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Compost-derived indole-3-acetic-acid-producing bacteria and their effects on enhancing the secondary fermentation of a swine manure-corn stalk composting

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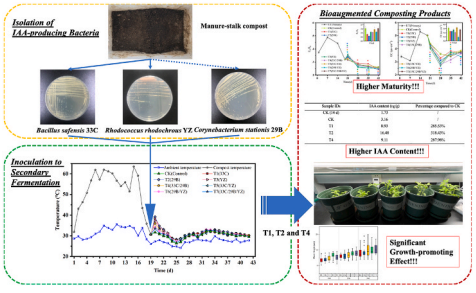
HIGHLIGHTS

- Two IAA-producing bacteria were isolated from swine manure composts.
- IAA-producing bacteria were amended in the secondary fermentation of composting.
- Only proper IAA-producing bacteria would increase the compost quality significantly.
- IAA accumulated in the composts with the inoculation of IAA-producing bacteria.
- IAA accumulation benefited the germination and early vegetative growth of tomatoes.

GRAPHICAL ABSTRACT



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ABSTRACT

Composting, as an effectively bio-oxidative process, has been widely used for converting organic waste to organic fertilizer. However, the low fertilizer efficiency of composting product limited its application in agriculture. To improve the growth-promoting effect of composting product, the present study investigated the bioaugmentation strategy of inoculating indole-3-acetic-acid (IAA)-producing bacteria. Firstly, two IAA-producing bacteria (*Bacillus safensis* 33C and *Rhodococcus rhodochrous* YZ) were isolated from composting products with high IAA yields of 39.18 and 16.32 μg mL^{-1}, respectively. Secondly, the microbial inoculants were prepared with 33C, YZ and a previously isolated IAA-producing strain *Corynebacterium stationis* 29B. To increase the accumulation of microbial secondary metabolites, microbial inoculants were amended at the secondary fermentation stage of composting. Physicochemical characterization showed that the maturity of composting product was significantly promoted by inoculating microbial inoculants prepared with 33C and 29B (single and combined inoculants). Finally, bioaugmentation with 33C and 29B increased the IAA contents of composting products by 2.9–5.2 times, which benefited the germination and early vegetative growth of plants. In summary, inoculating proper IAA-

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producing bacteria during secondary fermentation of composting could improve the quality of composting product and expand its application.

1. Introduction

With the development of breeding industry, more and more livestock and poultry wastes are produced and become potential threats to environment. These wastes are also great sources for nutrients urgently needed in agriculture (Apori et al., 2021). With the increasing approval of the "Zero-waste" concept (Awasthi et al., 2021), composting, as an efficient and practical method for organic waste treatment, has attracted much more attention recently (Li et al., 2020). It is a bio-oxidative process depending on the metabolism of microorganisms, which can convert organic matter into a humus-like stabilized product (Wang and Yuan, 2021). Generally, composting is a two-stage fermentation process (Bernal et al., 2009). During the first stage, bacteria and fungi quickly degrade the organics for nutrients and energy, leading to the dramatic increase of pile temperature. After the thermophilic stage, the pile gradually cools down and the second stage of fermentation begins (Yao et al., 2021). The remaining bio-liable organics are continuously broken down to stable bio-refractory products, which are rich in microbial secondary metabolites and nutrients for plants (Wang et al., 2018). Compared with chemical fertilizers, application of composting products from organic wastes in agriculture would not only benefit soil amendment (Wu et al., 2017), but also achieve growth-promoting and pathogen-resistant effects due to the existence of microbial secondary metabolites (Kavroulakis et al., 2005; Scaglia et al., 2015).

