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Comparison of the bacterial composition of two commercial composts with different physicochemical, stability and maturity properties

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ABSTRACT

Previously, two municipal solid waste commercial composts (MSW1 and MSW2) were characterized. Although sharing the same type of raw material, most of their physicochemical, stability and maturity properties differed. The present study aimed to characterize them at a microbiological level, and to infer on possible relationships between the composts properties and the structure of their bacterial communities. Both the 16S rRNA gene-based PCR-DGGE profiling and 454-pyrosequencing technology showed that the structure of the bacterial communities of these composts was distinct. The bacterial community of MSW1 was more diverse than that of MSW2. Multivariate analyses revealed that the high electrical conductivity, Cu content as well as the low phytotoxity of compost MSW1, when compared to MSW2, contributed most to shape its bacterial community structure. Indeed, high abundance of halophilic (*Halomonadaceae* and *Brevibacteriaceae*) and metal resistant organisms (*Brevibacteriaceae* and *Bacillaceae*) were found in MSW1. In addition, *Pseudonocardiaceae*, *Streptomycetaceae*, *Bacillaceae*, *and Brevibacteriaceae* may have contributed to the high humic-like acids content and low phytotoxicity of MSW1. In contrast, the high organic matter content and the high density of the cultivable fungi population were the parameters most correlated with the structure of the bacterial community of compost MSW2, dominated by *Corynebacteriaceae* and mainly *Aerococcaceae*, taxonomic groups not commonly found in composts.

1. Introduction

Composting is an ancient strategy to recycle a variety of organic wastes. It can be considered a biotechnological aerobic process where during the first phase (active phase) microbial communities transforme and partially mineralize the most easily biodegradable materials of organic matter (OM) present in the raw materials. The organic matter becomes stabilised as a consequence of the intense microbial activity occurring in this phase (Adani et al., 1997), which is also responsible for the rise of the compost temperature, inducing a transition from a mesophilic to a thermophilic stage (Tang et al., 2006). Then, a gradual decrease in the microbial activity leads to the cooling and the curing or maturation phase. In this phase occurs the conversion of part of the stabilised organic matter into humic-like substances (Chen and Inbar, 1993). Thus, while compost stability can be regarded as the result of high-rate microbial reactions occurring throughout the active phase of the process, compost maturity appears, on the contrary, as the effect of the curing phase. Given its high content in humic-like substances, compost is considered an added value product, allowing the recovery of degraded soil and the sustainable management of agricultural land when used as a soil amendment (Albrecht et al., 2011; Cayuela et al., 2009). However, only stable and mature composts should be used as a soil amendment (Castaldi et al., 2008; ECC, 2001; Gómez-Brandón et al., 2008). The application of unstable composts to soil may promote competition for oxygen between microorganisms and plant roots and/or seeds. In addition, it may promote nitrogen starvation of plants as microorganisms scavenge soil N as a consequence of the high C/N ratio of unstable composts. Unstabe composts may be also phytotoxic due to the emission of ammonia and the presence of other phytotoxic substances like phenolic compounds and ethylene oxide added to soil when the readily metabolizable organic matter of the compost is not fully degraded (Gómez-Brandón et al., 2008). Additionally, as referred to by Danon et al. (2008), some studies have emphasized the

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importance of achieving compost maturity to ensure balanced plant nutrition and the biological control of soil-borne plant disease.

Green waste, municipal solid waste, sewage sludge and manure are the raw materials commonly used in composting (e.g., Cahyani et al., 2003; de Gannes et al., 2013; Storey et al., 2015; Takaku et al., 2006), which is carried out in windrow or reactor systems. Independently of the raw material or the system, to obtain stable and mature composts, the operating conditions must favor the development of the microbial communities present at the beginning of the process. In fact, Partanen et al. (2010) have demonstrated that low initial pH and insufficient aeration limits microbial activity and delays the increase in temperature. Other abiotic parameter known to influence the composting process is the C/N ratio (Cayuela et al., 2009; Chroni et al., 2009; Guo et al., 2012; Tiquia, 2005), since without an adequate proportion of C and N, microorganisms are unable of growing (Maier, 2009). Indeed, the composition and function of the microbial communities depends on these and on other environmental factors, such as the temperature and the available nutrients (e.g., Blanc et al., 1999; Nakasaki et al., 2009; Ryckeboer et al., 2003; Tiquia, 2005). Hence, being the product of microbial transformations, the properties of the final compost are expected to depend on the operating conditions, including the type of the raw material and time of composting, which modullate the microbiological populations prevailing in each of the composting stages (Cayuela et al., 2009; Fracchia et al., 2006; Partanen et al., 2010; Storey et al., 2015).

