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Succession of bacteria diversity in the poultry manure composted mixed with clay: Studies upon its dynamics and associations with physicochemical and gaseous parameters

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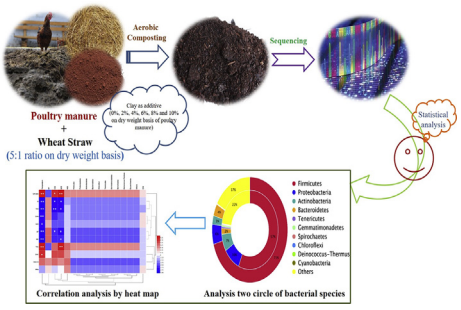
GRAPHICAL ABSTRACT



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ABSTRACT

In this study, the bacterial community succession and variations were investigated in poultry manure (PM) compost by the using high-throughput sequencing in six different concentration of clay [at 0% (T1), 2% (T2), 4% (T3), 6% (T4), 8% (T5) and 10% (T6) on PM dry weight basis] applied compost. The results indicated that dominant phylum were *Firmicutes*, *Proteobacteria*, *Actinobacteria* and *Bacteroidetes*, while *Bacillus*, *Paenibacillus*, *Virgibacillus*, *Oceanobacillus* and *Clostridium* were the dominant genera in all the treatments. Correlation analyses provided useful tools for insight into the bacterial interactions with environmental factors and also extension of the compost maturation and resistance of bacteria. During the course of study, the diversity of bacteria similar but relative abundance variable in each treatments. However, the average and the normalized (to bacterial RAs or copies of sequences) both remained greater in higher dosage of clay applied treatments. Finally, the RAs of various bacterial community composition was affected in PM compost by the clay application.

1. Introduction

Composting is widely adopted an effective organic waste recycling method, where numerous microbes involved to transformed organic substance in to stable compost and reduce the environmental risk (Cui et al., 2016; Liu et al., 2018). During composting, microorganisms (bacteria, actinomycetes and fungi) and environmental factors play significant role for rapid mineralization of organic matter (Song et al., 2014), however, bacteria are more proactive because of the facultative nature of their adaptation at adverse environmental condition and

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Table 1
Characteristics of composting materials used in this study.

Parameters	PM	WS	Clay	Mixture
Moisture content (%)	80.24 ± 2.34	8.89 ± 0.10	3.01 ± 0.02	58.64 ± 3.06
Total Kjeldahl nitrogen (%)	2.47 ± 0.06	0.42 ± 0.03	0.05 ± 0.01	1.93 ± 0.01
Total organic carbon (%)	46.84 ± 0.31	58.64 ± 1.42	1.34 ± 0.01	48.68 ± 0.01
pH	6.85 ± 0.07	7.14 ± 0.04	7.12 ± 0.03	7.12 ± 0.03
Electrical conductivity (μs/cm)	6214 ± 27	121.8 ± 6	94.31 ± 8	94.31 ± 8
Carbon/nitrogen ratio	18.96 ± 0.48	139.61 ± 12.04	26.8 ± 1.02	25.22 ± 0.46

PM – Poultry manure, WS – Wheat straw, Results are the average of three repeats ± standard deviation.

metabolism (Li et al., 2013; Fu et al., 2015). Therefore, the investigation of relative abundance and bacterial dynamics during the composting is great importance to understand the overall mechanism of organic waste mineralization. In order to identify the total bacteria dynamics and population during composting, last few years many classical isolation technique was commonly used such as PCR-DGGE, cloning and sequencing (Partanen et al., 2010; Chandna et al., 2013; Wang et al., 2015). But among the all techniques Illumina MiSeq high-throughput sequencing has been tested an impressive molecular tool which could unveil the detailed inside corresponding to bacterial dynamics with more accuracy (Cui et al., 2017).

