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Comparison of microbial communities and histological changes in Phase I rice straw-based *Agaricus bisporus* compost prepared using two composting methods

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ABSTRACT

Rice straw is an important raw material for preparing *Agaricus bisporus* compost in China, and the size mushroom yield is largely determined by the quality of compost, which in turn is dependent on the microbial processes occurring during composting and the microbial populations inhabiting the mature compost. In this study, we compared the traditional composting method used in China with an aerated tunnel-based composting system through Phase I of the process. Phospholipid fatty acid (PLFA) analysis, used to monitor changes in the respective microbial populations, demonstrated a significant difference in the structure of the microbial communities and bacterial biomass associated with the two compost types. Chemical analysis of the degraded rice straw (RS) revealed that hemicellulose and silicate levels decreased more strongly in the aerated compared than in traditional non-aerated compost. Transmission electron microscopy (TEM), used to assess histological changes occurring in the stem tissue and cell walls of RS subjected to the two composting methods, showed that the aeration treatment resulted in more pronounced effects on stem structure. Composting using the aerated method also facilitated faster mycelial growth rates and higher yields of mushroom fruit bodies. Our data indicated that aeration-assisted composting of RS leads to improved degradation and assimilation of breakdown products by *A. bisporus*, and suggest that introduction of the process on a commercial scale in China has huge potential.

1. Introduction

Agaricus bisporus, the white button mushroom, is a Basidiomycete fungus produced in large quantities for human consumption on a specific type of compost covered with a casing layer (De Groot et al., 1998). In South China, most *A. bisporus* compost is prepared from rice straw (RS), cow manure and gypsum. RS is a major agricultural waste product in many rice-growing countries, and composting is a valuable form of solid waste management that provides both mushroom cultivation substrate and fertiliser, and also generates additional income for farmers after the rice harvest.

Composting is a self-heating, aerobic, solid-phase process driven by the microbiological decomposition of organic materials (Gerrits, 1988). For many years, the traditional method for preparing mushroom compost was divided into two distinct phases: Phase I, during which the raw materials are mixed, moistened and stacked, resulting in considerable losses in dry matter, and Phase II, which involves a pasteurising action and conditioning treatments that together produce a selective and pathogen-free substrate (Randle and Hayes, 1972; Ross and Harris, 1982).

Microbiologically, composting can be considered to involve a succession of microbiota that are continuously adapting to changing nutrient supplies and variable environmental conditions (temperature, moisture content, carbon dioxide, oxygen and ammonium content) (Tuomela et al., 2000). The succession and diversity of microbial communities at different stages of composting have been studied by several culture-independent techniques including community-level physiological profiles (Carpenter-Boggs et al., 1998), phospholipid fatty acid (PLFA) analysis (McConkey et al., 1996; Carpenter-Boggs et al., 1998; Helgason et al., 2010), and amplified environmental deoxy ribonucleic acid (DNA)-based methods (Dees and Ghiorse, 2001; Takaku et al., 2006; Tiquia and Michel, 2002).

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Available information on the microbiology of a composting methodology as specific as that used for the cultivation of *A. bisporus* is relatively limited (Székely et al., 2009). Furthermore, this lack of knowledge is particularly pronounced for Phase I composting since this process occurs more slowly and is less well regulated than Phase II, which requires only a relatively short indoor heat treatment (pasteurisation) to prepare a pathogen-free growth substrate (Peters et al., 2000). The traditional Phase I of composting uses outdoor windrows, and the compost produced that way is often of poor quality due to the anaerobic conditions caused by poor oxygen transfer and temperature control (high inner and low outer temperatures). In the 1970s, an important development in *A. bisporus* cultivation was the introduction in Europe of bulk processes in composting technology (Klaver and Van Gils, 1988). Phase I and Phase II composting were performed in the same bulk which, after cooling to 25–30 °C, was inoculated with spawn and colonised by the fungal mycelium (Van Lier et al., 1994). This technology improved the quality of the compost and increased mushroom yields (Delcaire, 1978). However, despite the fact that China is one of the largest producers of *A. bisporus*, Phase I composting throughout most of the country still uses traditional outdoor windrow systems, resulting in lower yields compared with the United States and Europe.

In the US and Europe, compost for cultivating *A. bisporus* is produced from wheat straw, horse and chicken manure, and gypsum (Straatsma et al., 2000). In China, RS, cow manure and gypsum are widely used (Gong, 2011), and no studies on the microbial communities and histological changes associated with RS-based mushroom compost have been undertaken. In order to improve the conditions and efficiency of Phase I compost production, we designed a simple aerated, tunnel-like composting device that provides sufficient oxygen for microbiological growth and ensures consistency between the internal and external temperatures of the compost. Microbial changes in both the device-assisted aerated windrow and a traditional non-aerated control were monitored using phospholipid-derived fatty acid (PLFA) analysis. In addition, the degree of RS degradation was assessed by a chemical composition analysis, and histological changes in the stem tissue and cell walls were determined using transmission electron microscopy (TEM).

