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The Effect of Bacteria on the Formation and Polymerization of Humic Substance Precursors During Different Composting Periods

Junqiu Wu[1], Yingying Ma[1], Haishi Qi[1], Xinyu Zhao[2], Xu Zhang[1], Xinyu Xie[1], Yingqiu Du[3], Yue Zhao[1*] and Zimin Wei[1*]

[1]College of Life Science, Northeast Agricultural University, Harbin 150030, P.R. China
[2]State key laboratory of environment Criteria and Risk Assessment, Chinese Research Academy of Environmental Sciences, Beijing 100012, P.R. China
[3]Agricultural Products Quality and Safety Research Institute, Heilongjiang Academy of Agricultural Sciences, Harbin 150086, P.R. China

Research Article

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***For Correspondence**

Yue Zhao, College of Life Science, Northeast Agricultural University, Harbin, P.R. China. Tel: +86 45155190413.

E-mail: zhao1970yue@163.com

Zimin Wei, College of Life Science, Northeast Agricultural University, Harbin, P.R. China.

E-mail: weizimin@neau.edu.cn

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ABSTRACT

The aim of this work was to detect the formation and polymerization of humic substance (HS) precursors and put forward a staging regulating method to improve HS formation during different materials composting. Five materials composting of lawn waste, cabbage waste, corn straw, garden waste and chicken manure were conducted in this study. During composting, the HS precursors, including the polyphenols, carboxyl, amino acids, and polysaccharides and reducing sugars, conducted varied formation rules in the heating phase, thermophilic phase, cooling phase and maturity phase of composting. The dynamic of precursor's concentration and bacteria communities have been divided into five and three groups, respectively, by the non-metric multidimensional analysis, during different materials composting. Given that, a staging regulating method was proposed to promote HS amount during different materials composting.

INTRODUCTION

Humic substance (HS) as one of the most complex compounds in the composting has very important effect on improving soil fertility, complexing heavy metal and other environment remediation [1-3]. During composting, organic matter is decomposed and transformed by microorganisms to form HS precursors [4]. These precursors are polymerized in various ways, including lignin-protein pathways, polyphenols pathways and maillard reactions, to form HS [5,6]. The polyphenols, carboxyl, amino acids, reducing sugars and polysaccharide are regarded as the typical precursors to synthetize HS [7].

The formation of HS precursors can be divided into two aspects: decomposition and synthesis [6]. The microorganism can release the precursors when the organic matter is degraded to satisfy its growth. As the growth of microorganisms, they began to synthesize precursors by the shikimic acid and malonic acid pathway. The precursors are polymerized under the action of enzymes to form HS. Therefore, the formation of precursors is inseparable from the action of microorganisms. Composting process is facilitated by microorganisms through community succession and abundance changes [8,9]. At the beginning of composting, microbes drastically degrade organic matter to meet their metabolism and production, resulting in the intensely increased temperature. This process may lead to the accumulation of small molecular precursors. Furthermore, the microbial community structure will change with the heating temperature. At the thermophilic stage, composting material mainly dominant by thermophilic microorganisms to degrade recalcitrant materials [10]. Then, the composting temperature gradually goes down due to the microbial activity slows down and the organic matter stabilizes [11]. The rapid community succession is the main reason for precursor's formation. In addition, the bacteria are very important microbial populations that exist throughout the composting period [9]. Meanwhile, the key bacteria communities that influence the formation and polymerization of HS precursors have been investigated by Wu et al. [7]. However, it is obscure about the specific period of the precursor's formation and polymerization during different materials composting.

Among the microorganisms, the bacteria maintain the most abundant species and metabolically active [6]. Therefore, this paper mainly studies the effect of bacteria on the formation and polymerization of HS precursors during different materials composting. In this article, the effects of bacteria derived from different composting periods on the formation and polymerization of HS precursors are investigated based on the previous study of Wu et al. [7]. The differences among various materials composting have also been explored. Accordingly, this research provides a staging regulation method to promote HS formation, which will applicable to various types of composting materials.