Indole-3-acetic acid (IAA), as one of the best characterized plant hormones, regulates most of the growth and developmental processes of plants (Woodward and Bartel, 2005). Besides plants, many microorganisms including bacteria (Mohite, 2013) and fungi (Fu et al., 2015) are able to synthesize IAA. Various IAA biosynthesis pathways have been discovered in microorganisms with tryptophan as the main precursor (Spaepen et al., 2007). Most known IAA-producing microbes belong to the genera of *Rhizobium, Azotobacter*, *Azospirillum*, *Bacillus*, *Pseudomonas*, *Streptomyces* (Hayat et al., 2010). These IAA-producing microbes were mostly isolated from rhizosphere (Khalid et al., 2005), since IAA was an important signaling molecule regulating the interaction between plants and bacteria. Although IAA could be detected in the composting products (K. Miezah et al., 2008), only few cultivable IAA-producing microbes were successfully isolated from the composting products (Chin et al., 2017; Zouari et al., 2020). Moreover, there was almost no study concerning the application of IAA-producing microbes in the compost of manure, as a bioaugmentation strategy to improve growth-promoting effect. The amendment of most microbial inoculants into the compost was carried out at the very beginning of primary fermentation or even the pretreatment stage (Wu et al., 2017), targeting on improving the maturity of composting products. Some researchers also tried to endow the composting products with special properties, such as growth promotion or pathogen resistance (Kavitha and Subramanian, 2007; Sathianarayanan and Khan, 2008; Kumar and Shweta, 2013). But few of them chose the secondary fermentation stage for microbial inoculation. In fact, secondary fermentation was a more suitable timepoint for inoculating functional microbes capable of producing secondary metabolites, because most of them were mesophilic microbes that could not survive the thermophilic primary fermentation. Therefore, it was necessary and meaningful to investigate the effects of inoculating IAA-producing bacteria during secondary fermentation as a bioaugmentation strategy to improve compost quality.

In the present study, IAA-producing microbes were isolated from the composting products to ensure their proliferation ability during the secondary fermentation. These bacteria were further identified and prepared as single-strain or combined microbial inoculants following by testing their influences on the physiochemical and biological properties of composts. By IAA content quantification and pot experiment verification, this study proved that inoculating proper IAA-producing bacteria during secondary fermentation could be a promising technical routine to expand the application scope of composting products.

2. Materials and methods

2.1. Isolation and screening of IAA-producing bacteria from the compost samples

The compost samples were taken from the swine manure-corn stalk composting piles in the research greenhouse of the Institute of Urban Environment, Chinese Academy of Sciences (Xiamen City, Fujian Province, Geographical Location N 38°39′6.48″, E 104°04′35.11″). The samples were collected in a sterile plastic bag and stored in a refrigerator at 4 °C. The following strain isolation works were completed within 2 d.

10 g of compost sample was transferred into a 250 mL flask with 100 mL of sterile water before shaking at 120 rpm for 20 min. The impurities were then removed by filtration to obtain the suspension of compost sample, which was diluted 10^4–10^8 times before being spread on the Lysogeny Broth (LB) agar plates (yeast extract 5.0 g L^{-1}, sodium chloride 10.0 g L^{-1}, tryptone 10.0 g L^{-1}, agar powder 20.0 g L^{-1}, pH adjusted to 7.2–7.4, and sterilized at 121 °C for 20 min). These plates were incubated upside down at 30 °C for 1–2 d. Different types of single colonies were then picked and purified several times on the LB agar plates. Seed cultures were finally obtained by inoculating the purified strains into the LB liquid media (without agar powder) before shaking at 30 °C, 120 rpm for 1 d.

To screening the IAA-producing bacteria, seed cultures in the logarithmic growth phase (10^8–10^9 CFU mL^{-1}) were inoculated into the LB liquid media containing 3 mM L-tryptophan by 2% (v/v). After shaking at 120 rpm for 2 d, 100 μL of bacterial suspensions were mixed with 100 μL of Salkowski reagent (1 mL of 0.5 M $FeCl_3$ solution mixed with 50 mL of 35% $HClO_4$ solution) in a 96-well microtiter plate. The plate was then placed in dark for 30 min. If the color of the solution turned red or pink, the strain would be identified as an IAA-producing bacterium (Glickmann and Dessaux, 1995).