2. Materials and methods

2.1. Mushroom strain

A. bisporus, strain As2796, a major commercial cultivar grown in China, was obtained from the Fujian Academy of Agricultural Sciences and maintained at 25 °C on potato dextrose agar (PDA, 200 g l^{-1} potato extract, 20 g l^{-1} glucose and 20 g l^{-1} agar). Wheat grains (purchased from a local grocery store; cultivar unknown) were used to prepare *A. bisporus* spawn as described elsewhere (Cheng, 1999).

2.2. Composting and sampling

Composting was conducted at the Pinhu Experimental Station of the Horticulture Institute, Zhejiang Academy of Agricultural Sciences, during October and November, 2011. Two separate composting windrows (15 m × 2.0 m × 1.8 m), each consisting of a mixture of RS 3000 kg, rapeseed cake 200 kg, $CaSO_4{\cdot}2H_2O$ 75 kg, $Ca(H_2PO_4)_2{\cdot}H_2O$ 25 kg, $(NH_4)_2SO_4$ 20 kg and urea (CON_2H_4) 30 kg, were adopted. A simple aerated, tunnel-like composting device was designed consisting of two half-buried parallel PVC pipes (15 m long, 12 cm diameter), each fitted with centrifugal fans (CF, 2 kw) at one end and blocked at the other, with holes drilled in the upper surface to provide upward air flow. The diameter of the holes increased gradually (1–4 cm) from the open to closed ends, and the space between any two adjacent holes was approximately 20 cm. The surface of the windrow was covered with an imbricate-type plastic insulation layer (shaded area) (Fig. 1B). A conventional outdoor-composting windrow served as the control (Fig. 1A).

The mixture of substrate components was moistened by manually operated spraying, and a 1-kg sample (original RS) was taken before initiating the self-heating composting phase. Test and control windrows were separately composted and turned using a forklift on the 5th, 9th and 12th days before beginning the Phase II treatment in order to enhance the composting process and avoid the formation of anaerobic compartments. Phase I was divided into three stages (F-first turn, S-second turn and T-third turn) according to the number of times the stacks had been turned. During turning, water and $CaCO_3$ were added by manually operated to keep the moisture content (60–70%) and the pH (6–8) suitable for microbe growth. Before turning, samples (1-kg) were randomly collected from three different locations along the windrows corresponding to the inner (I), middle (M) and outer layers (O) respectively (Fig. 1C). At the end of Phase I, both conventional and aerated compost were transferred to a pasteurisation room. Phase II (6 days duration) was characterised by a rapid increase in temperature up to 60 °C for 8–9 h, stabilisation of the compost temperature at 45–50 °C for ~5 days, followed by gradual cooling to 25 °C. Samples were also collected for quality analysis after Phase II of the composting process was complete. Samples were dried (water content ~5%) at ambient temperature, and for a further 24 h using a vacuum freeze-drier (VirTis Company, New York, NY, USA) before storage at −20 °C prior to analysis.

2.3. Assay of physical and chemical properties

Throughout Phase I (12 d), the compost temperature was continuously monitored at depths of 20, 50 and 100 cm using tubular mercurial thermometers (Kai Longda Instrument Co., Tianjin, China), and water content was determined by drying 500-g sub-samples at 105 °C for 24 h. pH values of 10% (w/v) sample suspensions (in distilled water) were measured with an electronic pH metre (Mettler-Toledo Instruments Co., Ltd., Shanghai, China), and total nitrogen and carbon levels (g kg^{-1}) in three 5-g sub-samples were assayed adopting the Kjeldahl (Bremner and Mulvaney, 1982) and $K_2Cr_2O_3$ oxidation (Fan, 2007) methods, respectively and used to calculate C/N ratios. All the assays used blank controls to eliminate background effects. Crude fibre analyses were performed using alkali and acid treatments to isolate the cell wall residue representing the non-digestible parts of the sub-samples. Fibre profiles (cellulose, hemicellulose, total silicate, neutral detergent fibre [NDF] and acid digestible lignin [ADL]) were analysed as described by Van Soest et al. (1991).

2.4. Phospholipid-derived fatty acid (PLFA) analysis

All solvents were of HPLC or GC grade (Sigma–Aldrich, Milwaukee, WI, USA), and chloroform was stabilised with ethanol. Sodium phosphate, potassium hydroxide and acetic acid were of reagent grade. Glassware was washed with detergent, dried and heated at 500 °C overnight, centrifuge tubes were heated at 400 °C for 2 h and screw caps were sonicated with detergent, rinsed, dried and washed with chloroform. PLFA analysis was carried out on 1-g freeze-dried samples (see Section 2.2) as previously described (Bossio et al., 1998; Zelles, 1999; Córdova-Kreylos et al., 2006). Total lipids were extracted with a one-phase chloroform/methanol/phosphate buffer solvent (8:4:3.2). Phospholipids were separated from neutral and glycolipid fatty acids using a solid-phase extraction column (0.58 Si; Supelco Inc., Bellafonte, PA, USA). Fatty acids were then converted to methyl ester derivatives,