Inoculating exogenous bacterium Brevibacillus laterosporus ZR-11 in compost-maturing period could accelerate composting maturation by regulating physicochemical parameters and bacterial community succession

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Abstract

In this experiment, *Brevibacillus laterosporus* ZR-11, a bio-control strain with resistance to Fusarium wilt in cucumber, was inoculated in the mature manure prepared for maturing composting; conventional detection and Illumina Miseq high-throughput sequencing were adopted to investigate the changes of physicochemical factors and microbial communities in the composting process; correlation analysis was also conducted, with the purpose of providing some reference for improving the production technology of biological manure and upgrading the quality of products. According to experimental results, in the maturing period, bacterial community structures underwent substantial evolutions in both the inoculated group and the non-inoculated group. Microbial diversity analysis revealed that Firmicutes, Proteobacteria, and Actinobacteria were dominant bacterial phyla in both the inoculated group and the non-inoculated group in different periods, but their relative abundances were higher in the former. Through analyzing physicochemical indicators, it was found that ZR-11 could increase the temperature of maturing composting pile, accelerate compost maturation, and enhance the abundance of bacterial communities in the pile. The results of this study provided some reference for improving the maturing ING composting production technology of biological manure and upgrading the quality of products.

Introduction

In China, the rapid development of the livestock and poultry industry has increased the generation of animal wastes. Each year China produces 3.8 billion tons of livestock and poultry wastes in total, about 40% of which has caused serious environmental pollution and substantial loss of nutritional resources due to long-term piling and poor use (Bluemling & Wang, 2018; Wu et al., 2018). In addition, when animal wastes are directly applied in soil without harmless treatment and maturation, they not only cause groundwater pollution and introduce pathogens, animal-derived antibiotics, and hormones, but also may generate phytotoxic substances, affect plant growth, or even threaten human health (Pan & Chu, 2017; Van Epps & Blaney, 2016; Zhang et al., 2016). In this context, composting technology can be used to effectively realize the harmless treatment of livestock and poultry wastes, and the product of their harmless treatment, i.e., the mature manure, can be used in combination with biocontrol strains to prepare disease-resistant biological manure. This approach of combined use, in addition to solving the problems of instable effect and non-persistent action posed by the pure use of biocontrol strains, also helps to turn agricultural wastes into resources and alleviate their environmental pollution (Hao et al., 2019; Ventorino et al., 2016; Vida et al., 2017).

Biological manure, as a novel type of microbial fertilizers, has been extensively applied in agricultural production. As pointed out in many studies, when applied in crops, biological manure creates an inhibitory effect against indigenous pathogens that exceeds the inhibitory effect of ordinary microbial fertilizers, and the growth-promoting effect of biological manure is also superior to that of ordinary manure (Huang et al., 2011; Yuan et al., 2014; Yuan et al., 2016; Zhao et al., 2014). Clearly, biological manure has dual effects in agricultural production, which, to a large extent, depend on the properties of functional microbes. The key to preparing biological manure lies in inoculating functional bacteria with
nitrogen-fixing, phosphate-dissolving, potassium-dissolving, and disease-resistant effects in the mature manure (Bhardwaj et al., 2014; Han et al., 2016; Ma et al., 2018; Wang et al., 2013). However, the current practice is to inoculate functional bacteria in the late maturing period of manure, and the possibility of inoculating functional bacteria in the maturing period to prepare biological manure has not been explored by existing studies.

In the maturing period, with the decline of compost temperature and the basic maturation of organic matter, adding functional bacteria to prepare biological manure can effectively weaken the effect of indigenous miscellaneous bacteria on functional bacteria (Scheuerell & Mahaffee, 2005). According to some studies, by adding the complex bacterial community of N, P, and K into the materials at a reduced composting temperature and controlling the technological conditions of composting for massive reproduction, it is possible to not only achieve a high microbial count and a rational population structure, but also further enhance compost maturity (Wu et al., 2005). Therefore, clarifying whether the functional inoculant can colonize during compost maturation and its effects on the dynamics of microbial community structures and the physicochemical factors of compost products is of vital theoretical and practical guiding significance for regulating the production technology of biological manure and upgrading the quality of products.

