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Litter quality control of decomposition of leaves, twigs, and sapwood by the white-rot fungus *Trametes versicolor*

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ABSTRACT

Litter quality plays predominant roles in plant litter decomposition by modifying the activity of decomposer fungi, but little is known about the response of the decomposing activity of individual fungal strains to variations in litter quality. In the present study, the variability in the decomposing ability of a single fungal strain (*Trametes versicolor* IFO30340) was quantified under pure culture conditions to elucidate litter quality measures that control the decomposition. We used a total of 72 litters from 51 plant species, including leaves, twigs, sapwood, bark, heartwood, and petioles of broad-leaved trees, coniferous trees, and grass. Mass loss of litter caused by the fungus ranged from 0.9 to 59.8% of the original litter mass, was significantly higher in leaves, twigs, and petioles than in heartwood, and was significantly higher in broad-leaved than in coniferous litter. Tissue type (leaf, sapwood, or twig) and the relative amount of acid-unhydrolyzable residues to total nitrogen were selected as predictor variables of the mass loss of litters. Fourier transform infrared (FT-IR) spectroscopy showed that guaiacyl units of lignin negatively affected the fungal decomposition.

1. Introduction

Decomposition processes of plant litter in soil are controlled by interactions of climatic conditions, litter quality, and decomposer organisms [1]. Under a given climatic condition, litter quality plays predominant roles in regulating the decomposition by modifying the activity of decomposer microbes, including fungi [2,3]. For example, plant tissues richer in recalcitrant organic compounds, such as lignin and polyphenols, decompose more slowly [4], whereas higher nitrogen (N) and phosphorus contents in litter are associated with faster decomposition [5,6]. Such variations in decomposition in relation to litter quality are attributed to either changes in the species composition of decomposer fungal assemblages or plasticity of individual fungal species (or individual strains) according to litter quality. Of these two processes, previous studies mostly focused on the shifts of fungal assemblages in relation to the litter quality [7–9].

In contrast, less is known about the response of the decomposing activity of individual fungi to variations in the quality of different litters, including leaves, twigs, and sapwood of broad-leaved and coniferous trees with different levels of nitrogen and recalcitrant organic compounds. Only a few pure-culture experiments have documented the changes in decomposition in relation to litter quality. Mikola [10] showed that the mass loss of leaf litter varied when leaf litters of 25 plant species differing in chemical quality were inoculated with fungal isolates under constant conditions. Osono et al. [11] inoculated a total of 13 litter types with 10 *Xylaria* isolates under pure culture conditions and found that the fungal isolates caused greater mass loss of leaves than wood, and that the mass loss was related negatively to the lignin content and positively to the nitrogen content of the litters. Still, an integrative survey is lacking on the variability in the decomposing activity of individual fungal species or strains when inoculated to diverse litters from different plant species and tissues varied in chemical quality.

The purposes of the present study were to quantify the response of the decomposing ability of a single fungal strain when inoculated to diverse plant litters under pure culture conditions and to elucidate litter quality measures that control the decomposition. Specifically, the effects of recalcitrant organic compounds and N in

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litter were investigated as potential limiting factors of the fungal decomposition. We used a total of 72 litters from 51 plant species, including leaves, twigs, sapwood, bark, heartwood, and petioles of broad-leaved trees, coniferous trees, and grass, which varied in the content of recalcitrant organic compounds, polymer carbohydrates, and N. *Trametes versicolor* (L.) Lloyd IFO30340 was chosen as a test strain to achieve our purposes. *Trametes versicolor* is a common white rot fungus of wood producing a suite of extracellular enzymes degrading recalcitrant organic compounds including lignin, such as laccase and manganese peroxidase [12–14]. Strain IFO30340 registered in a culture collection has been used as one of the model strains for extracellular enzymatic systems responsible for decomposition [15,16] and also as a standard for the decomposition test of timber under the Japanese Industrial Standard (JIS). Finally, we applied Fourier transform infrared (FT-IR) spectroscopy [17] to the analysis of chemical structure of lignin and carbohydrate components in twigs and sapwoods, two major substrata of *T. versicolor* in the field, to obtain further insights into the litter quality control on decomposition.

2. Materials and methods

2.1. Source of litter and fungal isolate

A total of 72 litters from 51 plant species were used in the present study, including six tissues (27 leaves, 17 twigs, 19 sapwoods, 3 barks, 3 heartwoods, and 3 petioles) of three plant types (60 litters of broad-leaved trees, 11 of coniferous trees, and one grass) (detailed in Table A.1). Leaves in the present study denote lamina of newly shed leaves without obvious fungal or faunal attack, cut into pieces 1–1.5 cm in width. Twigs denote woody tissue of live trees less than 5 mm in diameter, cut into pieces 2–3 cm in length, and enclosed in thin bark. Sapwood and heartwood denote wood blocks of trees (approximately 10 × 10 × 5 mm) from live trees devoid of bark. Barks were derived from live trees and cut into blocks (approximately 10 × 10 mm, 5–10 mm in thickness).