MATERIALS AND METHODS

Composting Test

Five solid piles of cabbage waste (CW), lawn waste (LW), chicken manure (CM), garden waste (GW) and corn straw (CS) were conducted in the compost reactor and the changes in temperature of the composting reactor were proposed by Zhao et al. [12]. The basic properties of the raw materials have been reported by Wu et al. [7]. Before composting, the initial C/N ratio and moisture were adjusted to 30 and 60%, respectively, and maintain the moisture at around 60% [13]. The rate of 0.5 ml/min was chosen to compulsory ventilation. The composts were conducted for 50 days and samples were collected at day 0, 8, 20 and 32, which were regarded as the samples of heating phase (HP), thermophilic phase (TP), cooling phase (CP) and maturity phase (MP) to analysis bacteria communities. The temperature of the four phases were 35°C, 57°C, 43°C and 40°C, respectively.

DNA Extraction and PCR-DGGE Analysis

The total DNA extraction was conducted on the DNA kit (Omega Biotek, Inc), agarose gel electrophoresis was used to check DNA quality. For PCR-DGGE analysis, the prokaryotic 341F/534R was applied to amplify bacteria DNA **(Table 1)**. The system setting and method of polymerase chain reaction (PCR) were according to the proposal of Wei et al. [14] and the denaturing gradient gel electrophoresis (DGGE) followed the description of Zhao et al. [12].

Table 1. Primers used for actinobacteria PCR amplification.

Primer	Primer sequence (5'-3')
243-F	GGATGAGCCCGCGGCCTA
534-R	CCGCGGCTGCTGGCACGTA
GC-clamp	CGCCCGCCGCGCCCCGCGCCCGGCCCGCCGCCCCCGCCCC

Precursors and Humic Substance Analysis

HS precursors include polyphenols, carboxyl, amino acids, reducing sugars and polysaccharides were extracted and analysis in this study. Details of analytical methods have been reported in previous studies on HS formation aspects in Wu et al. [7]. Briefly, polyphenols were extracted according to the method of Baddi et al. [15]. Analysis of polyphenols was carried out on Agilent 1260, fitted with Agilent Zorbax Eclipse plus C18 reverse-phase column (250 mm × 4.6 mm) and detected with a UV diode-array detector (Agilent 1260) set at 280 nm. Carboxyl concentration was detected by the method of titration [4]. The amino acids, reducing sugar and polysaccharide concentration were detected by ninhydrin color liquid, anthrone reagent and dinitro-salicylate reagent, respectively [16].

HAs were extracted following the procedure of Zhou et al. [17]. The HAs concentration was analyzed by shimadzu TOC-VCPH, Japan.

Data Analysis

Data processing was performed using Quantity one software (version 4.5, Bio-Rad laboratories, USA) and Canoco for Windows (Version 5.0). The significant level of differences in the research was set as $p<0.05$.

RESULTS AND DISCUSSION

The Effect of Bacteria on the Formation of Humid Substance

Redundancy analyses (RDA) was employed to study the formation and polymerization rules of precursors under the effect of bacteria. **Figure 1** shows the relationships among the precursors, HS and bacteria communities derived from different composting periods. Every canonical axis is significantly correlated with the bacteria communities derived from different period of composting ($p<0.05$). This result illustrates that the bacteria communities are very important in explaining the formation of HS precursors at different composting periods [14]. Especially the bacteria derived from the HP and TP can promote the formation of different kinds of precursors. The bacteria diversity is most abundant at this stage [7], and will help confer to the formation of

HS precursors. López-González et al. reported that once the bio-oxidative stage is over, the rest of precursors derived from the bacteria degradation will polymerized to form HS [18]. Specifically, reducing sugars and polysaccharides positively correlate with species derived from LW1, LW4, CW2 and CW3. LW2, CM2, CS1 and CM4 also significantly promote the formation of reducing sugars and polysaccharides. Polyphenols and carboxyl are mainly promoted by communities in LW2, CM4, CM2, CS1, LW3 and GW1. Bacteria communities in HP and TP increase precursors concentration by degrading organic matter, but the species in CP and MP may promote polyphenols and carboxyl formation by biosynthetic pathway. This effect may be attributed to the dominant species in those periods. Nevertheless, the rest of the bacteria communities may also significantly promote the synthesis of other precursors and HS polymerization. The amino acids concentration was mainly increased by the bacteria species in CM1 and CM3. That may be due to that the protein is the primary component in CM, which is degraded as carbon and energy source for microbe metabolize [19]. As the composting process progressed, the species in the CP and MP move to HS, which indicate that these periods' populations can promote the synthesis of HS. However, the communities derived from GW2 and CS2 can also enhance the HS formation. This result demonstrates that the HS formation exists throughout the composting process. Due to the difficult degradation of CS and GW, the effect of bacteria on promoting precursor formation is not obvious. In general, precursor's formation is concentrated in HP and TP of composting and HS polymerization in CP and MP, which consisted with the dynamic of precursors and HS concentration [7].