The preliminary screened IAA-producing bacteria were then quantified for their IAA-producing capability. According to the method described by Apine and Jadhav (2011), 1 mL of the bacterial suspension was firstly centrifuged at 8000 rpm for 5 min; 100 μL of the supernatant were then taken and mixed with an equal volume of Salkowski reagent; the solution was finally placed in the dark for 30 min before measuring the absorbance (OD_{530}) at a wavelength of 530 nm with an ultraviolet–visible spectrophotometer. The content of IAA produced by bacteria could be calculated according to the standard curve of IAA (plotted by measuring the OD_{530} of a series of standard IAA solutions reacted with Salkowski reagent as described above).

2.2. Identification of IAA-producing bacteria

Colony morphological characteristics of IAA-producing bacteria grown on LB agar plates were observed for the size, shape, color, surface texture and edge. For SEM observation, samples were firstly collected by centrifugation (4 °C, 3000 rpm, 5 min) and washed by 0.1 M phosphate buffered saline (PBS, pH 7.4) twice; the bacterial pellets were then treated by 2.5% glutaraldehyde at 4 °C for 12 h and washed by 0.1 M PBS twice again; the pellets were dehydrated in 30%, 50%, 70%, 80%, 90% and 100% ethanol, and dried in critical point dryers for 24 h; the samples were finally loaded on the stage with conductive adhesive and coated with gold, before being observed with Hitachi S-4800.

Physiological and biochemical characteristics of IAA-producing bacteria were investigated by API 20NE kit following the instruction. To sequence 16 S rRNA gene, the genomic DNA was firstly extracted by thermal lysis; primers 27 F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492 R (5′-GGTTACCTTGTTACGACTT-3′) were then used to amplify the 16 S rRNA gene; PCR products were sent to Majorbio Co, Ltd. (Shanghai, China) for sequencing, and the sequences were submitted to EzBioCloud database for alignment; phylogenetic trees were constructed using MEGA7.0 software by Neighbor-Joining method with bootstrap analysis of 1000 replicates (Kumar et al., 2016).

2.3. Growth properties of IAA-producing bacteria

To determine the bacterial growth curve, the strain was firstly activated in the LB liquid medium and transferred to fresh LB liquid medium by 2% (v/v); the culture was then shaken at 30 °C, 120 rpm and regularly sampled; the absorbance (OD_{600}) of the bacterial suspension was finally measured at a wavelength of 600 nm with an ultraviolet–visible spectrophotometer.

To determine the antagonistic effects among different strains, plate confrontation assay was carried out. The strains were cross-streaked on the LB agar plate and cultured at 30 °C for 2 d. Bacteriostatic ring was used as the indicator for antagonistic effect.

2.4. Composting experiment

The swine manure used was freshly collected from the swine farm of Xiamen LeSen Ecological Agriculture Co, Ltd. Corn stalks (1 mm particle size) were purchased from Xiamen ChengHua Co, Ltd. The main physical and chemical properties of the composting raw materials were shown in Table S1.

Accordingly, the composting experiment was started up by mixing 1 kg (fresh weight) of swine manure with 0.45 kg (fresh weight) of corn stalks and 0.20 kg of deionized water to make sure the initial moisture content as 60% and the initial C/N ratio as 20. The primary fermentation was carried out in a large foam incubator following the windrow composting method. The pile body is stacked, the top is flattened, and the cross section is trapezoidal. In the early stage of composting (0–14 d), the moisture content was adjusted regularly to keep it above 50%. The oxygen was supplied by natural ventilation and regularly pile-turning, which was also conducted forcibly when the temperature of compost was higher than 65 °C. At the end of primary fermentation, the compost was fully turned over and divided evenly into 8 small foam incubators for secondary fermentation.

To bioaugment the secondary fermentation, the microbial inoculants were prepared by culturing the 33C, YZ and a previously isolated IAA-producing strain 29B (identified as *Corynebacterium stationis* (Zhou et al., 2021)) in the LB liquid media containing 3 mM L-tryptophan for 28, 18 and 36.8 h, respectively, according to the growth curves. These single-strain inoculants were further mixed together one by one to obtain the combined microbial inoculants. Seven of the small foam incubators mentioned above were marked as T1 ~ T7 and inoculated (2%, v/w) with 33C, 29B, YZ, 33C/29B, 33C/YZ, 29B/YZ and 33C/29B/YZ, respectively. The proportion of each strain in the combined microbial inoculants was kept at 1:1 or 1:1:1 (v/v). The eighth inoculator was inoculated with same volume of fresh LB liquid medium and marked as the control group (CK). Samples of both primary and secondary fermentation were taken regularly to determine the temperature, moisture content (MC), E_4/E_6, pH, electrical conductivity (EC) and germination index (GI), according to the literature (Wan et al., 2020).

