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Environmental control of lignin peroxidase, manganese peroxidase, and laccase activities in forest floor layers in humid Asia

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ABSTRACT

We investigated the activities of ligninolytic enzymes, lignin peroxidase (LiP), manganese peroxidase (MnP), and laccase (Lac), in forest floor layers collected from temperate and tropical regions in Japan, Thailand, and Indonesia. The effects of pH and Mn levels on the ligninolytic enzyme activities in the forest floor layers were analyzed. The activities of LiP, MnP, and Lac varied depending on the decomposition stage (i.e., depth) and pH of forest floor layers both in temperate and tropical regions. The activities of LiP, which has the lowest optimal pH, were higher in the highly acidic FH layers ($pH < 5.0$) than in the L layers. The activities of MnP and Lac were commonly detected in the less acidic L layers ($pH > 5.0$), whereas no MnP activity was detected in the highly acidic FH layers. These results indicate that MnP and LiP activities are dependent on pH and independent of the Mn concentrations in the forest floor layer. MnP and Lac play roles in the initial stage of litter decomposition, whereas acidification of forest floor layers enhances fungal activity and production of LiP in the FH layer.

1. Introduction

Lignin, as well as cellulose and hemicelluloses, is a major chemical component of woody and vascular plant materials. Lignin has a complex aromatic macromolecule structure and is thus refractory in forest soils (Martin and Haider, 1979). Removing the lignin shield around cellulose is a rate-limiting step in litter decomposition (Osono, 2007). Lignin degradation requires oxidative reactions by a complex set of ligninolytic enzymes and it cannot be cleaved by hydrolytic enzymes, which work for most other natural polymers (e.g., cellulose) (Kirk, 1984; Ten Have and Teunissen, 2001). A variety of fungi and some of ascomycetes and actinomycetes can typically produce ligninolytic enzymes (Kirk et al., 1978; Baldrian, 2006). Ligninolytic enzymes are extracellular and less substrate-specific due to the large and highly branched structure of lignin (Kirk, 1984).

Among soil microorganisms, white-rot basidiomycete fungi can decompose lignin most effectively by secreting the specific ligninolytic enzymes (Hatakka, 1994; Hofrichter, 2002). The major fungal oxidative enzymes involved in lignin degradation are lignin peroxidase (LiP), manganese peroxidase (MnP), and laccase (Lac) (Kirk and Farrell, 1987; Tien and Kirk, 1983; Leonowics et al., 2001), although a variety of the other ligninolytic enzymes (e.g., versatile peroxidase, aromatic peroxidase, and chloroperoxidase) have also been discovered to date (Wong, 2009; Hofrichter et al., 2010). These three enzymes, LiP, MnP, and Lac, have the capability to oxidize the phenolic substructure of lignin (Steffen, 2003). MnP and LiP can degrade lignin effectively because of their higher redox potential compared to Lac (Ten Have and Teunissen, 2001; Hofrichter et al., 2010). Specifically, MnP can oxidize the phenolic substructure of lignin effectively with eventual release of CO_2 (termed "enzymatic combustion") (Kirk and Farrell, 1987; Steffen, 2003), whereas LiP can oxidize the non-phenolic substructure that is most recalcitrant and abundant in lignin (Hatakka, 2001). The Lac was not considered to be the key enzyme in lignin degradation, however, the potential importance of Lac has been suggested in the co-presence of other enzymes and mediators (Hatakka, 2001; Leonowics et al., 2001).

The types and activities of ligninolytic enzymes are important factors affecting the processes of lignin degradation and litter decomposition rates (Tuomela et al., 2002; Berg et al., 2007). The activities of LiP, MnP, and Lac depend on the abiotic characteristics of

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the litter and soil environments (Criquet et al., 2000), as well as fungal species (Hatakka, 2001; Tuomela et al., 2002; Osono, 2007). The optimal pH and Mn concentrations differ between ligninolytic enzymes (Perez and Jeffries, 1990; Hatakka, 2001), with LiP exhibiting the lowest optimal pH among them (Marquez et al., 1988). MnP requires Mn^{2+}, which can form the Mn^{3+}-organic acid complex and attack lignin as a diffusible oxidant (Hofrichter, 2002). A high level of Mn was reported to stimulate MnP activity but to repress LiP activity (Rothschild et al., 1999). Thus, LiP and MnP activities are hypothesized to be influenced by the pH and Mn levels of forest floor layers.

The types and activities of ligninolytic enzymes may differ depending on the stages of lignin degradation. Perez and Jeffries (1990) suggested that MnP is important during the initial stages of lignin degradation, whereas LiP is more important in the later stages. The decomposition stages of litter also have a strong influence on the microbial community (fungal species or activity) and chemical properties of the litter (i.e., pH and Mn levels) (Osono, 2007). Therefore, the types and activities of ligninolytic enzymes could vary between fresh litter (L) and humus (F and H) layers. Although laboratory studies have identified the effects of pH, Mn level, and litter decomposition stage on the activities of LiP, MnP, and Lac (Steffen, 2003; Steffen et al., 2007; Singh and Chen, 2008), the roles of these enzymes remain unclear under the field condition, especially in tropical forest.

To analyze the environmental factors controlling the activities of LiP, MnP, and Lac in forest floor layers exhibiting a variety of pH and Mn levels in temperate and tropical regions, we tested (1) whether the activities of LiP, MnP, and Lac are dependent on pH, (2) whether MnP activities increase and LiP activities decrease with the Mn concentration in the litter, and (3) whether the distribution of enzymes differs between decomposition stages or the L, F, and H layers of forest floor.