Thus, in this experiment, ZR-11, a biocontrol strain with resistance to Fusarium wilt in cucumber, was inoculated in the compost maturation phase for maturing composting; conventional detection and Illumina Miseq high-throughput sequencing were adopted to investigate the changes of physicochemical factors and microbial communities in the composting process; correlation analysis was also conducted, with the purpose of providing some reference for improving the production technology of biological manure and upgrading the quality of products.

**Materials and Methods**

**Maturing manure**

In this study, the raw material source and composting site of the mature manure were both located in the Doudian Manure Factory of Beijing Hengsheng Fengnong Biotechnology Co., Ltd. During preparation, cattle manure, chicken manure, and mushroom residue were mixed by a ratio of 6:5:5 (C/N ratio = 30, moisture content = 65.77%), and the aerobic trough composting mode was adopted for composting. The maturing manure was composed of 29.41% of organic matter, 0.93% mg/kg of total N, 2.35 mg/kg of total P, 1.14 mg/kg of total K, and 59.12% of moisture content (pH = 8.7, maturity = 70.93%).

**Preparation of Brevibacillus laterosporus ZR-11**

*Brevibacillus laterosporus* ZR-11, the test strain, was acquired through isolating and screening in the laboratory from the compost samples of early raw materials in low-temperature, medium-temperature, and high-temperature period. ZR-11 is a member of Bacillus, and many existing studies show that it is a dominant bacterium in the high-temperature phase of composting and can serve as a functional
inoculant for improving the quality of composting (Kim et al., 2006; Sung et al., 2002). According to our preliminary experiments, ZR-11 has a significant antagonistic effect against four plant pathogens, i.e., *Fusarium proliferatum, Fusarium proliferatum Sheld, Fusarium proliferatum*, and *Fusarium oxysporum* (unpublished data).

In the experiment, preserved *Brevibacillus laterosporus* ZR-11 was activated for 24 h on an LB solid medium plate, and single colonies were picked, inoculated in 20 mL LB liquid medium, and shake-cultured for 24 h (37°C, 140 r/min) to prepare the seed solution. After that, it was inoculated in a 250 mL conical flask holding 100 mL LB liquid medium at the rate of 1%, followed by 24 h of shake culturing (37°C, 200 r/min) to prepare the culture solution of *Brevibacillus laterosporus* ZR-11. The culture solution was then diluted with sterile water until reaching a concentration of 1×10⁷ CFU/mL, followed by 10 min of centrifugation at 8,000 r/min. Finally, an equal amount of sterile water was used for bacterial re-suspension, and ZR-11 was inoculated in the mature manure at the rate of 0.5% (V/W).

**Maturing composting**

After taking 50 kg mature manure, the experiment designed two groups, i.e., the group inoculated with *Brevibacillus laterosporus* ZR-11 and the non-inoculated group. After uniform mixing, they were loaded into a 100 L small-sized aerobic high-temperature composting simulation installation; the pile was 0.6 m at height. The installation had an aeration time of 30 min/2h, an aeration rate of 4 L/min, from 22 July 2019 to 30 July 2019 (7 d). In the entire maturing composting process, samples were collected on D0, D1, D3, D6, and D8, respectively. An equal amount of samples were collected from the surface layer, center, and bottom layer of the pile in the fermenter, respectively. The samples collected were then mixed, and split several times by means of quartering (1.2 kg in total). After that, they were divided into three portions, one preserved in a refrigerator at 4°C, one refrigerated at -80°C, and the third crushed for testing after natural drying.