Of the 72 litters, leaves of 15 tree species were collected in a cool temperate forest located in northern Kyoto, Japan, in November 2000 [18]. Twigs, sapwood, and heartwood of 17, 13, and 3 tree species were collected in temperate forests near Kyoto city from January 2001 to March 2003. These materials were oven-dried at 40 °C for one week and preserved in vinyl bags until the experiment was started. Data of the decomposition of 10 other litters were derived from previous pure culture tests conducted in the same laboratory and published by the same authors [19–25], and those of the decomposition of 14 litters were derived from an unpublished dataset related to papers published by the same authors [11,26] (detailed in Appendix A). The strain of *Trametes versicolor* IFO30340 deposited in the Institute of Fermentation, Osaka (IFO), Osaka, Japan, was used in all pure culture tests.

2.2. Pure culture decomposition test

An individual pure culture decomposition test consisted of one fungal isolate inoculated to one of the 72 litters. Litters (0.2–1.0 g per dish, in most cases 0.2–0.4 g per dish) were sterilized by exposure to ethylene oxide gas at 60 °C for 6 h and used in the tests according to the methods described in Osono [27]. The sterilized litters were placed on the surface of Petri dishes (9-cm diameter) containing 20 ml of 2% agar. Inocula for each assessment were cut out of the margin of previously inoculated Petri dishes on 1% MEA with a sterile cork borer (6 mm diameter) and placed on the agar adjacent to the litters, one plug per dish. The dishes were incubated for 12 weeks in the dark at 20 °C. The dishes were sealed firmly with laboratory film during incubation so that moisture did not limit decomposition on the agar. After incubation, the litters were retrieved, oven-dried at 40 °C for 1 week, and weighed. The initial, undecomposed litters were also sterilized, oven-dried at 40 °C for 1 week, and weighed to determine the original mass. These initial litters were used to determine the initial proportion of dry mass and the initial chemical property. Three to four plates were prepared for each test, and four uninoculated plates served as a control. Mass loss of litter was determined as a percentage of the original mass, taking the mass loss of litter in the uninoculated and incubated control treatment into account, and the mean values were calculated for each litter. Prior to these tests, the sterilized litters were placed on 1% MEA, and after 8 weeks of incubation at 20 °C in darkness, no microbial colonies had developed on the plates. This assured the effectiveness of the sterilization method used in the present study.

2.3. Proximate chemical analyses

Litter materials used in the pure culture tests were ground in a laboratory mill (0.5-mm screen) and used for chemical analyses according to the method described in Osono et al. [28]. The amount of acid-unhydrolyzable residue (AUR) in the samples was estimated by means of gravimetry as acid-insoluble residue, using hot sulfuric acid digestion [29]. Samples were extracted with alcohol-benzene at room temperature (15–20 °C), and the residue was treated with 72% sulfuric acid (v/v) for 2 h at room temperature with occasional stirring. The mixture was diluted with distilled water to make a 2.5% sulfuric acid solution and autoclaved at 120 °C for 60 min. After cooling, the residue was filtered and washed with water through a porous crucible (G4), dried at 105 °C and weighed as AUR. This AUR fraction contains a mixture of organic compounds in various proportions, including condensed tannins, phenolic and carboxylic compounds, alkyl compounds such as cutins, and true lignin [30]. The filtrate (autoclaved sulfuric acid solution) was used for total carbohydrate (TCH) analysis. The amount of carbohydrates in the filtrate was measured by means of the phenol-sulfuric acid method [31]. One milliliter of 5% phenol (v/v) and 5 mL of 98% sulfuric acid (v/v) were added to the filtrate. The optical density of the solution was measured using a spectrophotometer at 490 nm, using known concentrations of D-glucose as standards. Total nitrogen content was measured by means of automatic gas chromatography (NC analyzer SUMIGRAPH NC-900, Sumitomo Chemical Co., Osaka, Japan).

The relative amount of AUR and total N (AUR/N) is a useful index of the substrate quality and was calculated according to the equation: AUR/N = AUR content (mg/g)/total N content (mg/g).

2.4. FT-IR analysis

FT-IR spectroscopy measures the absorbance versus wavenumber (or equivalently, wavelength) of light to detect the vibration characteristics of chemical functional groups in a sample (Table 1) [32]. A total of 30 litters, including twigs of 17 tree species and sapwood of 13 tree species, were used for FT-IR analysis in the solid phase (Table B.1). Each ground sample was embedded in KBr and compacted into a disc using a bench press. FT-IR spectra were recorded in the absorbance mode at a resolution of 4 cm^{-1} with wavenumber range of 400–4000 cm^{-1}, using FT/IR-4100 (JASCO Co., Tokyo, Japan). Each spectrum was composed of 100 scans.

FT-IR spectra were baseline-corrected and normalized using JASCO Spectra Manager, Version 2 (JASCO Co., Tokyo, Japan). A total of 15 peaks that reflect functional groups associated with the lignocellulose matrix in litter were selected in the fingerprint region (800-2000 cm^{-1}) (Table 1). Peak heights were expressed as