2. Materials and methods

2.1. Sampling of forest floor materials

Forest floor samples were collected from eight sites in October 2005. The L, F, and H layers were collected separately from each location in three replicates. Samples were collected from three sites in Japan (NG, TG, and KT), one site in Thailand (RP), and four sites (KRs, KRm, BS, and BB) in Indonesia. The climate, vegetation, geology, and soil type of sampling locations are summarized in Table 1. Sites were selected to include variability in soil pH in temperate and tropical forests. Within the temperate forests, Andisol in NG exhibited higher pH values than Spodosol (TG) and Inceptisol (KT). Within the tropical forests, Ultisol in KRs exhibited the highest soil pH due to the influence of ultramafic parent material (serpentine). Broad-leaved trees dominated the overstory vegetation at all sites. The sites have been described in detail by Fujii et al. (2008, 2009, 2011).

2.2. Chemical and biological properties of forest floor and soil samples

The forest floor pH was measured using a milled litter-to-solution (water) ratio of 1:20 (w/v). The Klason lignin concentration in forest floor samples was determined by digestion with sulfuric acid (Allen et al., 1974). The Mn concentration in the forest floor samples was determined using inductively coupled plasma atomic emission spectrometry (ICP-AES, SPS1500, Seiko Instruments Inc.) after nitric-sulfuric acid wet digestion.

To obtain the rough approximate estimates of fungal activity in the soils studied, the relative contribution of fungi to glucose-induced respiration in soil (0–5 cm depth) was measured by the selective inhibition method (Anderson and Domsch, 1973; Joergensen and Wichern, 2008). The field-moist soil samples were amended with a solution of ^{14}C-labeled glucose with/without cycloheximide (fungal respiratory inhibitor) (equivalent to 8 mg g^{-1}soil, respectively) and incubated at 22 °C for 24 h $^{14}C–CO_2$ was collected in 1 M NaOH solution and determined using liquid scintillation counting (Aloka, LSC-3050). The contribution of fungi to total microbial respiration (%) was calculated using the differences between $^{14}CO_2$ evolution rates of the glucose-amended soils with/without inhibitor. The experiments were carried out in three replicates.

2.3. Extraction of ligninolytic enzymes

According to Criquet et al. (1999), ligninolytic enzymes were extracted by shaking 80 g of litter in 700 ml of a 0.1 M $CaCl_2$ solution with 0.05% Tween 80 and 20 g polyvinyl polypyrrolidone at room temperature for 1 h on a reciprocal shaker. The suspension was filtered through a double layer of gauze to remove floating debris and then centrifuged at 12 000 *g* for 20 min at 4 °C. The supernatant was filtered through 3.0 μm and 1.0 μm filters and then dialyzed for 48 h at 4 °C in 14 kDa molecular mass cut-off cellulose dialysis tubing against frequently exchanged 2 mM bis-tris [bis (2-hydroxyethyl) imino-tris (hydroxymethyl) methane] buffer, pH 6.0. The supernatant of each extract was concentrated in a cellulose-dialysis tube with a 14 kDa molecular mass cut-off and covered with polyethylene glycol until obtaining a final volume of 1/10 to 1/20 of the initial volume. Most of the litter extract samples were brown-colored, however, the effects of turbid materials on enzyme assay were reported to be small (Archibald, 1992; Criquet et al., 1999).

2.4. Enzyme activity assay

For all of the ligninolytic enzyme activities assays, the reactions were allowed to proceed for 5 min at 30 °C. The enzymatic activities were measured at the optimal pH values of the respective enzymes (3.0, 4.5, and 5.7 for LiP, MnP, and Lac, respectively). The extracts boiled for 15 min served as controls for the activities of LiP and Lac,

Table 1
Site descriptions and characteristics of the surface soil.

Country	Site	Cordinates	Annual mean air temperature (°C)	Annual precipitation (mm)	Vegetation	Soil/Geology	Soil pH (H_2O)	Fungal respiration in soil (%)[a]
Japan	NG	N35°57′, E138°28′	6.9	1422	*Quercus crispula*	Andisol/Volcanic ash	4.6	36 ± 4
	TG	N35°37′, E1135°10′	10.7	1782	*Fagus crenata*	Spodosol/Sandstone	3.8	67 ± 9
	KT	N35°1′, E135°47′	15.9	1490	*Quercus serrata*	Inceptisol/Sandstone	4.2	71 ± 7
Indonesia	KRs	S1°49′, E115°59′	27.7	2256	*Harpullia arborea*	Ultisol/Serpentine	5.4	54 ± 4
	KRm	S1°49′, E115°56′	27.0	2256	*Harpullia arborea*	Ultisol/Mudstone	4.5	50 ± 5
	BS	S0°51′, E117°06′	27.0	2187	*Dipterocarpus* spp.	Ultisol/Sandstone	4.0	82 ± 3
	BB	S1°01′, E116°52′	25.0	2427	*Shorea leavis*	Ultisol/Sandstone	4.3	78 ± 8
Thailand	RP	N19°50′, E100°20′	25.0	2084	*Lithocarpus* spp.	Ultisol/Mudstone	5.0	36 ± 9

KRs and KRm denote KR serpentine site and KR mudstone site, respectively.
[a] Fungal respiration in soil indicates the relative contribution of fungi to glucose-induced respiration (mean ± SE ($n = 3$)).