2.5. Extraction and quantification of IAA from the composting products

The extraction steps were conducted according to the previous literature (Lebuhn and Hartmann, 1993). In detail, 3 g of samples of CK (14 and 42 d), T1, T2 and T4 (42 d) were added with 1 mL of 0.1 M PBS (pH 7.0), following by ultrasonic treatment (80 W) for 20 s and homogenization in the dark for 24 h. The samples were resuspended by 10 mL of 0.1 M PBS (pH 7.0) before additional ultrasonic treatment (80 W) for 1 min. The suspension was centrifuged at 5800 rpm for 30 min. The supernatant was then filtered by 0.45 μm filter membrane and stored in dark. The extraction steps described above were repeated twice. All extraction solution was mixed together and diluted to 500 mL with ultrapure water, following by adjusting the pH to 2.0–2.5 with H_3PO_4 solution. The solid phase extraction (SPE) column was activated with 10 mL methanol and ultrapure water, and equilibrated with 0.1 M PBS for 1 h. The extraction solution then passed through the column at a flow rate of 2–3 mL min^{-1}. After drying with nitrogen for 20 min, the SPE column was eluted with 5 mL ethanol. The eluate was dried under nitrogen, dissolved with 1 mL methanol and filtered by 0.45 μm filter membrane. Finally, the IAA content was quantified using High-performance liquid chromatography (HPLC, L-2000, Hitachi) equipped with an Extend-C18 column (250 mm × 4.6 mm, 5-μm 80 A) manufactured by Agilent (USA). The operation conditions of HPLC were set as follows: acetonitrile-0.1% formic acid aqueous solution (20:80, v/v) was used as mobile phase, injection volume was 10 μL, flow rate was 0.5 mL min^{-1}, column temperature was 30 °C, and UV detection wavelength was 254 nm. The elution time for IAA would be 17.5–17.7 min.

2.6. Pot experiment

100 g of composting products from CK, T1, T2 and T4 were fully mixed with 500 g of potting soil, and placed in 160 × 175 mm flower pots. Each pot was sown with 8 tomato seeds that had been soaked for 12 h, and cultured at 22 °C under a 12 h: 12 h light-dark cycle. Each pot was watered with 200 mL deionized water every 5 d. A blank group (BL) was also set by just culturing the tomato seeds in the potting soil without composting products, following the same planting operations described above. All treatments were carried out in triplicate. To evaluate the growth-promoting effect of the composting product, the heights of the germinated plants were measured on the 10, 20 and 30 d, while the dry weights of the biomass were measured after the plants were collected at 30 d, washed with deionized water and dried to constant weight at 105 °C.

3. Results and discussion

3.1. Isolation of IAA-producing bacteria 33C and YZ

The strains named as 33C and YZ were isolated and purified from the compost of swine manure in this study. After being inoculated in LB media and cultured for 48 h at 30 °C, 33C and YZ produced 39.18 and 16.32 μg mL^{-1} of IAA, respectively, which demonstrated similar IAA-producing capabilities as most known IAA-producing bacteria (Table 1). As shown in the table, most IAA-producing bacteria were isolated from the environment that coexisted with plants. Our study was the first time that IAA-producing bacteria were isolated from the composting products of swine manure, which not only preliminarily ensured their growth during fermentation, but also showed great potential on enhancing the fertilizer efficiency of the composting products.