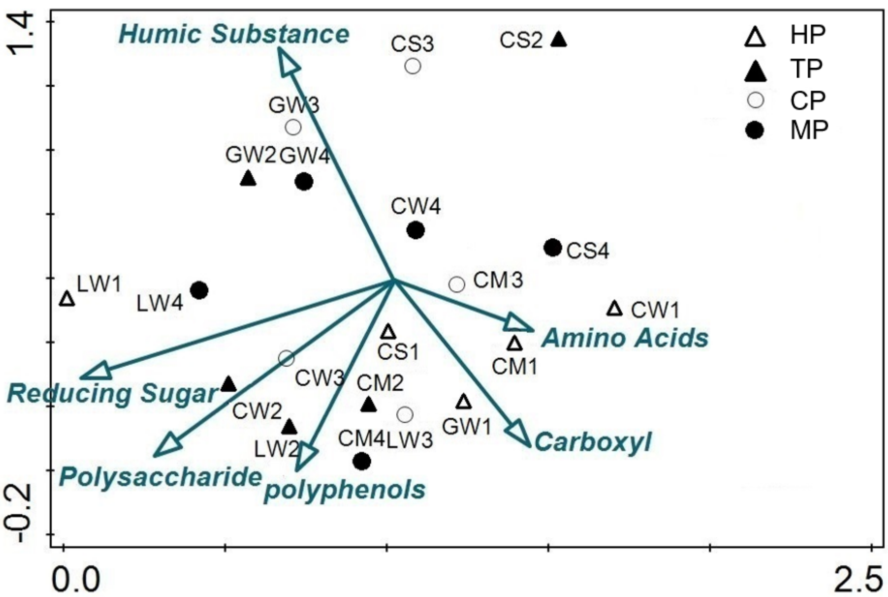

Figure 1. Redundancy analyses of the formation and polymerization of humic substance and precursors (LW1: bacteria communities in HP; LW2: bacteria communities in TP; LW3: bacteria communities in CP; LW4: bacteria communities in MP; the bacteria communities in other materials is marked in the same way).

The Differences among Various Composting Materials

The non-metric multidimensional (NMDS) was utilized to detect the difference and similarity of the changes of precursor's concentration and bacteria species information during different materials composting. It is of great significance to explore the formation of HS in different materials composting. The NMDS of precursors concentration shows that the five materials are divided into five groups **(Figure 2)**, this may be due to the different properties of the raw materials. The results in **Figure 1** illustrate that the bacteria in LW composting mainly produce reducing sugars and polyphenols. That may be caused by the large amount of easily decomposed cellulose in LW, which provide adequate substrate for the production of polysaccharides and polyphenols during composting. The cellulose is also the main structural substance of CW and CS; however, they are not classified as the same group with LW. During the composting process, the polysaccharides, polyphenols and carboxyl are mainly produced by CW and CS, which was similar to LW. The main structure of GW is lignin, which is difficult to be degraded by microorganisms during composting [19]. Therefore, during the GW composting, protein and any other simple organic matters primarily decomposed by microbe. Nevertheless, the lignin degradation may mainly conduct in the later stage of composting combining with the HS formation. Whereas in CM composting, the amino acids are the main precursors of HS, because protein is the main structure of CM. Besides, the bacteria communities in varied composting periods were different. The bacteria information in the five composting materials was mainly be divided into three categories: 1) Bacteria in HP; 2) Bacteria in HP and CP, 3) Bacteria in MP. This result demonstrates that the bacteria in the same period of different materials perform similar functions. But there are also some functional bacteria communities which play key roles in the formation of precursors [7]. In conclusion, the formation and polymerization of HS precursors can be regulated by staging during different materials composting. The key bacteria communities related to the evolution of HS precursors had been discussed in previous study of Wu et al. [7]. Therefore, it can be combined with this study to provide a new staging regulating method.

