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Research article

Chestnut green waste composting for sustainable forest management: Microbiota dynamics and impact on plant disease control

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ABSTRACT

Making compost from chestnut lignocellulosic waste is a possible sustainable management strategy for forests that employs a high-quality renewable organic resource. Characterization of the microbiota involved in composting is essential to better understand the entire process as well as the properties of the final product. Therefore, this study investigated the microbial communities involved in the composting of chestnut residues obtained from tree cleaning and pruning. The culture-independent approach taken highlighted the fact that the microbiota varied only slightly during the process, with the exception of those of the starting substrate and mature compost. The statistical analysis indicated that most of the bacterial and fungal species in the chestnut compost persisted during composting. The dominant microbial population detected during the process belonged to genera known to degrade recalcitrant lignocellulosic materials. Specifically, we identified fungal genera, such as *Penicillium, Fusarium, Cladosporium*, *Aspergillus* and *Mucor*, and prokaryotic species affiliated with *Bacilli*, *Actinobacteria*, *Flavobacteria* and *γ-Proteobacteria*. The suppressive properties of compost supplements for the biocontrol of *Sclerotinia minor* and *Rhizoctonia solani* were also investigated. Compared to pure substrate, the addition of compost to the peat-based growth substrates resulted in a significant reduction of disease in tomato plants of up to 70 % or 51 % in the presence of *Sclerotinia minor* or *Rhizoctonia solani*, respectively. The obtained results were related to the presence of putative bio-control agents and plant growth-promoting rhizobacteria belonging to the genera *Azotobacter, Pseudomonas*, *Stenotrophomonas*, *Bacillus*, *Flavobacterium*, *Streptomyces* and *Actinomyces* in the chestnut compost. The composting of chestnut waste may represent a sustainable agricultural practice for disposing of lignocellulosic waste by transforming it into green waste compost that can be used to improve the fitness of agricultural plants.

1. Introduction

The chestnut (*Castanea sativa* Mill.) is an important species for nut and wood production in Italy. Chestnut trees produce large quantities of waste residue, such as burrs, leaves and wood, that is often burned (Guerra-Rodriguez et al., 2006), resulting in forest fires. For this reason, the composting of chestnut residues obtained from cleaning and pruning and their utilisation in agriculture would represent a sustainable management practice for chestnut forests; moreover, the practice could be a source of added value for the sector. While current composting methods are well known, very few studies have reported composting processes for chestnut green waste and their use in agricultural systems (Guerra-Rodri;guez et al., 2006; Ventorino et al., 2013). Recently, the development of on-farm composting processes has elicited great interest, especially for the recycling of biomass residues of agricultural origin (Brito et al., 2012; Tuomela et al., 2000). Importantly, the compost obtained from these “clean biowastes” is more harmless than the compost obtained using other starting matrices because it originates from a pre-selected organic matrix that is generally free of xenobiotics and heavy metals (Ntougias et al., 2008; Pepe et al., 2013a). These properties ensure a high-quality end product that can be used as an all-purpose fertilizer and represents an interesting carrier for solid-based inoculants used in

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crop systems (Ben Rebah et al., 2007).

Composting is a low-cost method that is widely used to stabilize agricultural organic wastes through the degradation of organic materials by a consortium of microorganisms (Chen et al., 2014). A succession of different microbial populations are involved in this bio-oxidative process, and therefore, characterization of the microbiota present throughout the composting process and in the mature product is important (Cahyani et al., 2004). The beneficial effects of compost applications in agriculture have been recognized due to their physicochemical properties and microbiological composition, and numerous studies have demonstrated its potential as a growth substrate to stimulate plant growth and suppress soil-borne diseases (Martin and Brathwaite, 2012). In fact, the incorporation of microbial populations that characterize the compost into the soil could result in increased soil fertility (nitrogen-fixing and nitrifying bacteria), improved structural stability (exopolysaccharide producers), the synthesis and excretion of hormones, and the addition of chelators and nutrients, such as amino acids and vitamins (Boulter et al., 2002), and may contribute other effects to increase the biological activity and soil quality (Buyer et al., 2010). Castillo et al. (2013) demonstrated the importance of the use of agro-industrial waste as a profitable, sustainable solution to reduce the high price of fertilizers. Moreover, composting has achieved a reputation not only as a means of resource management by sustainable nutrient recycling and as an environmentally friendly method for the production of fertilizer but also for providing a product that can be used to suppress soil pathogens that affect indigenous plants (Hagn et al., 2008). The use of compost to suppress plant diseases has great potential among biological control practices (Noble and Conventry, 2005). Several reports have demonstrated the suppressive effect of compost from different matrices against a wide range of vegetable diseases caused by a variety of soil-borne plant pathogens, including *Rhizoctonia solani*, *Sclerotinia* spp., *Pythium* spp., *Fusarium* spp., and *Phytophthora* spp. (Cotxarrera et al. 2002; Martin and Brathwaite 2012). The suppression characteristics of composts are due to the presence of specific antagonistic microorganisms that are mainly responsible for the observed disease control (Huang et al., 2012) by different mechanisms, including antibiotic production, parasitism, competition for nutrients, antibiosis, the production of lytic and other extracellular enzymes, root surface colonization sites and the induction of systemic resistance in the plants (Hoitink and Boehm, 1999).

