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Enzymatic characterization of microbial isolates from lignocellulose waste composting: Chronological evolution

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Successful composting is dependent upon microbial performance. An interdependent relationship is established between environmental and nutritional properties that rule the process and characteristics of the dominant microbial communities. To reach a better understanding of this relationship, the dynamics of major metabolic activities associated with cultivable isolates according to composting phases were evaluated. Ammonification (72.04%), amylolysis (35.65%), hemicellulolyis (30.75%), and proteolysis (33.61%) were the more frequent activities among isolates, with mesophilic bacteria and fungi as the prevalent microbial communities. Bacteria were mainly responsible for starch hydrolysis, while a higher percentage of hemicellulolytic and proteolytic isolates were ascribable to fungi. Composting seems to exert a functional selective effect on microbial communities by promoting the presence of specific metabolically dominant groups at each stage of the process. Moreover, the application of conglomerate analysis led to the statement of a clear correlation between the chronology of the process and characteristics of the associated microbiota. According to metabolic capabilities of the isolates and their density, three clear clusters were obtained corresponding to the start of the process, including the first thermophilic peak, the rest of the bio-oxidative stage, and the maturation phase.

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1. Introduction

Composting is defined as a biotransformation process in which solid organic matter turns into mature and stabilized material by means of microbial action in aerobic conditions. The final product, compost, is a noteworthy soil amendment (Ros et al., 2006), a valuable substrate for soilless culture (Martínez et al., 2013), and an efficient tool for bioremediation processes (d'Errico et al., 2013) or soil restoration (Tejada et al., 2009b). Such applications are related to compost capability for improving soil's physical, chemical and biological properties (Ozores-Hampton et al., 2011), which in turn results in higher soil fertility and productivity.

Microorganisms play a key role both in the composting process and in compost properties. On the one hand, most of the transformations that organic matter undergo, particularly during the bio-oxidative stage, are ascribable to microbial action (Federici et al., 2011), and, on the other hand, effects derived from compost incorporation into soil strongly depend upon microbial populations in the final product (Farrell et al., 2010). It can be said, therefore, that proper performance of the process and quality of the final product lie on qualitative and quantitative composition of microbial communities associated to different stages (Pepe et al., 2013).

Several reports concerning dynamics and characterization of composting microbiota have been published throughout the years. Nevertheless, available information is not as thorough as would be desirable. On the one hand, diversity regarding composting substrate is limited, since most reports are focused on the valorization of municipal solid wastes and, to a lesser extent, in biosolids (Bonito et al., 2010; Khalil et al., 2011). Taking into account the influence that nature and properties of substrate have cause on microbial populations in composting (Ishii and Takii, 2003), processes with different starting materials represent unique environments from a microbiological point of view. Thus, a universal microbial pattern representative of every condition is not available. Knowledge of a specific process demands individual analysis.

On the other hand, there is a paucity of literature describing the complete biochemical characterization of the microbiota involved in the process. Some reports monitor the enzymatic evolution of the process (Tejada et al., 2009a), and others identify specific microbial communities (Li et al., 2013b). A reduced number of studies

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set up a connection among various profiles (Wei et al., 2012). Finally, some studies characterize one or a limited group of isolates (Eida et al., 2012). To our knowledge, no papers monitoring the enzyme dynamics of the process according to biochemical characterization of isolates exist.

Relevant metabolic capabilities of microorganisms in relation to organic-waste transformation are diverse. On the basis of the specific nature of the residual material, particular enzymatic features will be more significant in the biodegradation reactions. Nevertheless, general enzymatic systems involved in the transformation of main polymeric macromolecules always take part in the process. Thus, metabolic activities such as protein degradation, lipid modification, or even lignocellulose transformation are associated with the composting process in relation to whatever the properties of the starting material are, since they are of universal distribution. By contrast, other activities depend upon the properties of the starting material (Vaz-Moreira et al., 2008). In a plant-waste-based composting processes, activities such as pectin degradation or starch hydrolysis potentially gain importance on account of residue composition, becoming as predominant as more common enzymes (Ajila et al., 2012). The characterization of the dynamics of microbial functionality during the different stages of composting not only would provide a better understanding of the evolution of the process from a microbiological perspective and a more complete overview of connections between biotic and abiotic factors, but would also provide a tool for mining microorganisms useful in different biotechnological fields (Amore et al., 2013; Vargas García et al., 2012).

The aim of this work was to study the dynamics of culturable microbiota according to its metabolic capability profile during composting of lignocellulosic waste. A detailed analysis of metabolic potential of microorganisms throughout the different stages in the biotransformation process could contribute to gaining knowledge and understanding of the microbial role in composting and the influence of process conditions in the composition of the microbial population.