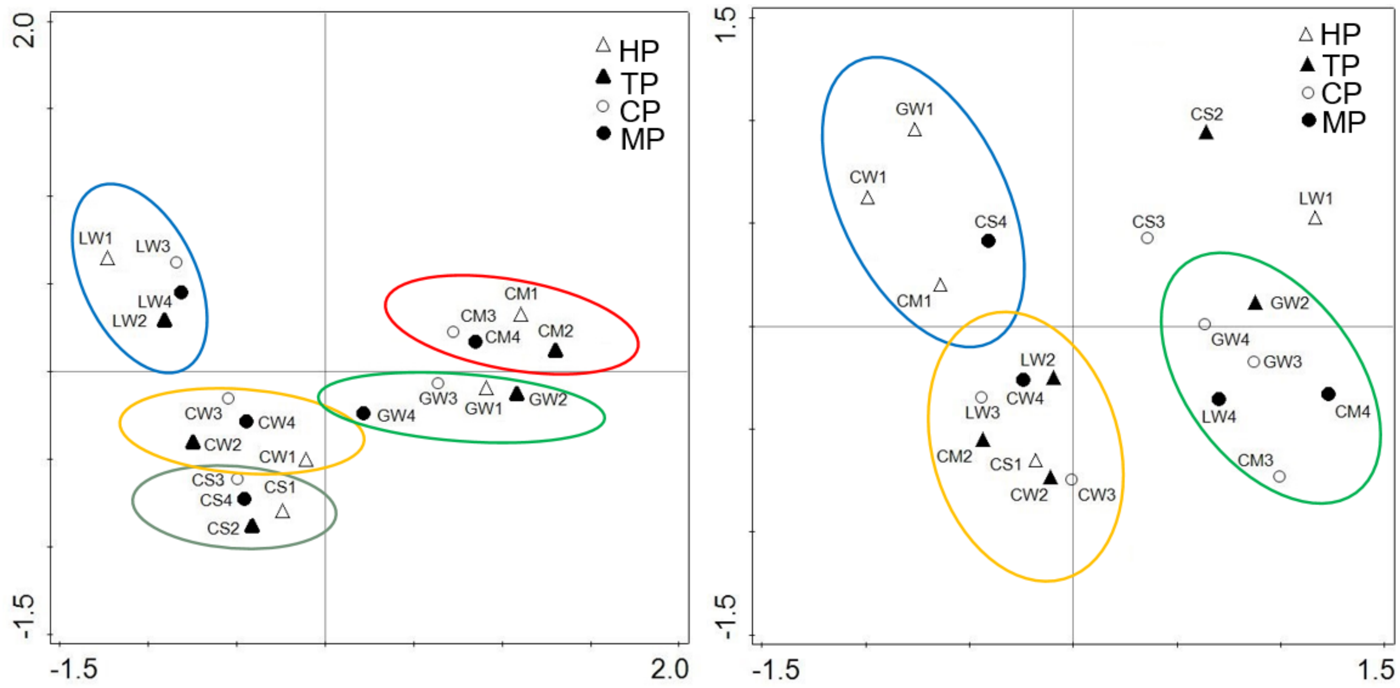

Figure 2. The non-metric multidimensional of a: precursors concentration and b: bacteria communities during different materials composting (LW1: precursors concentration and bacteria communities in HP; LW2: precursors concentration bacteria communities in TP; LW3: precursors concentration bacteria communities in CP; LW4: precursors concentration bacteria communities in MP; precursors concentration the bacteria communities in other materials is marked in the same way). Ellipses demonstrate the groups of different materials.