Table 1 summarizes the bacteria described as the most predominant in composts. This revision was based on studies published after 1999 in journals indexed to the ISI – Web of Knowledge, in which the composting system was windrow or reactor. In addition, only studies where the bacterial identification was based on 16S rRNA gene sequence analysis were included, independent of using culture-dependent and/or independent methods. Based on Table 1 it is possible to conclude that, in general, composts harbor a wide diversity of bacteria. Indeed, organisms belonging to ten different phyla have been shown to be abundant in composts. As expected, some groups are only abundant when mesophilic (e.g. *Enterococcaceae*) or thermophilic (e.g. *Rhodothermaceae*) conditions prevail, while others occur during the cooling or maturation phases (e.g. *Cytophagaceae*). However, several bacterial groups have been detected in composts, independent of the raw material, composting system or phase. Examples are bacteria affiliated to phylum *Chloroflexi*, and to the families *Flavobacteriaceae* and *Sphingobacteriaceae* (*Bacteroidetes*), *Bacillaceae* and *Paenibacillaceae* (*Firmicutes*), *Alcaligenaceae* (*Betaproteobacteria*) and *Xanthomonadaceae* (*Gammaproteobacteria*).

Although several studies revealed the composition and dynamics of the microorganisms during the composting (Table 1), little is known about the correlation between the microbial composition and the compost quality. In a previous study, based on an exhaustive physicochemical, stability and maturity characterization, it was shown that two commercial composts (MSW1 and MSW2) differed greatly (Silva et al., 2013). This variation was mainly due to differences on the content of organic matter, moisture, electrical conductivity, heavy metals and humic-like acids, degree of stability and germination index. Regarding these differences, in the present study, we intended to verify if this dissimilarity was also observed at the bacterial community level and if there was a relationship between these parameters and the bacterial community structure. We also intended to identify the common and unique populations and to evaluate if the physiological characteristics of the most abundant groups were compatible with the physicochemical, stability and maturity properties of the studied composts.

2. Materials and methods

2.1. Composts

Two commercial composts (MSW1 and MSW2) were analyzed in the present study. MSW1 and MSW2 were produced from municipal solid waste using windrow and reactor composting process, respectively. For both composts, very large components were manually removed from municipal solid waste. For MSW1, the organic fraction was further separated applying a sieve (7.5 cm) and then the organic matter was mixed with green wastes and composted in piles with air supply, water addition and periodic turning. To produce MSW2 compost, the non-separated municipal solid was composted in a rotating drum reactor with forced aeration and water addition, followed by mechanical removal of remaining large particles of plastic, glass, metal, etc. Maturation of MSW2 occurred in a static pile. For both composts, the time of the composting process was 6 months. The physicochemical, stability and maturity parameters of these composts were previously determined (Silva et al., 2013) and are presented in Supplementary Table S1. The stability was assessed using the Dewar self-heating test and the respiration activity after 4 days (AT4) (Silva et al., 2013).

The compost sampling to carry out the microbial analyses was made with sterilized material from five different points (~10 g each) of the compost bag. For MSW2, visible glass, plastic, and metal particles were asepticaly removed with forceps. After pooling and homogenization by mixing, it was immediately analyzed for the enumeration of cultivable microbial populations and frozen at −80 °C before processing for bacterial community analyses.

2.2. Enumeration of cultivable microbial populations

The enumeration of the cultivable populations was carried out using the membrane filtration method as previously described (Ferreira da Silva et al., 2006). Briefly, ten grams of compost were suspended in 90 mL of a sterile solution (0.85% (w/v) NaCl), shaken for 30 min and were sequentially diluted in 10-fold series until a final dilution of 10^{-7}. One millilitre of each dilution of compost suspension was filtered through a 47 mm membrane with 0.45 μm porosity and placed on Plate Count Agar (PCA, Merck) for total heterotrophs, Actinomycete Isolation Agar (AIA, Difco) for actinobacteria and Rose Bengal Agar (Rose Bengal, Merck) for fungi enumeration. Total heterotrophs and actinobacteria were enumerated after incubation at 30 °C for 24 h and 7 days, respectively. Fungi were enumerated after incubation at 22 °C for 7 days. Data from triplicates were expressed as CFU g^{-1} dry matter (oven-dried compost basis).

2.3. Bacterial community analysis

Total DNA of each compost was extracted using Power Soil™ DNA Isolation Kit (MO BIO) with an additional cycle of freeze-thawing with liquid nitrogen and an incubation step at 65 °C during 30 min, after 40 min horizontal agitation at 1300 rpm. To obtain 1 μg of pure and good quality total DNA for 454 pyrosequencing analysis, total DNA from six independent DNA extractions of the same compost (6 × 0.25 g, wet basis) was pooled into one sample. For PCR-DGGE profiling, which requires a lower amount of DNA, total DNA was obtained from 3 independent DNA extractions as described above, which were analyzed independently (I/II/III).

For PCR-DGGE profiling, a 500 bp fragment (based on the reference strain *Escherichia coli* bases 984 and 1378) was amplified using the 16S rRNA gene primers forward F984GC, containing a