3.2. Identification of IAA-producing bacteria 33C and YZ

The morphologies of 33C and YZ were shown in Fig. 1. The colonies of 33C on LB agar plate were circular, rough, matt, light-yellow and semitransparent. The cells of 33C were straight rods with polar flagella without neither visible endospore nor capsule. The colonies of YZ were also circular and semitransparent, but the surface was smooth and the color changed from pink to orange-red. The cells of YZ also lacked endospore and capsule, but the shape was long rod with slight curve.

In terms of physiological and biochemical characteristics (Table 2),

Table 1
IAA yield of IAA-producing bacteria.

Strains	IAA yield ($\mu g \cdot mL^{-1}$)	Sources	References
Halomonas aquamarina DB01	8.97	Saline-alkali soil	Sun et al. (2020)
Burkholderia gladioli JF-8	57.48	*Bidens pilisa* L	Hu et al. (2019)
Leucobacter tardus 4-3	94.30	Button mushroom compost	Yoo et al. (2017)
Streptomyces 31SSS	~50	Marine sediment	Rashad et al. (2015)
BAN74, BAN86, BAN87	/	Active volcano sites	Amaresan et al. (2014)
Proteus vulgaris TK-2	13.84	Heavy metal-polluted soils	Tian et al. (2014)
Arthrobacter pascens ZZ21	59.81	Rhizosphere soil	Zhang (2014)
Bacillus flexus A60	29.17	Rhizosphere soil	Liu (2017)
Agrobacterium vitis A48	41.55	Rhizosphere soil	Lee et al. (2012)
Bacillus aryabhattai B20	37.08	Rhizosphere soil	Zhao et al. (2016)
29B	54.73	Swine manure compost	Zhou et al. (2021)
33C	39.18	Swine manure compost	This study
YZ	16.32	Swine manure compost	This study

both 33C and YZ were Gram-positive and catalase-positive. Both of them could utilize tryptophan for indole production, which was highly correlated with IAA production. They could also assimilate most of carbon sources tested in this experiment, except capric acid and phenylacetic acid for 33C and capric acid for YZ. In addition, they both lacked the ability of glucose fermentation, arginine dihydrolase and urease, suggesting the incapability of anaerobic metabolism. However, only YZ owned the ability of reducing nitrate, while only 33C showed catalytic activities of β-glucosidase, protease and β-galactosidase correlated with the capacity of utilizing complex substrates. In summary, both 33C and YZ were typical aerobic heterotrophic bacteria using a wide spectrum of organic carbon sources, which suggested their great potentials of colonizing during composting.

33C and YZ were further identified based on their 16 S rRNA genes. The phylogenetic trees (Fig. 2) produced by the neighbor-joining analysis revealed that the closest relative of 33C was the strain *Bacillus safensis* subsp. *safensis* FO-36 b with the similarity of 99.25%, while YZ

Table 2
Physiological and biochemical characteristics of the two isolated strains.

Items	33C	YZ
Gram staining	+	+
Catalase	+	+
Nitrate reduction	+	–
Indole production	+	+
Glucose fermentation	–	–
Arginine Dihydrolase	–	–
Urease	–	–
β-glucosidase	+	–
Protease	+	–
β-galactosidase	+	–
Glucose assimilation	+	+
Arabinose assimilation	+	+
Mannose assimilation	+	+
Mannitol assimilation	+	+
N-Acetyl-Glucosamine assimilation	+	+
Maltose assimilation	+	+
Gluconate assimilation	+	+
Capric acid assimilation	–	–
Adipic acid assimilation	+	+
Malate assimilation	+	+
Citrate assimilation	+	+
Phenylacetic acid assimilation	–	+

