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Successions and diversity of humic-reducing microorganisms and their association with physical-chemical parameters during composting

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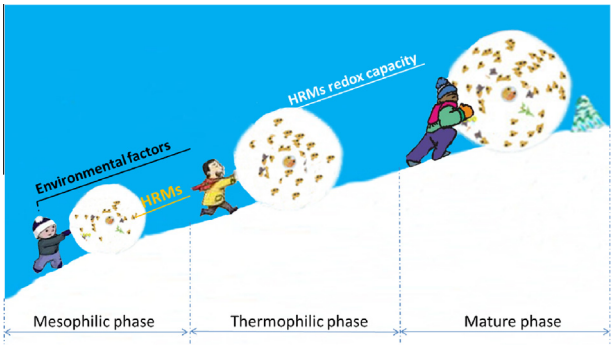
HIGHLIGHTS

- Humic-reducing microorganisms (HRMs) during composting were studied.
- Composting increased the abundance and diversity of the HRMs.
- The HRMs community composition was related significantly to DOC, DON and GI.
- Composting process increased the Fe (III)–citrate reduction capacity of the HRMs.

GRAPHICAL ABSTRACT



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ABSTRACT

Humic-reducing microorganisms (HRMs) could utilize humic substances (HS) as terminal electron mediator to promote the biodegradation of recalcitrant pollutants. However, the dynamics of HRMs during composting has not been explored. Here, high throughput sequencing technology was applied to investigate the patterns of HRMs during three composting systems. A total of 30 main genera of HRMs were identified in three composts, with *Proteobacteria* being the largest phylum. HRMs were detected with increased diversity and abundance and distinct patterns during composting, which were significantly associated with dissolved organic carbon, dissolved organic nitrogen and germination index. Regulating key physical-chemical parameters is a process control of HRMs community composition, thus promoting the redox capability of the compost. The redox capability of HRMs were strengthened during composting, suggesting that HRMs of the compost may play an important role on pollutant degradation of the compost or when they are applied to the contaminated soils.

1. Introduction

Humic-reducing microorganisms (HRMs) were the microorganisms with the capacity for promoting the bioremediation of contaminated environments by transferring electrons from bacterial cell surfaces to humic substances (HS) or quinoid analogues (Lovley et al., 1996). The microbe-reduced HS as exogenous

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electron shuttles could transfer electrons to insoluble terminal electron acceptors such as heavy metals and refractory organics (Lovley et al., 1996; Wolf et al., 2009; Wu et al., 2014). This biochemical pathway driven by microbial reduction of HS has been recognized as the main pathway in soils and sedimentary environments (Lovley et al., 1996). Previous studies indicated that a wide diversity of microorganisms was observed with the capacity of reducing HS or quinone model compounds (Coates et al., 2002; Cervantes et al., 2000). Most of the HRMs are Fe(III)-reducing bacteria, and also include denitrifying sulfate-reducing, fermentative and methanogenic microorganisms (Martinez et al., 2013). A recent review presented that the biochemical pathway involved in the HS reduction by HRMs has a number of similarities with the biochemical mechanisms that microbial reduction of Fe(III) (Bird et al., 2011). Thus, the capacity of HRMs for Fe(III) reduction is responsible to the release of some toxic metal such as manganese (Lovley, 1991), chromium (Francis et al., 1989) and uranium (Lovley, 1991) in the polluted soils.

Compost is widely used as amendment to improve soil properties, during the process, various organic solid waste materials are degraded into humus-like materials by microorganisms (García-Jaramillo et al., 2016). Considering HS could be served as terminal electron acceptor for microbial respiration, it is seasonable to speculate that the formation of HS could induce the breeding of HRMs during composting. Composting process is generally divided into three stages, i.e., mesophilic-, thermophilic- and mature-phases. Variation during the composting process was an important factor to influence the successions of dominant microbial groups (López-González et al., 2015). Nevertheless, to data, none of studies have been recognized about the composition, diversity, and succession patterns of HRMs communities in various compost and types of succession patterns occurring through the three main stages.

A complete understanding of HRMs community during the composting process is essential for developments of biotechnological applications to soil remediation. However, microorganisms isolated from composts were unknown microflora, leaving a 'black box' of undiscovered microbial composition. Various culture-independent methods have made attempted to provide a comprehensive analysis of microbial community composition, such as 16S rRNA gene (16S rDNA) diversity via terminal restriction fragment length polymorphism (T-RFLP), denaturing gradient gel electrophoresis (DGGE) (de Gannes et al., 2013; Dees and Ghiorse, 2001; Zhang et al., 2011). However, the resolution indicated by these molecular techniques is limited to influence the community accurately and incomplete estimate of phylogenetic diversity. For this purpose, the V3–V4 regions of 16S rDNA gene were sequenced via Illumina MiSeq 2500 platform which is a high-throughput sequencing technology has greatly advanced the understanding of microbial communities in various environments (Martinez et al., 2013).