2. Material and methods

2.1. Composting procedure and sampling

Sun-dried tomato plants and pinewood chips, ground to ≤3 cm size, were mixed in 1:1 (w/w) proportion (C/N ratio: 25) and disposed as a trapezoidal pile on a cement platform equipped with a basal-forced aeration system. The pile was built up using about 500 kg of starting material and distributed according to following dimensions: 1.5 m width × 3 m length × 1 m height. Throughout the process, temperature was monitored using a long-handled (50 cm) thermometer probe PT100 model MPT2 (Lexitron-Guemisa, Madrid, Spain), while moisture content and aeration status were kept at prearranged levels, between 45 and 50% and over 10% oxygen, respectively, during the bio-oxidative stage. Moisture content was corrected by watering when necessary, and an air supply was provided through the basal system (perforated PVC tubes connected to a pump Lowarda CEAM-7013, Montecchio Maggiore, Italy) with a regime of aeration of 0.6 m s^{-1} every 4 h for 5 min. Additional mechanical-turning operations were carried out on the basis of the thermal profile to promote the mixing of the material and reactive microbial activity.

A thorough sampling strategy was programmed in order to achieve the most accurate scenario. According to thermal values, 19 samples were collected at different times of the process (Table 1). At every sampling time, nine locations were set and distributed along the three spatial dimensions (width: 25, 75, and 125 cm; length: 50, 150, and 250 cm; height: 10, 50, and 90 cm). Subsamples were equally mixed to a final weight of 1 kg and split into three replicates. Replicates were fractionated and treated according to specific protocols: air-dried at 40 °C overnight, ground to <1 mm for chemical analyses, and freshly processed for microbial isolation, moisture, and pH determination.

2.2. Chemical and physical analyses

The moisture content was determined by drying at 105 °C for 24 h. The pH was analyzed in a 1:10 (w/v) water extract. Total carbon (C) and nitrogen (N) were determined in solid samples by dry combustion at 950 °C using a LecoTruSpec C–N Elemental Analyzer (Leco Co., St. Joseph, MI, USA). Organic-matter content was assessed by determination of loss on ignition at 550 °C to a constant weight.

2.3. Microbial analysis

Mesophilic and terrmophilic microbiota (bacteria, actinobacteria, and fungi) were quantitatively estimated using a 10-fold serial dilution method. Initial suspension was obtained by adding 10 g of compost to 90 mL of sterile saline solution (NaCl, 0.9% w/v) and shaking for 30 min at room temperature. APHA and Rose Bengal Agar (Cultimed, Spain) were respectively used for bacteria and fungi, while actinobacteria was grown on Sodium Caseinate Agar (SCA) (composition in g L^{-1}: sodium caseinate 2.0; asparagine 0.1; sodium propionate 4.0; K_2HPO_4 0.5; $MgSO_4$ 0.1; $FeSO_4$ 0.001; glycerol 5.0; agar 15.0). Bacteria were incubated for 48 h and fungi and actinobacteria for 96 h, at 30 or 50 °C for mesophilic or thermotolerant microorganisms, respectively. Results were reported as colony-forming units (CFU) per gram of compost dry weight.

All different morphotypes identified in the plates were isolated in the conditions previously described for media and incubation. Isolates were regularly transferred to fresh agar slant and cryopreserved at −80 °C in cryoballs (Cryoinstant™, Deltalab, Barcelona, Spain) for long-term conservation.

2.4. Enzymatic analyses

All the isolates were investigated regarding their metabolic capability in relation to starch, pectin, lipid, and protein hydrolysis; lignocellulose fractions degradation; ammonification; and phosphate solubilization. Media used for the assay of each activity were as follows: starch agar for amilolytic activity (Vedder, 1915), Winogradsky medium with asparagine for ammonifying microorganisms (Pochon and Tardieux, 1962), Janshekar basal medium added to microcrystalline cellulose 0.5% and aniline blue-black 0.005% for cellulolytic activity (Janshekar et al., 1982; Kauri and Kushner, 1988), Janshekar basal medium added to xylan 0.5% for hemicellulolytic activity (Janshekar et al., 1982), basal medium added to poly R-478 dye 0.02% (Freitag and Morrell, 1992), basal medium added to tributyrin 1.0% (Leuschner et al., 1997), basal medium added to polygalacturonic acid 1% (Cotty et al., 1990), Janshekar basal medium added to sodium caseinate 1.0% for proteolytic activity (Janshekar et al., 1982) and NBRIP medium for phosphate-solubilizing activity (Nautiyal, 1999). All reagents were purchased from Sigma–Aldrich (St. Louis, Mo, USA). All assays were carried out on agar plates, except ammonification (MPN tubes). Every agar plate was used for testing eight different isolates. Bacteria and actinobacteria were assayed adding 25 μL droplets of microbial suspensions resulting from biomass grown on an agar slant for 48–72 h. In the case of fungi, pieces of 6 mm of diameter made with a hole puncher were disposed on each of the eight similar divisions in the plate. Tubes for ammonifying MPN were

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