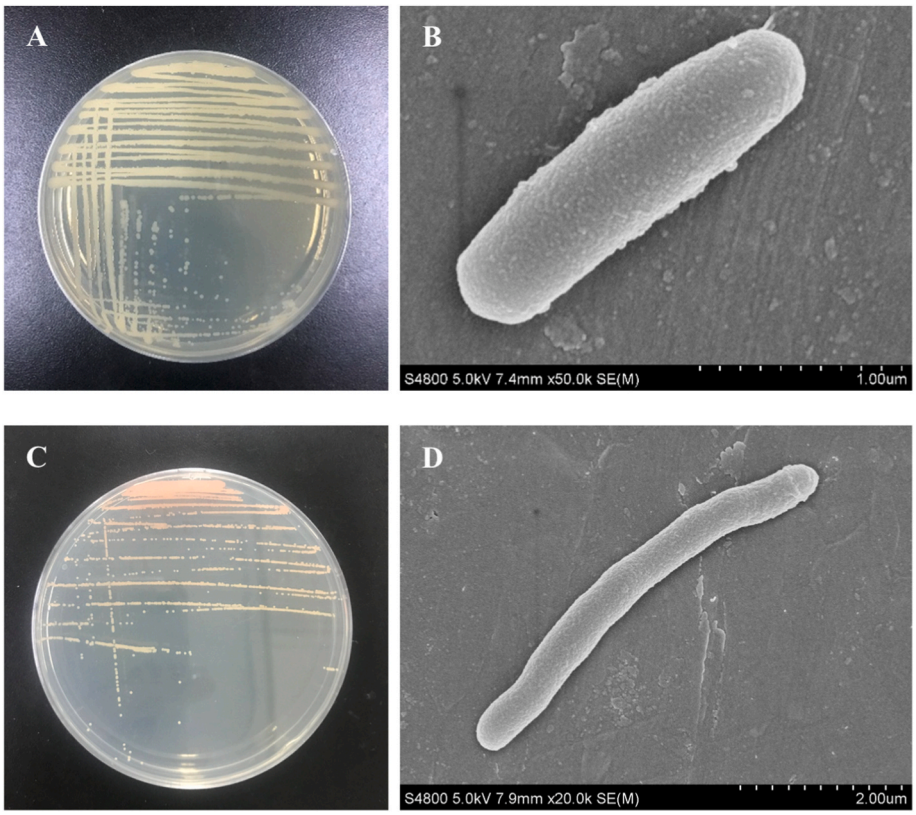

Fig. 1. Colony morphology and SEM images of 33C (A, B) and YZ (C, D).

was closest with the strain *Rhodococcus rhodochrous* NBRC 16069 according to the similarity of 99.28%. What was more, all *Bacillus* including 33C and all *Rhodococcus* including YZ were classified into the same branches, respectively. It strongly suggested that the most probable affiliations of 33C and YZ were to *Bacillus* and *Rhodococcus*, respectively.

The morphology, physiological and biochemical properties of 33C and YZ also matched most of the description of *Bacillus safensis* (Satomi et al., 2006) and *Rhodococcus rhodochrous* (Goodfellow and Alderson, 1977), respectively. Therefore, the strain 33C was identified and named as *Bacillus safensis* 33C and the strain YZ was identified and named as *Rhodococcus rhodochrous* YZ. Interestingly, *Bacillus safensis* subsp. *safensis* FO-36 b was described as a strain incapable of producing indole, which was different from 33C. However, it was well known that multiple environmental isolates of *Bacillus* species have the capability of producing IAA (Kumar et al., 2014). Nevertheless, gene expression concerning IAA biosynthesis was usually regulated by environmental conditions (i.e. osmotic stress and the existence of antagonistic microbes) (Molina et al., 2018). Therefore, it was highly possible that the complex composting environment could drive the expression of IAA biosynthesis genes as a weapon for microbes against abiotic and biotic stresses (Khare and Arora, 2010).

3.3. Growth properties of the IAA-producing bacteria

To prepare the combined microbial inoculants for the secondary fermentation of swine manure, growth properties of 33C and YZ, as well as another IAA-producing strain 29B (Zhou et al., 2021) were preliminarily studied. Firstly, plate confrontation assay was used to determine the antagonistic effects among these three strains. As shown in Fig. S1, no bacteriostatic ring at the intersections of neither 33C/29B, 29B/YZ nor 33C/YZ was observed, and all the colonies showed normal morphologic characters on the LB plates. It suggested that the cultures of all these three strains could be mixed randomly to prepare different combined inoculants.

