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Prokaryotic successions and diversity in composts as revealed by 454-pyrosequencing

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HIGHLIGHTS

- Pyrosequencing was applied to analyze compost prokaryotic communities.
- Communities were analyzed at each of the three major compost phases.
- In-depth census of community composition was attained and succession patterns revealed.
- Mesophilic- and mature-phase communities were distinct.
- This data on community composition may help determine biomarker assays for compost maturity.

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ABSTRACT

In this study, 454-pyrosequencing was applied to analyze prokaryotic patterns in three lignocellulosic composting systems across the three main phases. In all composts, diversity expanded as composting progressed. Communities in the mesophilic- and mature-phases of all composts were distinct, which did not support the concept that organisms present in the mesophilic phase enter dormancy during thermophilic period, and re-colonize the compost at the mature phase. Analysis of similarity revealed compost phase was a significant source of dissimilarity ($p = 0.011$), compost type was not ($p = 0.401$). Analysis of variance also showed significant phase effects on the abundance of (p-value): *Archaea* (0.001), *Planctomycetes* (0.002), *Chloroflexi* (0.016), *Deltaproteobacteria* (0.027), *Bacteria* (0.046) and *Gammaproteobacteria* (0.056). Mature-phase compost was a preferred niche for the *Archaea*, *Planctomycetes*, *Chloroflexi* and *Deltaproteobacteria*, while *Gammaproteobacteria* were predominant in earlier phases. Thus, the mature phase pattern could have implications in the development of biomarker assays for compost maturity.

1. Introduction

Composting is the aerobic, microbial decomposition of organic materials into humus-like material. Generally three stages are recognized, namely the mesophilic-, thermophilic- and curing/mature-phases. Variation in the processes occurring in these phases reflects variation in the constituent microbial communities. Thus, a thorough understanding of microbial communities throughout the composting process is crucial for understanding the system and optimizing compost product quality. The composition and dynamics of microbial communities in composts have been examined by culture-dependent and independent techniques. Nevertheless, our knowledge of the prokaryotic diversity (bacterial and archaeal) associated with various composts and the types of succession patterns occurring through the three main stages are limited. From culture-based analysis, at least 150 different bacterial genera/species have been identified in various composts (Ryckeboer et al., 2003). But, the restrictions imposed by any given set of growth conditions preclude a comprehensive analysis of community composition. For example, in a study of rice hull-food waste compost using culture-dependent and -independent techniques, the cultures were consistently dominated by various *Bacillus* species, even in cases where the culture-independent analysis indicated *Bacillus* (*Firmicutes*) to be negligible components of the community (Takaku et al., 2006).

Culture-independent investigations have focused on analysis of 16S rRNA gene (16S rDNA) diversity *via* terminal restriction fragment length polymorphism (T-RFLP), denaturing gradient gel electrophoresis (DGGE) and sequencing (Dees and Ghiorse, 2001; Nakasaki et al., 1985; Partanen et al., 2010; Takaku et al., 2006; Thummes et al., 2007; Tiquia, 2005; Yamamoto et al., 2010). However, relatively few studies have examined prokaryotic communi-

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ties in composts across all three phases (Partanen et al., 2010; Tiquia, 2005). Furthermore, the resolution achievable by DGGE or T-RFLP and the throughput of organism identification by 16S rRNA gene cloning and Sanger sequencing are limited compared to the potential diversity of microbial communities in composts. Thus, the composition and diversity of these communities remain ill-defined.

Pyrosequencing of 16S rRNA libraries has provided insights of unprecedented depth into microbial community composition and diversity in a wide variety of biomes, and has been particularly useful for analysis of soil. But, pyrosequencing of prokaryotic communities in composts has thus far been limited to analysis of biosolids stabilized by composting (Bibby et al., 2010), and 16S rRNA libraries were dominated by the *Bacteroidetes*, *Chloroflexi*, *Firmicutes*, *Proteobacteria*, and *Actinobacteria*. Many questions remain regarding the composition and dynamics of microbial communities in compost, which pyrosequencing can begin to address. For example, it remains largely unknown how prokaryotic community composition changes with the progression of the process. Finally, a broader question is whether any unifying patterns in prokaryotic community composition or dynamics can be identified across compost types that might be informative of processes important in composting.

In the present study, we investigated prokaryotic diversity in compost by applying pyrosequencing to analyze bacterial and archaeal 16S rRNA gene amplicons from lignocellulosic composting systems. Based on the literature, hypotheses tested in this study were: (1) thermophilic communities in compost are predominantly *Bacillus*, and (2) organisms present in the mesophilic phase enter dormancy during the thermophilic period, and re-colonize the compost at the mature phase. In order to test these hypotheses our goal was to determine the prokaryotic successions in three lignocellulosic composting systems, and to develop a census of the diversity of prokaryotes inhabiting these systems.