**Determination of physicochemical parameters**

A DTSW-2 digital electronic thermometer (Tai’an Detu Automation Instrument Co., Ltd.) was used to measure the temperatures at the top, middle, and bottom of the pile. An SH1 0A moisture meter (Shanghai Jinghai Instrument Co., Ltd.) was adopted to measure the moisture content of compost samples. After fresh samples and distilled water were mixed by a ratio of 1:10 (mass concentration), a PB-10 pH meter (Sartorius, Germany) was employed to measure the pH of compost samples. After sample grinding and sieving (40-mesh sieve), TOC was measured using the potassium dichromate oxidation spectrophotometric method in accordance with the *Soil Determination of organic carbon-Potassium dichromate oxidation spectrophotometric method* (HJ 615–2011); total N content was measured using the Kjeldahl method in accordance with the *Solid wastes-Determination of volatile halohydrocarbons- Headspace gas chromatography mass method* (HJ 714–2014); total P content and total K content were measured using vanadium-ammonium molybdate colorimetry and flame photometry respectively in accordance with the *Organic fertilizers* (NY 525–2012). A TOC analyzer (MultiN/C3100,
Jena, Germany) was used to measure the organic matter content of compost samples. Seed germination index was measured using the method proposed by Sun (Sun et al., 2016).

**DNA extraction and 16s rDNA high-throughput sequencing**

After quick-freezing about 0.4 g fertilizer sample with liquid nitrogen and grinding it, a soil DNA kit (E.Z.N.A. ® Soil DNA Kit, Omega Bio-tek, Norcross, GA, USA) was used for DNA extraction. A Nanodrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) was used to detect DNA concentration and purity. The PCR amplification of V3-V4 regions was performed using bacterial 16s rDNA primer pair 319F (5’-ACTCCTACGGGAGGCAGCAG-3’) and 806R (5’-GGACTACHVGGGTWTCTAAT-3’) (Fadrosh et al., 2014). Amplicon sequencing was conducted on an Illumina MiSeq PE300 platform.

**Sequencing data analysis**

The quality control of original data was carried out using FASTX-Toolkit v0.0.13 software (http://hannonlab.cshl.edu/fastx_toolkit/), and the proportion of high-quality bases (Q value ≥ 30) was ≥ 90% of reads. Based on the overlap between PE reads after quality control, PE reads were assembled using Flash v1.2.11 software (Magoc & Salzberg, 2011). QIIME v1.9.1 software (Caporaso et al., 2010) was adopted for processing, and VSEARCH v2.14.1 software (Rognes et al., 2016) was used for detecting chimera sequences. Based on a sequence similarity level of 97%, the Uclust method in QIIME software package was employed to perform OTU clustering analysis. On the basis of Silva reference database (Release138), taxonomic annotations were made for the OTUs of each sample. The Shannon, Simpson, Chao1, and ace indices of microbial communities in the pile were calculated, respectively. LEfSe software (https://bitbucket.org/nsegata/lefse/src/default/) was used to estimate the abundance differences among microbial species in pile samples.

Redundancy analysis (RDA) was performed using CANOCO v 5.02 software (Šmilauer & Lepš, 2014), with the purpose of investigating the correlations of the microbes in samples with both samples and environmental physicochemical factors. The BioEnv program in R v 3.6.0 software package was used to identify the physicochemical factors having significant effects on community changes; after that, Vegan program package (Dixon, 2003) was employed to perform variance partitioning analysis (VPA), thus identifying the factors driving community changes. STAMP v2.1.3 software was adopted to compare the microbes showing significant differences in relative abundance in different samples (Parks et al., 2014).

**Real-time fluorescence quantitative PCR (qPCR) analysis**

To verify the colonization status of the inoculant in the secondary composting phase, qPCR analysis was adopted. Both qPCR reaction primers and experimental system referred to the method of Marche MG et al. (Marche et al., 2019). Each group was prepared with three sample repetitions, and each sample with three technical repetitions. On the platform of Applied Biosystems 7500 Fast Real-Time PCR System, TB Green Premix Ex Taq (Takara) was used for PCR reactions. PCR amplification conditions: 10 min of pre-degeneration at 95°C, followed by 15 s of degeneration at 95°C, 1 min of annealing at 60°C, a total of 40 reaction cycles, and finally 1 min of extension at 60°C.
Statistical analysis

Statistical analysis was performed using GraphPad prism 8 software (GraphPad 8, San Diego, CA, USA); analysis of variance was conducted based on ANOVA; significance of difference analysis was carried out according to the Duncan method (p value < 0.05); correlation analysis followed the Spearman method.

