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An investigation of the biodiversity of thermophilic and thermotolerant fungal species in composts using culture-based and molecular techniques

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ABSTRACT

In this study, the biodiversity of thermophilous fungi in two different commercial composts was investigated using culture-based methods, denaturing gradient gel electrophoresis (DGGE) and tag-encoded pyrosequencing. 454 pyrosequencing of the internal transcribed spacer (ITS) region recovered a total of 175 OTUs between the two composts. The Ascomycota was the dominant phylum in both composts (90 % of all sequences recovered) with the thermophilic-rich orders Sordariales and Eurotiales being the most numerous. Molecular studies demonstrated the frequent presence of several thermophilic (*Scytalidium thermophilum*, *Myriococcum thermophilum*) and thermotolerant (*Pseudallescheria boydii*, *Corynascus verrucosus* and *Coprinopsis* sp.) fungi in the composts, despite the absence of these species from the culture-based analysis. Conversely, *Aspergillus fumigatus* and *Mycocladus corymbifer*, which were the dominant species in cultivation analyses, had very low representation in molecular studies. The results show that the previous picture of the dominant thermophilous fungi in compost communities derived from culture-based analysis has been biased, and that composting environments represent a potentially rich resource of novel fungi.

Introduction

Composting is a self-heating, aerobic process in which organic matter is decomposed under controlled conditions by the action of micro-organisms (Finstein & Morris 1975; Kutzner 2000). In recent years, this practice has gained worldwide importance as a way of managing large-scale waste and decomposing it into a beneficial humus-like product that can be used for improving soil quality and fertility (Epstein 1997; Kumar 2011). From a microbiological perspective, composting is an exceptionally complex process in which many different micro-organisms participate. The composting process generally proceeds in predictable stages (mesophilic, thermophilic and curing) that can be recognised by rises and declines in temperature (de Bertoldi et al. 1983; Ryckeboer et al. 2003). These temperature phases reflect the activities of successive microbial populations performing the degradation of increasingly recalcitrant organic matter. As different environmental factors (nutrient availability, water activity, temperature, pH, etc.) change during the course of

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composting, the microbial populations responsible for the degradative activities also change (Epstein 1997; Kutzner 2000).

The main groups of micro-organisms participating in the composting process are bacteria and fungi (Kutzner 2000; Hultman et al. 2010). Fungi are particularly important as they play a central role in composting due to their ability to attack organic residues that are too dry, acidic or low in nitrogen for bacterial decomposition (Finstein & Morris 1975; Ryckeboer et al. 2003). This activity is carried out as a result of extensive hyphal networks that penetrate throughout the composting material and decompose the more recalcitrant organic fractions chemically and mechanically. In addition, the hyphal network physically stabilises the compost, providing improved aeration and drainage (Singer et al. 2000).

A large number of fungal species can be found in composting materials. During the mesophilic stage, most of the fungal species appear to be those already present in the original substratum prior to the composting process (Kutzner 2000). By contrast, thermophilic and thermotolerant fungi from compost are usually well adapted to the process conditions and can be found in composting piles from different sources (Johri et al. 1999; Kutzner 2000). Thermophilic fungi have been defined as those fungi that show no growth below 20 °C and have a maximum growth temperature at or above 50 °C (Cooney & Emerson 1964; Maheshwari et al. 2000). The optimum growth temperature for these fungi usually ranges between 40 and 50 °C (Morgenstern et al. 2012). In contrast, thermotolerant fungi can grow at temperatures below 20 °C but can also grow at temperatures above 50 °C (Cooney & Emerson 1964; Mouchacca 2000). The term "thermophilous" has been used to designate both thermophilic and thermotolerant fungi (Mouchacca 1997; Johri et al. 1999). Thermophilous fungi have been suggested as important biodegradation agents in composts due to the high temperatures occurring in these systems (Ryckeboer et al. 2003) and they have been linked to the degradation of recalcitrant substrates such as cellulose, hemicellulose and lignin during the process (Sharma 1989; Tuomela et al. 2000).

Despite the clear importance of thermophilic and thermotolerant fungi in composting, most of the research on these species was conducted over thirty years ago. Due to the limited availability of molecular techniques at that time, enumeration and isolation of thermophilic fungi was mostly performed using classical culture-based methods on rich organic complex media (Cooney & Emerson 1964; Tansey 1971; Kane & Mullins 1973; Tansey & Jack 1977). Moreover, only a few publications have concentrated on their diversity and role in composting environments (Kane & Mullins 1973; Klamer & Sochting 1998). Even though culture-based approaches have been useful in identifying several species of thermophilic fungi in compost, they are known to detect only a small proportion of all fungi present in environmental samples (Ishii et al. 2000; Ryckeboer et al. 2003; Novinscak et al. 2009). Thus, thermophilic fungi playing important roles in the degradation process and producing enzymes of potential commercial interest may have remained hitherto undetected. The need to investigate previously studied substrata to unearth overlooked fungal diversity has been highlighted several times (Hawksworth & Rossman 1997; Blackwell 2011) and recent estimates suggest that only about 100 000 fungal species are known from at least 1.5 million, but possibly as many as 3 million, total fungal species on Earth (Hawksworth 2012).

Recent studies on microbial diversity in composts have begun to utilise culture-independent approaches such as phospholipid fatty acid analysis (PLFA), denaturing gradient gel electrophoresis (DGGE) of PCR-amplified DNA fragments combined with the sequencing of relevant bands, terminal restriction fragment length polymorphism analysis (T-RFLP) and more recently, next-generation sequencing (Anderson & Cairney 2004; Hultman et al. 2010; Lindahl et al. 2013). These techniques have successfully determined microbial diversity by targeting taxonomically relevant molecular markers such as the 16S rRNA gene in bacteria and the 18S rRNA gene or the internal transcribed spacers (ITS region) in fungi (Mitchell & Zuccaro 2006; Novinscak et al. 2009). Next-generation sequencing technologies (such as 454 pyrosequencing, Illumina, SOLiD and IonTorrent) offer the opportunity to analyse microbial communities at a very high level of detail (Tedersoo et al. 2010; Monard et al. 2013).

To date, studies of microbial diversity in composts using molecular techniques have focused on the bacterial community (Ishii et al. 2000; Poulsen et al. 2008; Szekely et al. 2009; De Gannes et al. 2013), with only a few recent studies investigating the fungal populations using DGGE or clone libraries (Hultman et al. 2009; Novinscak et al. 2009; Bonito et al. 2010). No studies, however, have focused on thermophilic fungi or attempted to compare compost biodiversity using culture-based techniques and molecular methodologies. The aim of this study was to use culture-based methods, DGGE and tag-encoded pyrosequencing to assess the biodiversity of thermophilic fungi that participate in the composting process and compare the results obtained using these techniques.

Materials and methods

Compost samples

Mature compost samples were obtained from two different commercial composting facilities (A and B). The compost obtained from site A (The Compost Shop, Hightown, Merseyside, UK) utilised forestry and botanical cuttings as starting material. Compost from site B (Colima city council's composting site, Colima, Mexico) used a mix of botanical cuttings and municipal solid waste. Both composts were produced using the open windrow method with occasional turning to ensure aeration of the process. Compost samples were obtained at the curing (final) stage of the process and were considered by their manufacturers as ready-to-use. Composts were sieved (4 mm), mixed thoroughly to ensure homogeneity before chemical analyses, culture-based experiments and DNA extractions were performed.

Compost chemical analysis

Percentage water content (% w/w) in compost samples was determined by measuring the difference in mass of a 5 g sample