However, the relationship between microbial communities and the degradation process in green waste composting is relatively poorly understood (Yu et al., 2007). The potential suppressive effect of compost on soil-borne plant pathogens has to be evaluated on a case-by-case basis because it cannot be predicted purely on the basis of the compost characteristics (Huang et al., 2012). Compost quality depends on the diversity and dynamics of the microbial community during the composting phases for specific wastes. Thus, molecular techniques (e.g., PCR-DGGE) that allow the direct determination of the genetic diversity in different samples have been developed to widen the knowledge and investigate the microbiota from natural environments (Alfreider et al., 2002; Belyaeva et al., 2012).

In this study, the succession of bacterial and fungal populations was evaluated during chestnut green waste composting and in the finished compost to detect and characterize the dominant genera and/or species that mediated this process and their potential beneficial effects on plants. Moreover, the suppressive activity of compost as a soil supplement was estimated by determining its effect on soil-borne plant pathogens in *in vitro* and *in vivo* experiments with tomato plants.

2. Material and methods

2.1. Composting process and sampling

Chestnut residues consisting of leaves, branches, bark, and hulls were placed in piles in a composting bin, as previously described (Ventorino et al., 2013). The degradation of lignocellulosic waste was performed in a chestnut forest in Roccamonfina (Caserta, Italy; 41°18′94″N, 13°55′84″E) for 345 days. Composite samples of approximately 1 kg were obtained immediately after pile preparation (T0) and after 15, 30, 45, 60, 75, 90, 105, 135, 165, 195, 225, 255, 285, 315 and 345 days of composting (T1-T15) from three different points in the pile core.

2.2. Genomic DNA extraction

Microbial cells were desorbed from the lignocellulosic matrices as previously reported (Ventorino et al., 2013). The total microbial DNA was extracted using the FastDNA Spin Kit for Soil (MP Biomedicals, Illkirch Cedex, France) according to the manufacturer's specifications.

2.3. PCR-DGGE analysis and sequencing of the dominant bands

The primers V3f (5′-CCTACGGGAGGCAGCAG-3′) and V3r (5′-ATTACC GCGGCTGCTGG -3′) (Muyzer et al., 1993) were used for prokaryotic DGGE analysis. The PCR mixture and conditions were performed as previously described (Pepe et al., 2013b). The primers NL1 (5′-GCATATCAATAAGCGGAGGAAAAG-3′) (Kurtzman and Robnett, 1998) and LS2 (5′-ATTCCCAAACAACTCGACTC-3′) (Cocolin et al. 2000) for the 26S rRNA gene were used to analyse the eukaryotic population. The PCR mixture and conditions were performed as previously reported (Palomba et al., 2011). Based on the method of Muyzer et al. (1993), a GC-clamp was added to the forward primers.

DGGE analyses were performed with a polyacrylamide gel [8 % (wt/vol) acrylamide-bisacrylamide (37:5:1)] using a Bio-Rad DCode Universal Mutation System (Bio-Rad Laboratories, Milan, Italy). The denaturant gradient was 30–60 %, and the electrophoresis was run at 60 °C and 200 V for 240 min for both the bacterial and fungal analyses. After electrophoresis, the gels were stained with SYBR Gold (20 min) (Invitrogen, Milan, Italy) and rinsed in distilled water (5 min).

Dominant bands were excised from the gel, and the eluted DNA was re-amplified using the PCR conditions described above. The amplicons were verified by DGGE using DNA amplified from compost samples as the control. The products that migrated as a single band and at the same position with respect to the control were purified and sequenced. The DNA sequences were determined and analysed as previously reported (Pepe et al., 2011) and compared to the GenBank nucleotide data library using the BLAST program at the National Center of Biotechnology Information web site (http://www.ncbi.nlm.nih.gov) to determine their closest phylogenetic relatives.

2.4. Physico-chemical characteristics

Main physico-chemical characteristics of peat and compost were evaluated and resumed in Table 1. The pH was determined suspending samples in distillate water 1:10 (wt/vol). Porosity (%) and water holding capacity (% vol/vol) were measured according to methods described by Zucconi et al. (1981). Total and organic nitrogen contents (%) were determined by Kjeldahl method, while total organic carbon content (%) was determined by volumetric method redox (DM 13/09/1999). Available potassium (mg/kg K_2O)