Results

Analysis on physicochemical indicators of composting materials

On the whole, the temperatures of the two piles presented similar dynamic change trends, that is, they first rose and then declined. Relative to the non-inoculated pile, the inoculated pile saw a fast and intense temperature rise, and was heated on the third day to 52°C, 19°C above initial temperature; in contrast, the non-inoculated pile experienced a slow and mild temperature rise, with a maximum rise of 5.5°C only (Fig. 1A).

The moisture contents of the two piles both presented a declining trend in the maturing composting process (Fig. 1B). On D1 of maturing composting, the moisture contents of the inoculated group and the non-inoculated group were 52.16% and 56.19%, respectively; at the end of maturing composting, they declined to 47.63% and 51.13%, reductions of 4.53% and 5.06%, respectively. Meanwhile, the pH values of the two piles declined from 8.7 at the beginning to about 7.20 at the end of maturing composting (Fig. 1C). The pH value of the inoculated pile was slightly lower than that of the non-inoculated pile.

The organic matter contents of the two piles both presented a gradually declining trend in the maturing composting period. To be specific, the organic matter contents of the inoculated group and the non-inoculated group declined from 29.41% at the beginning to 24.21% and 23.51% at the end of maturing composting, reductions of 5.2% and 5.9%, respectively (Fig. 1D). With the progress of compost maturation, the C/N ratios of the two piles both presented a declining trend; in particular, the C/N ratio of the inoculated group declined more dramatically, and, at the end of maturing composting, the C/N ratio of the inoculated group (19.74) was obviously lower than that of the non-inoculated group (22.65) (Fig. 1E).

In the maturing period, the GI indices of the two piles presented a gradually rising trend. The GI index of the inoculated group rose quickly, and reached 91.56% at the end of maturing composting; in contrast, the GI index of the non-inoculated pile rose slowly, and only reached 88.94% at the end of maturing composting (Fig. 1F). Clearly, in the maturing composting process, the GI index of the inoculated pile was significantly higher than that of the non-inoculated group.

The total N and TOC contents of the inoculated group and the non-inoculated group uniformly presented a declining trend; in contrast, their total P and total K contents experienced no significant change on the whole in the maturing composting process.

Analysis on the diversity of microbial communities
High-throughput sequencing acquired a total of 403,219 assembled sequences, and each sample contained 57,115 – 76,575 sequences; a total of 25,695 OTUs were generated at the sequence similarity level of 97% (Table 1). OTUs number that plotted versus sequencing number (depth) had leveled off to reach a plateau when > 50,000 reads were sequenced. Thus, sequencing number here was sufficient to cover overall composition for each sample, and the obtained information was reliable for downstream analyses.

Table 1
Analysis on the sequences of High-throughput sequencing in different samples of composting materials

<table>
<thead>
<tr>
<th>Sample</th>
<th>Day</th>
<th>Sequences</th>
<th>Average Length(bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inoculated group</td>
<td>0</td>
<td>64004</td>
<td>477</td>
</tr>
<tr>
<td>Non-inoculated group</td>
<td>0</td>
<td>59117</td>
<td>479</td>
</tr>
<tr>
<td>Inoculated group</td>
<td>3</td>
<td>72643</td>
<td>478</td>
</tr>
<tr>
<td>Non-inoculated group</td>
<td>3</td>
<td>57115</td>
<td>480</td>
</tr>
<tr>
<td>Inoculated group</td>
<td>6</td>
<td>76575</td>
<td>475</td>
</tr>
<tr>
<td>Non-inoculated group</td>
<td>6</td>
<td>73765</td>
<td>474</td>
</tr>
</tbody>
</table>

Figure 2A and 2B provide the Alpha diversity indices of microbial communities in various samples. As can be known from Chao1 and ACE, relative to the initial point of composting, on D3 of maturing composting, the abundances of bacterial communities declined significantly in both piles; later they remained relatively stable, and did not experience any dramatic change.

