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Effect of thermo-tolerant actinomycetes inoculation on cellulose degradation and the formation of humic substances during composting

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ABSTRACT

The inoculum containing four cellulolytic thermophilic actinomycetes was screened from compost samples, and was inoculated into co-composting during different inoculation phases. The effect of different inoculation phases on cellulose degradation, humic substances formation and the relationship between inoculation and physical–chemical parameters was determined. The results revealed that inoculation at different phases of composting improved cellulase activities, accelerated the degradation of cellulose, increased the content of humic substances and influenced the structure of actinomycetic community, but there were significant differences between different inoculation phases. Redundancy analysis showed that the different inoculation phases had different impacts on the relationship between exogenous actinobacteria and physical–chemical parameters. Therefore, based on the promoting effort of inoculation in thermophilic phase of composting for the formation of humic substances, we suggested an optimized inoculation strategy to increase the content of humic substances, alleviate CO_2 emission during composting.

1. Introduction

Recently, as the large quantity of agriculture waste is increased day by day all over the world, the disposal of agriculture waste has become a major problem, which may result in environmental pollution. Agriculture waste is an important part of organic solid wastes which have been treated by landfilling, incineration, pyrolysis, biogas processes and composting (Kulcu and Yaldiz, 2004). Composting is a complex biological decomposition process, which could transform organic matter into humus-like and stable product. The material may be used as soil conditioners or organic fertilizers (Jurado et al., 2015; Kulcu and Yaldiz, 2004). Given that microorganisms play a crucial role in all the events related to biotransformation of organic substrates during composting, inoculating exogenous microbes is usually proposed to activate the biodegradation of organic matter, shorten the composting period and improve the maturity of the composting (Jurado et al., 2015; Zhou et al., 2015).

Cellulose-rich crop residues account for 50% of the dry weight in agriculture waste, and the biodegradation of cellulose is a vital part of the carbon cycle in the biosphere (Haruta et al., 2002). Therefore, cellulose is a valuable and enormous resource for cyclic utilization. Cellulose as an unbranched homopolysaccharide is composed of β-D-glucopyranose units linked by (1–4) glycosidic bonds (Antoinette et al., 1997). Microbes can secrete several types of cellulase based on the different catalytic reactions, including carboxymethyl cellulose (CMCase), exoglucanase (such as FPase), etc. The main carbon sources in cellulosic organic material are polymers such as cellulose that are slowly decomposed (Yu et al., 2007). Substantial studies have been shown that the inoculation of exogenous microorganisms, such as bacteria and fungi, could secrete cellulases and accelerate the biodegradation of cellulose during composting (Wang et al., 2014; Zeng et al., 2010). However, few studies were focus on the role of actinomycetes inoculation in the composting process.

Actinobacteria play a key role of microbal community during composting. Although actinobacteria has a slower growth rate than fungi, actinobacteria may have more important features, for example, thermo-tolerance, adaptability to extreme environments, much more amenable to genetic modification, etc. (Budihal et al., 2016; Chang et al., 2014; Saritha et al., 2013). Meanwhile, actinobacteria could form spore to resist harsh environments at high temperature stage of composting. Moreover, the ability of actinobacteria to decompose lignocellulose into soluble carbohydrate has been confirmed in soil (Adulla and El-Shatoury, 2007). Zhao et al. (2016) reported that the inoculation of actinomycete acceler-

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ated the degradation of cellulose in composting. The study by Jurado et al. (2015) showed that inoculation of actinomycete promoted the humification process. Therefore, it is expected to explore the possibility of untilization of cellulolytic actinomycetes strains in cellulose-rich material of composting. Furthermore, prior studies revealed that various phases of inoculation during composting could cause diverse influences on the quality of composting products, especially the obvious efficiency of inoculation in the cooling stage (fermentation phase) (Zhao et al., 2016; Zeng et al., 2010). Considering the critical roles of thermophilic stage and thermophiles in the biodegradation of composting (Sarkar et al., 2010; Xi et al., 2005), it is of interest to determine the effect of thermophilic actinomycetes inoculated in the thermophilic phase.

Humic substances play an important role in carbon sequestration and can increase the fertility and stability during composting process (Zhou et al., 2014). During composting, polymeric organic matter is transformed and stabilized into humic substances by microorganisms and enzymes (Wu et al., 2017; Jurado et al., 2015). The hypotheses of humic substances formation have been disputed. However, a view is widely held that cellulose could be decomposed into some compounds by microbes, such as polysaccharides which could be the substrates for the humic products formation (Harrison, 2008). Many studies have shown that the relationship between indigenous microbes and the formation of humic substances (Wu et al., 2017; Villar et al., 2016). Previous research also indicates that the correlation between microbial diversity and lignocellulose degradation (Zhao et al., 2016; Jurado et al., 2015; Jurado et al., 2014). However, few available reports focus on the relationship between the conversion of cellulose into humic substances and microbial community in the case of inoculating exogenous microbes. Thus, it is necessary to comprehend the relationship between succession of microbial communities, cellulose degrading, humic substances formation and inoculation period during composting.