2. Methods

2.1. Compost composition and analyses

The compost study was described previously in de Gannes et al., (2012). Briefly, composting was done with in-vessel rotary type drums, in a randomized 3×2 factorial design with six treatments and two replications per treatment. Substrates used for composting were plant wastes (*viz.* rice straw, sugar cane bagasse, coffee hulls) mixed with either cow- or sheep-manure to obtain a C:N of 25–35:1. The rice composts attained a thermophilic temperature of *ca.* 57 °C for a period of 3–4 days, which then decreased to 31 °C by day 12. However, the coffee composts had attained a peak thermophilic temperature of 64 °C that was followed by a cooling period of *ca.* 30 days. The sugar cane bagasse compost reached a peak temperature of *ca.* 38 °C, which was not considered a thermophilic stage. At the completion of the study on day 82, the C:N of the composts were: 12 (rice), 15 (coffee) and 18 (bagasse).

2.2. DNA extraction and pyrosequencing

Sampling and DNA extraction was described previously in de Gannes et al., (2012). Briefly, the composts were sampled at the mesophilic stage (day 0), thermophilic stage (day 2 for rice straw composts, day 3 for coffee hulls composts, no sample for sugar cane bagasse) and completion of the study (mature stage, day 82). At each time point *ca.* 50 g of composite sample was removed from each compost system with sterilized tongs, and placed into sterile Nasco Whirl Pack bags (Lakewood Biochemical Co., Inc., Dallas, TX) that were subsequently stored at −20 °C until used for DNA extraction. The Power Soil DNA Isolation kit (MO BIO Laboratories, Inc., West Carlsbad, CA) was used to extract DNA and post-extraction clean-up was done with the Power Clean DNA Clean up kit (MO BIO Laboratories), with both procedures following the manufacturer's instructions. Aliquots of the treatment replicates were pooled, and an aliquot from each of these samples was then used for PCR. For the mature phase of the bagasse and coffee composts, two replicate aliquots were used in PCR, giving a total of ten samples analyzed by pyrosequencing.

Primers were F515/R806 that targeted the V4 region of the 16S rRNA gene, which amplifies both bacterial and archaeal sequences (Caporaso et al., 2011). Each forward primer contained the A linker for sequencing, and one of ten unique Roche multiplex identifiers (MID) 83–91. A unique 10-base MID was used to tag PCR amplicons from each compost sample (Table S1). The mature phase of the bagasse and coffee were selected for replicate PCR because in prior analysis of ammonia oxidizing *Archaea* in these samples revealed strongly contrasting patterns of diversity (de Gannes et al., 2012). PCR was done by using conditions described by Caporaso and coworkers (Caporaso et al., 2011). Amplicon concentrations were measured with a Qubit fluorometer (Invitrogen, Grand Island, NY), the libraries were pooled in equimolar concentrations and then cleaned by $5\times$ passage over AMPure beads (Breckman Coulter, Brea, CA). The cleaned samples were clonally amplified *via* emulsion PCR using the GS FLX Titanium Lib-L LV emPCR Kit (Roche Applied Science, Indianapolis, IN) according to the manufacturer's recommendations, with the exception of using 25% of the recommended volume of amplification primer. DNA library capture was done using a ratio of 1 DNA molecule per capture bead. Following amplification, reactions were collected, emulsions were broken and beads containing clonally amplified DNA were enriched according to the manufacturer's protocol. Enriched DNA beads were counted using the CASY Model DT cell counter (Roche Applied Science). Enriched DNA beads were deposited into the wells of a GS FLX Titanium Pico Titer Plate fitted with a 2-region gasket and sequenced using a GS FLX Titanium Sequencing Kit XL+ according to the manufacturer's instructions. Image analysis and signal processing were done using GS Run Processor software v. 2.6 *via* the shotgun/paired end signal processing pipeline (Roche Applied Science).

2.3. Bioinformatics and statistical analyses

Sequence data processing was done using QIIME v. 1.4 following a procedure similar to that of Caporaso et al., (2011). Sequences shorter than 250 bp and longer than 400 bp were eliminated from the data set, and those remaining with quality scores >25 were grouped into operational taxonomic units (OTU) at a similarity level of 97% by using Cd-hit. Taxonomic assignments of OTUs was done by BLAST-N with a representative sequence from each OTU against the Greengenes core set and the highest level of taxonomic resolution determined by BLAST-N at a confidence of $\geqslant 0.80$ was applied to the OTU. The QIIME package was also used for OTU-based alpha diversity analyses (10 sampling repetitions without replacement at each sampling depth) including rarefaction and computation of the Chao1 metric. To assess beta diversity, the weighted UniFrac metric was used for principle coordinate analysis (PCoA) and jack knife analysis of phylogenetic distances. Analysis of similarity (ANOSIM) was done with the R programming environment (www.R-project.org) examining pairwise UniFrac distances as a function of compost type and phase (999 permutations). Differences in abundance of taxa as a function of compost type or compost phase were assessed for significance by analysis of variance (ANOVA) and Tukey's test by using R. Two replications were used for each treatment during the experiment, with subsequent pooling of DNA extractions from both replicates prior to PCR and