According to Shannon index, the diversities of bacterial communities in the two piles both presented a trend of declining first and rising afterwards in the maturing composting process. Compared to original materials, the diversities of bacterial communities in the two piles were much lower. On D3 of maturing composting, the diversity of bacterial community in the non-inoculated pile reached the lowest level; on D6 of composting, it rose significantly. In the late phase of maturing composting, there was no significant difference between the two piles in the diversity of bacterial communities (Fig. 2C).

Simpson index showed that there was no significant difference between the two piles in bacterial species diversity in the maturing composting process, except the significant rise of bacterial species diversity in the non-inoculated pile on D3 of maturing composting. This was possibly caused by the particularities of individual samples in the pile (Fig. 2D).

Figure 3 provides the results of comparison in terms of the diversity of microbial communities based on PCoA. The contribution rate of the two axes PCoA1 and PCoA2 to the difference in bacterial community changes was 47.59%, and that of the first axis PCoA1 was 34.12%. On PCoA1, the bacterial communities in the original piles could be satisfactorily distinguished from those in pile samples in the composting process. Meanwhile, the bacterial communities in the inoculated pile could also be satisfactorily
distinguished from those in the non-inoculated pile, suggesting that the introduction of maturing composting and exogenous bacteria exerted significant effects on original bacterial communities.

**Analysis on microbial community structure**

Figure 4A shows the bacterial community composition at phylum level in different pile samples. Clearly, in the maturing period there was no obvious difference between the two piles in bacterial community composition at phylum level. To be specific, in the composting period, the relative abundance of Firmicutes presented a trend of increasing first and decreasing afterwards in the two piles; the relative abundances of Proteobacteria and Bacteroidetes presented a trend of declining first and rising afterwards; the relative abundance of Actinobacteria presented a rising trend. In the initial phase of compost (D3), the dominant bacterial communities in the inoculated pile and the non-inoculated pile were Firmicutes (accounting for 75.54% in the former, and 64.82% in the latter), and Actinobacteria (accounting for 14.39% in the former, and 29.90% in the latter). In the late phase of compost, the dominant bacterial communities in the inoculated pile and the non-inoculated pile were Firmicutes, Proteobacteria (accounting for 40.19%-45.82% in the former, and 25.13%-29.90% in the latter), Actinobacteria (accounting for 25.13%-27.49% in the former, and 29.23%-30.90% in the latter), and Bacteroidetes (accounting for 10.34%-15.30% in the former, and 17.00%-25.24% in the latter).

Figure 4B shows the bacterial community composition at genus level in different pile samples. Clearly, bacterial community composition experienced significant changes at genus level. *Bacillus* and *Ignatzschineria* were dominant bacterial communities in the initial materials of composting, and accounted for 37.60% and 15.25%, respectively. The relative abundance of *Bacillus* declined from 37.60% at the initial point of composting to 15.18% (the inoculated pile) and 6.29% (the non-inoculated pile) respectively in the initial phase of composting; in the subsequent composting process, it continued to decline gradually, and eventually maintained at the level of 1.00%-2.44%. The relative abundance of *Ignatzschineria* declined from 15.25% at the initial point of composting to 0.01%-0.05% in composting period. The relative abundance of *Saccharomonospora* increased rapidly from that in the initial phase of composting to 16.77% (the inoculated pile) and 15.48% (the non-inoculated pile) respectively; in the subsequent composting process, it declined gradually. The relative abundance of *Ammoniibacillus* increased rapidly to 13.64% in the initial phase of composting (D3) in the inoculated pile, but was 1.86% in the non-inoculated pile; in other composting period, it was uniformly below 1%. *Oceanobacillus* was the dominant bacterial community in the non-inoculated pile in the initial phase of composting, with an abundance of 11.11%. *Actinomadura* maintained a high abundance in the non-inoculated pile in the entirecomposting process, which gradually rose with the extension of composting time (accounting for 28.79%-34.76%). The relative abundance of *Actinomadura* in the inoculated pile increased to 30.55% on D6 of composting, but declined slightly at the end of composting (accounting for 28.16%).

