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Succession of organics metabolic function of bacterial community in swine manure composting

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ABSTRACT

Organics metabolic function of bacterial communities was evaluated in 60 days composting of swine manure and pumice by using MiSeq sequencing, PICRUSt and Biolog tools. The diversity of bacterial communities significantly decreased during the first 10 days, and gradually increased in the cooling and curing phase. The PICRUSt and Biolog analysis indicated that carbohydrate, lipid and amino acids metabolisms were relatively higher in the thermophilic phases. Xenobiotics biodegradation and metabolism, lipid metabolism, terpenoids and polyketides and biosynthesis of other secondary metabolites were mainly detected in the curing phases. Canonical correspondence analysis (CCA) indicated that the succession of bacterial community and organics utilization characteristics were highly affected by the temperature, moisture and oxidation reduction potential (ORP) in the swine composting system.

1. Introduction

Annual production of swine manure has been estimated about 776 million tons in China [1]. Swine manure contains abundant of humus and nutrients, which could be used to improve the quality of soil and the quantity of crop [2]. However, fresh swine manure could not be directly applied to soil, because unstable organic matters and pathogens in the swine manure could be detrimental to plant and human health. Composting is a cost-effective and environmental-friend bio-treatment method of organic solid wastes, which is widely used to stabilize organic matters, reduce pathogens and phytotoxic substances in animal manures before land application.

Bacteria are dominating decomposer in aerobic composting system. Previous literature mainly focused on bacterial composition and phylogenetic in composting system by using culture dependent method, denaturing gradient gel electrophoresis (DGGE), terminal restriction fragment length polymorphism (T-RFLP), phospholipids fatty acids analysis (PLFAs) and high-throughput sequencing, respectively [3,4]. The composition of bacterial community was significantly different in the previous literature of swine manure composting. The abundances of *Aeromonas* and *Clostridium*_sensu_stricto_1 were the highest in the thermophilic phase of swine manure composting with wheat straw, but the abundance of *Bacillus* apparently increased after adding superabsorbent polymers [5]. Issatchenkia (11.92%) was the main genus in the thermophilic phase of swine manure composting amended with spent mushroom substrate and rice husks [6]. *Bacteroides* and *Sporosarcina* were the dominant genera in the thermophilic phase of swine manure composting with sawdust, while *Psychrobacter* became one of the main genus after adding bamboo biochar [7,8]. It illustrated that various organic bulking agents prominently impacted the composition of microbial community in swine manure composting system.

The function of microbial community dominated organics degradation, nutrients transformation, biological heat production, humic-like substances formation in composting process [9], but the information on the functional characteristics of microbial communities was limited in previous composting literature. Recently, Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) was developed to predict the function of microbial community based on the data from high-throughout sequencing. Wei et.al found that microbial metabolic functions, cellular processes and environmental information processing were the main functions of microbial community in the composting process of maize straw [10]. Microbial carbohydrate metabolism decreased during the first 30 days composting of sewage sludge, but the sequences associated with oxidative phosphorylation and fatty acids synthesis were enhanced in the curing phase [11]. Lipid and lignin metabolism were relatively high in the initial period of coconut leaf vermicomposting, but the biosynthesis of secondary metabolites and plant beneficial properties were improved in the curing period [12]. Cell motility and membrane transport had tight relationship with pathogen infection on root, while the

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metabolism of carbohydrate, energy and biosynthesis of secondary metabolites may inhibit the spread of disease in soil and keep host plant healthy [13]. In addition, the PICRUSt was applied to investigate microbial metabolic function in wastewater treatment and medical field [14,15]. However, microbial behavior not only depended on the composition of microbial community but also was influenced by their metabolic activities in a particular environment. The predict results of PICRUSt tool could not comprehensively reflect the actual metabolic function of microbial community in composting system, because the microbial activities were impacted by the expression of function genes.

Biolog method could provide more information on the substrates utilization capacity of microbial community under mixed cultivation condition with larger number of replicates [16]. Biolog microplate contained environmental applicable substrates, including amines/amides, amino acids, carbohydrates, carboxylic acids, miscellaneous and polymers [17]. The biolog method was applied to assessment bacterial and fungal diversity in soil after organic amendment application. It was found that the bacterial community in soil had the highest carbon sources utilization ability after amendment with the vermicompost from household solid waste compost, followed by chicken manure and the vermicompost from horse and rabbit manure [18]. Bacterial community displayed the highest utilization capacity of β-methyl-D-glucoside and D-mannitol in thermophilic phase of cow manure composting but a steady downtrend on carboxylic acids and amino acids utilization during the incubation process [19]. Fungal communities rapidly degraded carboxylic acids and polymers at the thermophilic phase of cow manure composting, while the fungal community had stronger ability to degrade amines, amino acids and carbohydrates in the mature phase [20]. The climate change had a significant effect on microbial substrate utilization patterns. Warming improved the carbon source utilization capacity of microbial community, especially for the biodegradation of amine and carboxylic acids in soil [21]. However, the biolog method cannot provide the comprehensive information of microbial community, such as genetic information, replication and repair, translation transcription, energy metabolism, secondary metabolism, cofactors and vitamins metabolism. Therefore, the PICRUSt and Biolog could be considered as the mutual complementary methods to evaluate the function of microbial community in composting system.