This study aimed: (1) to investigate the evolution of abundance and diversity of HRMs community composition in three organic wastes composting systems across the three main phases, (2) to identify the relationship of HRMs community composition with physical-chemical parameters, and propose the regulating method for the distribution of HRMs community composition by the key physical-chemical parameters, and (3) to evaluate redox capacity of HRMs by reducing Fe(III)–citrate. This may be informative to evaluate the contribution of compost in contaminated soils.

2. Materials and methods

2.1. Compost collection and analyses

Compost samples were obtained from Shanghai Songjiang composting factory. Three compost sources in this study were broiler chicken manure (CM), dairy cattle manure (DCM) and corn straw waste (SW). Each compost piles was 1.5 m tall, with 2 × 3 m wide, and supplied with oxygen every week. SW was mixed with 1% urea with an initial C/N ratio of 31.4 ± 2.53. SW was also used to adjust the C/N ratio of CM and DCM, which was 20.32 ± 1.73 and 23.97 ± 1.29, respectively. The moisture was adjusted to 70%. Composting was considered to be finished when the temperature of the compost became stable and the germination index approached 80%. Each sample (about 2 kg) contains triplicate composite samples that were collected from the top, center, and bottom of the piles at three main stages, i.e., mesophilic phase (1d), thermophilic phase (7d) and mature phase (23d), respectively, and was mixed thoroughly before further experiment. The composts at three phases in SW, DCM and CM were labeled as SW1, SW7, SW23, DCM1, DCM7, DCM23, CM1, CM7 and CM23.

A part of samples were processed for the isolation of HRMs immediately, and the rest were stored at 4 °C for physical-chemical analysis. Physical-chemical parameters were shown in Table S1. Physical-chemical parameters included temperature, C/N ratio, dissolved organic carbon (DOC), dissolved organic nitrogen (DON), organic matter (OM), ammonia nitrogen (NH_4^+-N), nitrate nitrogen (NO_3^--N), moisture, pH and biological indicator included germination index (GI). Details of analytical methods have been reported in previous studies on the environmental parameters aspects (Wang et al., 2015). Briefly, Temperature was measured by a digital thermometer which was placed in each pile to record continuously. pH was measured by a pH meter. Moisture content was assessed by drying samples at 105 °C for 24 h. Total nitrogen (TN) was tested using Kjeldahl method. Ammonia nitrogen (NH_4^+-N) and nitrate (NO_3^--N) were extracted using 2 M KCl (1:10 ratio) at 200 rpm for 1 h. Samples were then filtered using a 0.45 lm mesh, and concentrations were determined using NaRSH'S colorimetry and spectrophotometry, respectively. Total carbon (TC), dissolved organic carbon (DOC) and nitrogen (DON) were measured using an organic carbon analyzer (TOC-Vcp, Shimadzu, Japan). The germination index (GI) was determined in accordance with (Tiquia et al., 1996).

2.2. Enrichment of HRMs

The humus analog anthraquinone-2, 6-disulfonate (AQDS) has been used extensively as a humus analog in studies on humus for microbial respiration (Lovley et al., 1996; Wu et al., 2014). Piepenbrock et al. (2014) have studied that 16S rRNA gene based diversity were rather versatile between enrichment cultures reducing humics or AQDS. Therefore, AQDS was selected as electron acceptor to enrich the HRMs in this study. The basal medium was performed according to Xu et al. (2014). In addition, lactate (5 mmol L^{-1}) and AQDS (0.5 mmol L^{-1}) was added as carbon substrates and electron acceptors. The media were sterilized by autoclaving for 20 min and cooled to room temperature under a constant stream of 80% N_2 and 20% CO_2. 1 g compost sample suspended in 9 mL of medium or from 10 mL of water sample was transferred into sterilized serum bottles containing 50 mL lactate-AQDS medium, capped with butyl rubber stoppers. The bottles were cultured at 30 °C in the dark. Positive enrichment for AQDS reduction was visually for color change from transparent to orange. The mixture was transferred into fresh medium with 6% inoculum. A stable microbial culture was performed by reducing ferric citrate assay plus ferrozine assay (see below). After almost 7–8 transfers, a stable potential to reduce ferric citrate by the microbial culture was obtained. Standard anaerobic techniques were used throughout and the samples incubated in an anaerobic chamber with a N_2 stream.