5 g L^{-1} $MgSO_4$, 0.01 g L^{-1} $FeSO_4$ and 200 mg L^{-1} 4-HBA as carbon source, pH 5.0) contained in 250 mL Erlenmeyer conical flasks.

Two milliliters of inoculum containing fungal mycelium at 1.1 mg L^{-1} was added to the 4-HBA degradation test medium with the same composition as the liquid mineral salt medium mentioned above, and the expected concentration of the 4-HBA carbon source ranged from 200 to 1200 mg L^{-1}. The cultures were incubated at 28 °C in a shaker rotating at 160 rpm for 48 h. The fermented liquid was then collected to determine the concentration of 4-HBA. Meanwhile, fungal mycelia were washed twice with sterilized distilled water and the biomass analyzed after drying at 60 °C for 24 h. In all experiments, cultures were grown in triplicate, and results are expressed as means ± standard deviations. Control cultures without fungus were processed in an identical manner to account for abiotic factors.

An Agilent 1290 Infinify with UV detector and Agilent ChemStation Software were used for the quantitative and qualitative analysis of 4-HBA. An Agilent C18 column, 250 mm × 4.6 mm, with particle size 5 μm was used for high performance liquid chromatography (HPLC). The mobile phase contained acetonitrile and 1.3% acetic acid at a ratio of 17:83 (v:v). The flow rate, injection volume, detection wavelength and column temperature were 0.6 mL min^{-1}, 20 μL, 260 nm and 35 °C, respectively.

2.4. 4-HBA degradation in liquid cultures

Based on the maximum fungal biomass and high 4-HBA degradation in Section 3, the optimal concentration of 4-HBA for endophytic fungal degradation was 600 mg L^{-1}. Thus, in the following liquid cultures, we used 600 mg L^{-1} 4-HBA as the sole carbon source to evaluate the ability of the fungi to degrade 4-HBA. The other components of the medium and culture conditions were identical to those described in Section 2.3. Control cultures without fungal mycelium were handled in an identical manner.

Additionally, in order to determine whether glucose in the environment would affect the ability of the fungus to degrade 4-HBA,

Download English Version:

https://daneshyari.com/en/article/4382680

Download Persian Version:

https://daneshyari.com/article/4382680

Daneshyari.com