The aims of this study were to investigate the succession of organics metabolic function of bacterial community in swine manure during aerobic composting process. Pumice was used as a reused inorganic bulking agent to eliminate the interference of organic bulking agents on analysis on microbial diversity and metabolism characteristics in the swine manure. Potential metabolic function and carbon sources utilization capacity of bacterial community were evaluated using PICRUSt and Biolog as the complementary methods. The relationships among environmental factors, bacterial communities and metabolic function were assessed in this system. It extended our understanding on the metabolic function of bacterial community in swine manure composting process.

2. Materials and methods

2.1. Composting process and sample collection

Fresh swine manure was collected from the livestock and poultry farm in Harbin, northeast China. Temperature, moisture, pH, the ratio of carbon and nitrogen (C/N), dissolved organic carbon (DOC), dissolved total nitrogen (DTN) and oxidation reduction potential (ORP) of the swine manure were detected during the 60 days composting process. Pumice (Hong Qiao mineral plant, China) was adopted as amendment to avoid the effect of organic bulking agents on microbial composition in swine manure composting process. The particle size and volume weight of the pumice were around 1–3 mm and 0.34–0.49 g/cm^{-3}, indicating that pumice had high porosity and low density. In addition, pumice had outstanding water-absorbing capacity, for the initial moisture and water-absorbing ability were 8% and 51–72%, respectively [22].

The initial moisture of the mixed composting materials was around 56%, for 8 kg swine manure (moisture around 76%) amended with 4 kg pumice (moisture 8%). The mixed composting materials was put into cylindrical plexiglass reactors (40 L volume, 40 cm diameter × 100 cm height), which was placed in a water bath to avoid excessive loss of heat in the 60 days composting. A temperature control system was used to regulate the temperature of the interior and exterior of reactors [23]. 1 L/min of the fresh air was continuously charged into the reactor by a pipe at the bottom to keep composting process at aerobic condition. Samples were equally collected at 9 different sites in three depths. Three samples were mixed from 3 sites of 3 levels [24]. The composting samples were subsequently used for DNA extraction and chemical and physical analyses. The extracted DNA was stored at −80 °C until use.

2.2. Chemical and physical analyses

The moisture content of swine manure samples was detected by the weight loss, which were dried at 105 °C in an oven until the later mass loss gets inferior to 0.5% of the previous mass loss. Elemental Analyzer (Vario EL, German) were used to determine TC, TN and C/N of the dried swine samples after grounding and screening through a 200 mesh screen. 10 g of swine manure samples were extracted by using 100 mL dH_2O, and then shook at 180 rpm for 10 min. The DOC and DTN concentration of aqueous extract were detected by TOC analyzer after the filtration through a 0.45 μm membrane [9]. The pH and ORP of the extraction were detected by using a pH/ORP meter (PHSJ4F, China).

2.3. Biolog EcoPlate inoculation and analyses

One gram (dry weight) of swine manure samples were suspended into 100 mL sterilized NaCl solution (0.85%, w/v), and shaken for 20 min at 20 °C. Ten-fold serial dilutions were made and the 10^{-3} dilution was used to inoculate the plates [25]. 150 μL of the diluted mixture was added to 96 plate wells by using eight channel pipettes. Subsequently, the plates were cultivated at 25 °C in darkness. The absorbance of plates was detected and recorded at 590 nm and 750 nm by using automatic microorganism identification instrument (Biolog, USA) per 12 h for 144 h [26]. Optical density (OD) value from each well was adjusted by subtracting the absorbance of the control (blank well) value. Optical density value obtained at 120 h of incubation represented the optimal range of optical density readings, so 120 h of incubation results was used for the assessment of microbial function diversity and statistical analyses [16].

2.4. DNA extraction and MiSeq sequencing

0.5 g of the samples (dry weight) was used to extract DNA by using the Power Soil DNA Isolation Kit (MO BIO Laboratories, Carlsbad, CA, USA). NanoDrop ND-1000 Spectrophotometer (Thermo Fisher Scientific, USA) was applied to detect the concentration of extracted DNA. The DNA extraction of the samples was pooled and kept at −80 °C until used. The variable region V4–5 of the 16S rRNA gene was selected for the construction of the bacterial community library for MiSeq sequencing. The process of polymerase chain reaction and the amplification products recycle were described as Hao et al. [24]. The recycled amplification products of the samples were sent to Majorbio-Biopharm Biotechnology Co., Ltd. (Shanghai, China) for Illumina MiSeq sequencing using Illumina PE 300 (Illuminna Inc., San Diego, CA).

2.5. Data and statistical analyses

Average well-color developments (AWCD), McIntosh index (U) and Shannon diversity index (H) were calculated by analysis of variance