In the study, thermophilic actinomycetes strains were isolated from composts, characterized with the ability of cellulosic degradation, and subsequently inoculated into agriculture wastes composting at different stages. The aims of this study are (1) to explore the effect of inoculating exogenous actinomycetes on cellulose degradation in diverse phases, (2) to analyze the relationship of actinomycetic community structure with physical–chemical parameters, and (3) to evaluate the effect of inoculation on the humification degree in different stages of composting. The results may help to preliminarily understand the way of humic substances formation and provide a useful strategy for industrialized agriculture waste composting.

2. Materials and methods

2.1. Microbial source of inoculum

Microorganisms of inoculum was composed of four actinomycetes strains, which were screened from samples of organic wastes composting from different sources including poultry manure, agriculture waste and municipal solid waste. The greatest cellulosic enzymatic strains with tolerance to high temperature (60 °C) were chosen and they were *Streptomyces* sp. H1, *Streptomyces* sp. G1, *Streptomyces* sp. G2 and *Actinobacteria bacterium.* T9, respectively (Supplementary Table S1). The phylogenetic tree of these four actinomycetes was shown in Supplementary information (Fig. S1). They all showed the highest value of cellulose activity at 40 °C or 45 °C. The cellulose decomposition composite inoculum was the blend of these four actinomycetes at the proportion of 1:1:1:1, which was grown at 45 °C for 5 days.

2.2. Raw material preparation and composting process

The raw materials for composting were corn straw and dairy manure which were collected from Xiangfang farm in Heilongjiang province. Corn straw belonging to a kind of recalcitrant organic materials was chopped into 20–30 mm as bulking agent.

Composting experiments were carried out in experimental compost reactors which have been described previously by Zhao et al. (2016). The analogous experimental reactor was widely used in substantial studies and easier to control, and all treatments could have multiple repetitions at the same time (Zhao et al., 2016; Wang et al., 2015; Zhou et al., 2015; Zeng et al., 2010). The changes of reactor temperature see Supplementary information (Fig. S2). The descriptions of composting materials are provided in Supplementary Table S2 of the Supporting Information (SI). Approximately 8 kg fresh weight of mixture of material was composted in the reactor. The proportion of the dairy manure and corn straw in the mixtures was 2:1. The ratio of moisture content was maintained at 60%, and C/N ratio was approximately 30:1. To supply aeration, each pile was turned twice a week in the first week and once a week later.

One control and four other treatments were set up in three stages composting process. No microbes were inoculated during composting in the control group (CK) and microorganisms were inoculated in Run 1, Run 2, Run 3 and Run 4. All of the treatments were replicated three times. Compound actinobacteria agent was inoculated in the initial stage (day 0) at 35 °C as Run1. The second group was inoculated in high temperature stage (day 6) at 55 °C as Run2. The third group was inoculated in the cooling stage (day 15) at 45 °C as Run3. The fourth group was inoculated in all day 0, 6, 15 as Run4. For every group, each of inoculant was at the level of 2% in dry weight. The concentration of inoculant in the piles was 10^9 CFU mL^{-1}. The control piles received the same amount of distilled water. The whole time of composting in this study is 60 days for each group. Approximately 80 g of samples in each pile was taken from various stages of composting on day 0, 4, 10, 17, 24, 30, 40, 50 and 60. A part of experimental samples are used for physical–chemical analysis and others are stored at -20 °C until further analyses.

2.3. Cellulase enzymes assay

Cellulase enzymes activities were determined using with fresh samples. Cellulase activities (CMCase, FPase) were assayed with glucose as the standard. FPase was determined using the method described by Lan et al. (2013) with Whatman No. 1 filter paper as substrate. The reducing sugar released was determined by the DNS method (Miller, 1959). The CMCase activity was measured following the method of Nadia et al. (2015) and the solution of sodium carboxy-methylcellulose was used as substrate. The activity of CMCase was determined by measuring the reducing sugar according to the DNS methods of Miller (1959). One unit of cellulose enzymes activity was defined as the enzyme required to produce 1U of glucose per g of sample per min (Nadia et al., 2015).

2.4. Physicochemical analyses

Compost samples were air-dried and ground for physicochemical analyses. Organic matter (OM) was measured by dry combustion and the samples were put into muffle furnace at 550 °C for 6 h. The remaining ash was weighed and used to determine the carbon content of the samples. Total nitrogen (TN) was measured by the Kjeldahl method (Bremner, 1965).

The cellulose and lignin content were estimated by Van Soest's method (Van Soest et al., 1991). The difference between acid detergent fibre (ADF) and acid detergent lignin (ADL) was the content of

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