A new staging regulating method to promote the formation of HS precursors

There is no doubt that HS and its fractions have very important effect in environmental remediation [20,21]. As large of efforts availably improved HS production by adding lignin, biochar and any other waste materials, their fundamental theory is to provide precursors for HS formation [17,22,23]. However, little research has been done to promote the production of HS precursors by regulating key bacteria activity during different materials composting. Given that, we focused on the relationships between bacteria communities and precursors, especially the formation and polymerization rules of the precursors in different composting periods under the effect of key bacteria. According to **Figure 2**, the dynamics of precursor's concentration are various in different materials composting. Therefore, based on the previous study of Wu et al. [7], we provide a staging regulating method to enhance the formation and polymerization of HS precursors during different materials composting. For example, **Figure 3** shows the staging regulating method of LW for promoting the production of precursors to formation HS. The relationships between the environment factors, bacteria and precursors are positive in **Figure 3**. In the HP of LW composting, the activities of band 7 and 19 can be improved by adding NH_4^+-N, which lead to the accumulation of reducing sugar. The increased reducing sugar concentration can not only satisfy the growth of microorganisms, but also provide substrate for microbial synthesis of HS precursors. In addition, the band 7 and 19 can also promote the formation of polyphenols and reducing sugars in TP of LW composting. Accordingly, in the first two stages of LW composting, the NH_4^+-N concentration can be increased by the ammonia containing wastewater, CM and even the ammonia gas obtained from composting gas recovery. It not only benefits for the waste disposal, but also reduces the toxic gas pollution. During the CP of LW composting, the key bacteria communities have evolved to band 9, 14 and 19, which mainly promote the formation of polyphenols, carboxyl, amino acids and HS. Band 19 still promotes the production of reducing sugars, but other precursors are more dominant. In this period, the precursors can be promoted by increasing the NO_3^--N concentration, pH or C/N. Among them, raising the compost C/N is the easiest to achieve. Therefore, the crop waste may be one of the best choices. Especially in China, as a large agricultural country, a large number of agricultural straw need reasonable treatment. In the MP of LW composting, the key bacteria are just as before in addition to band 9, which activity can also be enhanced by NO_3^--N concentration, pH or C/N in MP. Nevertheless, due to the decline in microbial activity and diversity in the MP of composting, the easily decreased materials may be a good choice to improve the C/N, for instance the fruit and vegetable or sugar-moll wastes. In this way, the wastes can be reasonably disposed and the emission of waste gas can also be decreased.

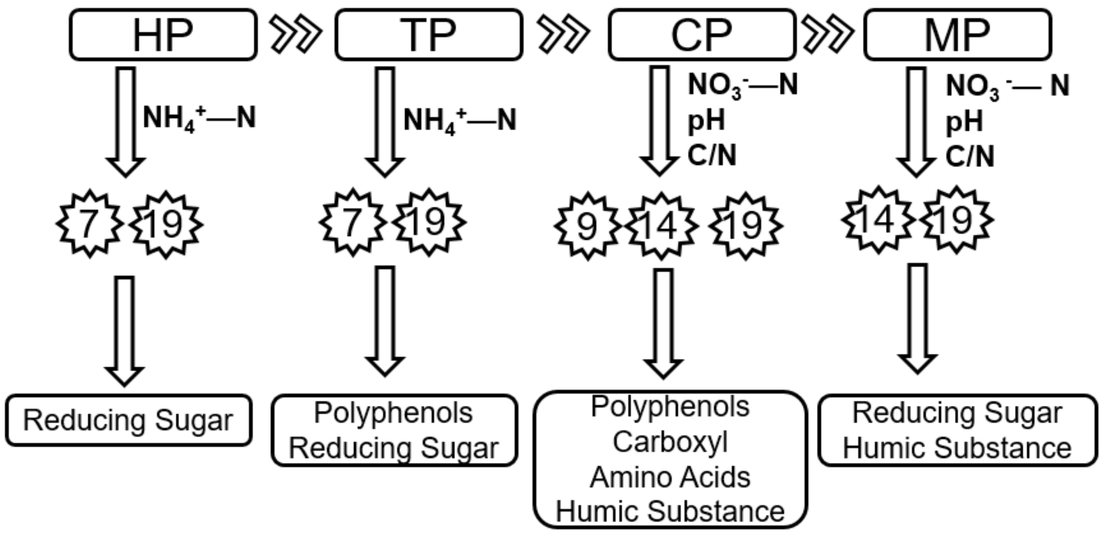

Figure 3. A staging regulating method for promoting the formation and polymerization of HS precursors was proposed by controlling the environmental factors and bacteria communities during LW composting. The twelve pointed star and rectangle meant different key bacteria communities and precursors and HS. The key bacteria communities could be affected by different physical-chemical parameters. In addition, the key bacteria communities positively affected the precursors and HS, which indicated by the arrows.

CONCLUSION

This study illustrates that the HS is formed throughout the period of composting, whereas the precursors are mainly formed in the HP and TP. The results of NMDS show that the dynamics of precursor's concentration in various materials composting are different from each other, and the bacteria communities are divided into three groups based on the different periods of composting: 1) Bacteria in HP; 2) Bacteria in HP and CP and 3) Bacteria in MP, respectively. Finally, a staging regulating method is proposed to promote the HS amount according to different materials.

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