It is well known that organic matter transformation includes many bio-oxidative methods like cellulolysis, proteolysis and amylolysis etc., which is essential for rapid composting (Dees and Ghiorse, 2001; Jindo et al., 2012). Xi et al. (2016) and Awasthi et al. (2017b) reported that organic matter transformation is govern by group of bacteria and their enzyme activities during the composting. There are some recent studies have revealed that interactions between bacterial diversity with different kinds of organic matter transformation (Takaku et al., 2006; Wang et al., 2007). However, most of previous literatures also confirmed that microbial diversity and enzymatic activities have a mutually influence on organic matter transformation during the composting and its stability (Wakase et al., 2008; Insam et al. 2010; Zhang et al., 2016). However, composting has been proven that as an most effective bioremediation technology for the removal of toxic and persistent organic compounds from organic waste (Gou et al., 2018; Selvam et al. 2012), but in this consideration has been obstacle was noticed by Su et al. (2015), who observed that the bacterial abundance and diversity were increased during composting of sewage sludge, although, its change with progress of composting and applied bulking agents as well as amendment. Similarly, Wang et al. (2015) investigated variation of RAs of bacteria during manure composting and found that biochar amendment was significantly affect to nitrifying bacteria abundance and mechanism of organic matter degradation. Tkachuk et al. (2014) also confirmed that different dosage of additives amendment (depletion, stasis, or increment), may be considerably interrelated with the composition of the composting mixture, environmental factors (temperature and pH), or the bacterial community dynamics during the composting.

Hence, the diversity of specific phylum, class, family, order, genes and species of bacteria in organic matter transformation is not clear. There is still little information about quantitative estimation of RAs of the key bacteria and its correlation with organic matter transformation, which is essential to identification of bacterial dynamics and its importance during poultry manure (PM) composting. In this study, we identify the impact of different dosage of clay amendment in relative abundance of bacteria diversity in PM compost using high-throughput sequencing technology. In addition, the correlation of gaseous emission and physicochemical properties were also investigated by Redundancy analyses (RDA) which is essential to understand the overall mechanism of PM composting.

2. Materials and methods

2.1. Description of composting materials collection and processing

The experiment was carried out at Northwest Agriculture and Forestry University campus. The PM used in this study was obtained from a local poultry farm (Yangling, Shanxi Province, China) and the wheat straw (WS) was collected from local market. The WS is mixed with PM as bulking agent to harmonies the standard C/N ratio (25:1) for rapid composting. WS was cut into 1 cm^{-1} length before mixing by the using of a mechanical grinder to get uniform raw materials size. The clay used in this experiment was obtained from the local area. The depth of collection is 10–15 cm in the middle of the composting mass and then crushed to pass through a 0.15 mm sieve and mixed prior to used. The major mineral components of clay are montmorillonite and kaolinite and the clay particles percent content is about to 70%, thus this kind of nature clay can be considered to represent clay material to be used as composting amendment to improve the PM composting. Selected characteristics of each substrate are given in Table 1.

2.2. Experimental setup and compost sample collection

The pilot-scale composting reactor was used in present investigation, while reactor volume, shape and size with functional process were already mentioned in our previous study (Awasthi et al., 2017c). Six composting mixtures were design in triplicate to identify the influence of different dosages of clay [at 0% (T1), 2% (T2), 4% (T3), 6% (T4), 8% (T5) and 10% (T6) on PM dry weight basis] on taxonomic variation of bacterial abundance and diversity. While treatment (T1) PM + WS was run as control for comparison purpose. Approximately 100-L of each composting mixture was filled in each reactor and composted for 50 days. The moisture level of composting mixture was maintained about 55% and reset periodically on turning days 0, 3, 7, 14, 21, 28, 35, 42 and 50. The turning reduced the compaction and maintained the homogeneity of composting mixture as well as enhanced the bacterial abundance and its succession. The temperature, pH, electrical conductivity profile changes and variations of gaseous (CO_2, CH_4, NH_3 and N_2O) emission among the all treatments is not illustrated in this study but ~250 g compost samples were collected from each reactor for further analysis as previously referred (Awasthi et al., 2017a). For meta-genome analysis, the final compost samples was randomly taken from each treatment separately and process according to our previous experience (Awasthi et al., 2017a).

2.3. DNA extraction, PCR amplification and 16S rDNA sequencing

The DNA was extracted from fresh compost samples using Fast DNA kit (Omega Biotek, Inc.) for soil as per the manufactures instruction. The purity of DNA and its concentration were determined by nano - spectrophotometer and 1% (w/v) agarose gels electrophoresis. Standard Q-PCR was employed using specific full length universal forward and reverse primers. The details of primers and standard PCR conditions are given in the supporting information. In addition, the PCR products were refine using Qiagen Gel Extraction Kit and PCR Clean-up