**Bacterial community difference analysis at genus level**

To identify the bacterial species showing significant differences in bacterial relative abundance in the maturing composting process, significance of difference analysis was carried out on the bacterial species
composition of microbial communities at genus level, followed by screening based on the criterion of LDA (linear discriminant analysis) Score > 4.0, as detailed in Fig. 5. On D3 of composting, significant differences were shown by a total of four genuses, that is, two in the inoculated group (i.e., *Ammoniibacillus* and *Bacillus*) and two in the non-inoculated group (i.e., *Actinomadura* and *Longispora*) (Fig. 5A). According to bacterial influence analysis, *Actinomadura* had the greatest influence, with LDA Score = 5.18, followed by *Ammoniibacillus*, with LDA Score = 4.86 (Fig. 5B).

On D6 of composting, significant differences were shown by a total of nine genuses, that is, six in the inoculated pile (i.e., *Thermovum*, *Phyllobacteriaceae*, *Persicitalea*, *Luteimonas*, *Longispora*, and *Sphingobacterium*) and three in the non-inoculated pile (i.e., *Flavobacterium*, *Saccharomonospora*, and *Halomonas*) (Fig. 6A). According to bacterial influence analysis, *Flavobacterium* (the non-inoculated pile) had the greatest influence, with LDA Score = 4.78, followed by *Thermovum*, with LDA Score = 4.74 (Fig. 6B).

Similarly, at the end of composting phase, significant differences were shown by a total of eight genuses, that is, five in the inoculated pile (i.e., *Luteimonas*, *Pusillimonas*, *Persicitalea*, *Thermovum*, and *Sphingobacterium*) and three in the non-inoculated pile (i.e., *Flavobacterium*, *Halomonas*, and *Galbibacter*) (Fig. 7A). According to bacterial influence analysis, *Flavobacterium* had the greatest influence, with LDA Score = 4.91, followed by *Luteimonas*, with LDA Score = 4.80 (Fig. 7B).

**Correlation between microbes and habitat physicochemical factors**

CCA was adopted to analyze the correlations between the physicochemical parameters (temperature, pH, N, P, K, moisture content, and organic matter content) of piles and the dominant bacterial genuses. As shown in Fig. 8, the dominant bacterial genuses were divided into two clusters, and the included angle between a physicochemical parameter and a bacterial community relative to the origin showed the correlation between them. To be specific, in the first cluster, three bacterial genuses were positively correlated with temperature, moisture content, N, P, TOC, and organic matter content; in the second cluster, five bacterial genuses were positively correlated with pH, and two bacterial genuses were positively correlated with K. On D3 of inoculation, the total relative abundance of the three bacterial genuses correlated with temperature in samples (i.e., *Ammoniibacillus*, *Bacillus*, and *Saccharomonospora*) in the inoculated group was significantly higher than that in the non-inoculated group. This also explained why the temperature of the inoculated group was higher than that of the non-inoculated group in the composting process, and reached its peak on D3. The two groups used the same composting raw materials, with the only difference being the addition of the inoculant or not. Thus, the exogenous inoculant was the primary reason why the temperature of the inoculated group was higher than that of the non-inoculated group.

**Change of ZR-11 in the maturing composting process**
Our analysis on bacterial diversity data showed that the abundance of the inoculated biocontrol strain ZR-11 presented a change trend of high, low, and high with the progress of the maturing composting process (Fig. 9A). Meanwhile, the results of qPCR analysis (Fig. 9B) also indicated that ZR-11 could colonize in the secondary compost maturation phase.

Discussion

Composting is the spontaneous biodegradation of organic matter in an aerobic environment, affected by multiple physiochemical conditions and microbial activity (Bernal et al., 2009). pH reflects the acid-base environment where microbes function in the composting process; an excessively high or low pH affects both the growth of microbes and the degradation of organic matter, and consequently influences the composting process. At the beginning of maturing composting, some easily degradable nitrogenous organic compounds experience ammoniation under the action of microbes, resulting in the rise of pH (Liu et al., 2017). With the progress of maturing composting and the weakening of the ammoniation action of microbes, the two piles had a basically stable pH in the late phase (Liu et al., 2019). Temperature, on the other hand, reflects the microbial activity of the composting system and the degradation rate of organic matter, and signals the success of composting. On D3 of inoculation, the inoculated pile quickly entered the high-temperature phase (> 50 °C), mainly because the rapid degradation of organic matter by aerobes produced a lot of heat (Wei et al., 2018). The high temperature hereby caused could once again kill mature organic pathogens, thus reaching the hygienic standard on composting (Wu et al., 2017). With the consumption of easily degradable matter and the decline of microbial metabolism intensity, the compost began to enter the cooling phase. In the manure composting process, C/N ratio is generally recognized as an important indicator of compost maturation. It is generally assumed that the decline of C/N ratio below 20 from 25–30 at the beginning is a sign of compost maturation. In addition, C/N ratio varies slightly with composting materials. In this study, at the end of maturing composting, the C/N ratio of the inoculated group (19.74) was obviously lower than that of the non-inoculated group (22.65), suggesting that the inoculant might have promoted compost maturation. GI is an important biological indicator that reflects the phytotoxicity and maturity of compost (Zhang et al., 2018). As far as plants are concerned, GI comprehensively mirrors the low toxicity (long plant roots) or high toxicity (germination rate) of compost; when GI is above 80%, it can be held that the compost is completely mature (Wu et al., 2010). At the end of maturing composting, the GI index of the inoculated pile reached 91.56%, which suggested that the inoculant could speed up compost maturation and ensure the compliance of composting products with the standard on biological manure production.

In the maturing composting process, the composition and diversity of microbial communities both changed obviously (Zhang et al., 2009). In this study, the abundance of microbial communities in the maturing composting composting process presented a declining trend, while the diversity of microbial communities presented a trend of declining first and rising afterwards. Similar to other studies, this study also identified Firmicutes, Bacteroidetes, Proteobacteria, and Actinobacteria as the dominant bacterial communities in all samples (Cesarano et al., 2017; Wang et al., 2016). Some studies have reported Firmicutes as the most dominant bacterial phylum in the high-temperature phase, mainly because
Firmicutes can survive at high temperature (> 55°C) and participate in all kinds of metabolic activities (Rainisalo et al., 2011; Ren et al., 2016). With the progress of maturing composting, the relative abundance of Actinobacteria presented a rising trend; Actinobacteria could also inhibit pathogenic microorganisms through excreting all kinds of antibiotics (Ren et al., 2016), so the increase of Actinobacteria reduced the biotoxicity of compost products. At genus level, in the materials of the compost maturation phase, the abundances of the microbes of Bacillus, Ignatzschineria, Caldicoprobacter, Sinibacillus, Thiopseudomonas, and Tepidimicrobium dropped abruptly after experiencing maturing composting. To be specific, the abundance of the microbes of Ignatzschineria declined rapidly from 15.25% at the initial point of composting to 0.01%-0.05% in composting period. Ignatzschineria is the second dominant bacterial community in initial materials, isolated from adult flies; it is reported to be correlated with human wound infection (Barker et al., 2014; Mejias et al., 2016; Tian et al., 2013). This also demonstrates the necessity of providing maturing composting and maturation. In the late phase of composting in the two piles, the Actinomadura of Actinobacteria had the largest proportion, and was the dominant bacterial community in the late phase of composting in both piles (Fig. 4b). Some studies have reported the occurrence of Actinomadura in the late phase of composting, and proposed to adopt Actinomadura as one of the relevant indicators for evaluating pile maturity (Roudiere et al., 2007).

In this study, in the inoculated group on D6 of maturing composting, Actinomadura was the dominant bacterial genus, with a proportion of 30.54%; the same proportion was observed in the non-inoculated group on D8 of maturing composting, which suggested that the inoculant might have promoted pile maturation. Thus, the addition of the inoculant affects the proportions of dominant bacteria, but does not change their species.

In the composting process, physicochemical parameters are correlated with the changes of microbial community structures. Different physicochemical parameters have different effects on bacterial community structures, and, in particular, pH exerts the most significant effect on them (Xu et al., 2013). In this study, RDA was adopted to analyze the correlations between the physicochemical parameters (temperature, pH, N, P, K, moisture content, TOC, and organic matter content) of piles and the dominant bacterial genuses. Analysis results showed that pH had the most significant influence on bacterial community structures, followed by temperature in the second place; in contrast, moisture content, N, P, TOC, K, and organic matter content had weaker effects on them. This suggests that, on the one hand, microbes lead to the accumulation of organic acids in the composting process (Awasthi et al., 2019); on the other hand, the change of pH changes the contents of Ca and Mg in the compost, thus indirectly affecting microbial communities (Lopezgonzalez et al., 2015). For this reason, pile pH can be changed to modify microbial community structures, thus improving compost quality. Some researchers have tried to reduce nitrogen loss by adding sawdust of different pH values into sludge compost (Lucas et al., 2011). The correlation between nitrogenous substances and bacterial community structures indicates that the N content of compost varies with the continuous conversion of microbial metabolites (Li & Li, 2015); in addition, the quality and quantities of nitrogen sources also control the changes of bacterial community structures (Zhou et al., 2019). As shown in Fig. 8, although temperature was not the environmental factor having the highest correlation with the changes of microbial community structures, it could still modify
microbial community structures through affecting microbial activity (Wallenstein et al., 2007). The two piles used the same composting raw materials, with the only difference being the addition of the inoculant or not. Thus, the exogenous inoculant was the primary reason why the temperature of the inoculated group was higher than that of the non-inoculated group. The rise of temperature caused intense changes in bacterial community structures.

Notably, our analysis on bacterial diversity data showed that the abundance of the inoculated biocontrol strain ZR-11 presented a change trend of high, low, and high with the progress of the maturing composting maturation process. Meanwhile, the results of qPCR analysis also indicated that ZR-11 could colonize in the maturing composting maturation phase; however, its relative content was lower than the additive amount recommended for biological manure, 0.2E + 08 cfu/g (NY_884–2012). For this reason, it is necessary to further explore the additive amount of the biocontrol strain in maturing composting and its regulation mechanism in the maturation process.

**Conclusions**

In this study, ZR-11, a biocontrol strain with resistance to Fusarium wilt in cucumber and acquired through isolation and screening in the laboratory, was inoculated in the compost maturation phase; conventional detection and Illumina Miseq high-throughput sequencing were adopted to analyze the effects of its inoculation in the compost maturation period on bacterial community structure and the correlation with physicochemical factors. Through analyzing physicochemical indicators, it was found that the exogenous inoculant could increase the temperature of an maturing composting pile, accelerate compost maturation, and enhance the abundance of bacterial communities in the pile. In the entire maturing composting process, bacterial community structures underwent substantial evolutions; in the same composting group, bacterial community structures of different period had a low similarity; in the same phase, bacterial community structures of different composting groups had a high similarity. Microbial diversity analysis revealed that Firmicutes, Proteobacteria, and Actinobacteria were always dominant bacterial phyla in different period, and the addition of the inoculant increased the relative abundances of the three bacterial phyla in the pile. The addition of the inoculant affects the proportions of dominant bacteria, but does not change their species. Different physicochemical parameters have different effects on bacterial community structures, and, in particular, pH exerts the most significant effect on them.

**Declarations**

**Ethical Approval**

This paper has no potential conflicts of interest and does not involve human participants and/or animals. All authors are aware and have agreed to submit it.

**Consent to Participate**
Informed consent was obtained from all individual participants included in the study.

Consent to Publish

This publication has been approved by all co-authors.

Declaration of conflicting interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Author Contribution

Li REN conducted the formal analysis, investigation, and contributed to the writing of the original draft. Jieming LI was involved in conceptualization, formal analysis, investigation, resource allocation, writing the original draft, reviewing and editing, visualization, supervision, and securing funding. Huifen LI contributed to the formal analysis. Zhonghui GUO, Ji LI, and Yizhong LV were also involved in the formal analysis.

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Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References


Figures
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Analysis on physicochemical indicators of composting materials (A, B, C, D, E, and F denote the change levels of the temperature, moisture content, pH, organic matter content, C/N ratio, and GI of compost samples on D0, D1, D3, D6, and D8, respectively)
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