To guarantee an optimal physiological condition for inoculation, the growth curves of 33C, 29B and YZ were measured (Fig. S2). Among these three strains, 33C experienced the longest lag phase (24 h) and the shortest exponential growth (24–40 h), while YZ and 29B adapted to new environment much faster (8 and 12 h, respectively) and grew exponentially for a longer period of time (8–28 and 12–32 h, respectively). To balance the activity and the abundance of microbes, the time points of 80% exponential growth were chosen as the best culturing time lengths for inoculant preparation (36.8, 28, and 18 h for 33C, 29B and YZ, respectively).

3.4. Parameters during the composting of swine manure

To investigate the effect of microbial inoculants on fermentation promotion, the pure and mixed cultures of 33C, 29B and YZ (7 microbial inoculants in total) were inoculated respectively at the beginning of secondary fermentation of swine manure. Compost temperature is a key index reflecting the microbial activity of degrading organics (Liang et al., 2003). Therefore, the end of primary fermentation could be determined as the 19 d, when the compost temperature dropped closely to the ambient temperature. Accordingly, the 19 d was chosen as the time point for inoculation. As shown in Fig. 3 (A), the compost temperatures of all treatment groups with microbial inoculants were much higher than that of CK at the 20 d, indicating the high metabolic activity of the inoculated IAA-producing bacteria. Although the temperatures of

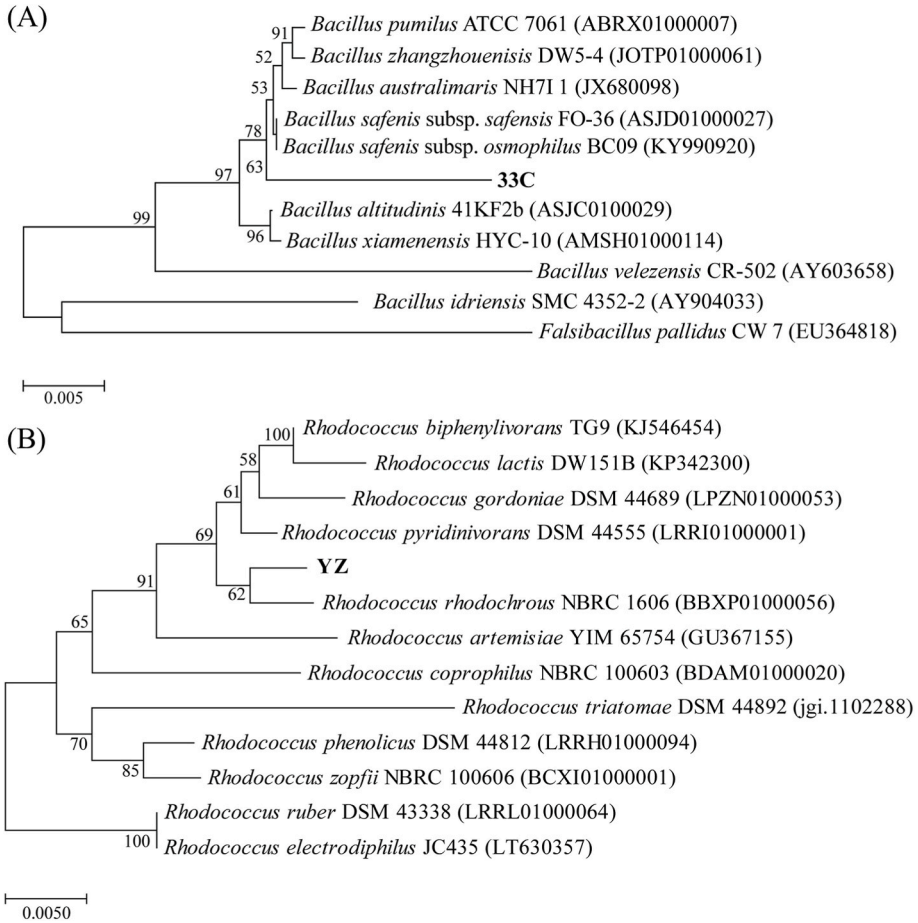

Fig. 2. Neighbor-joining phylogenetic trees of 33C (A) and YZ (B).

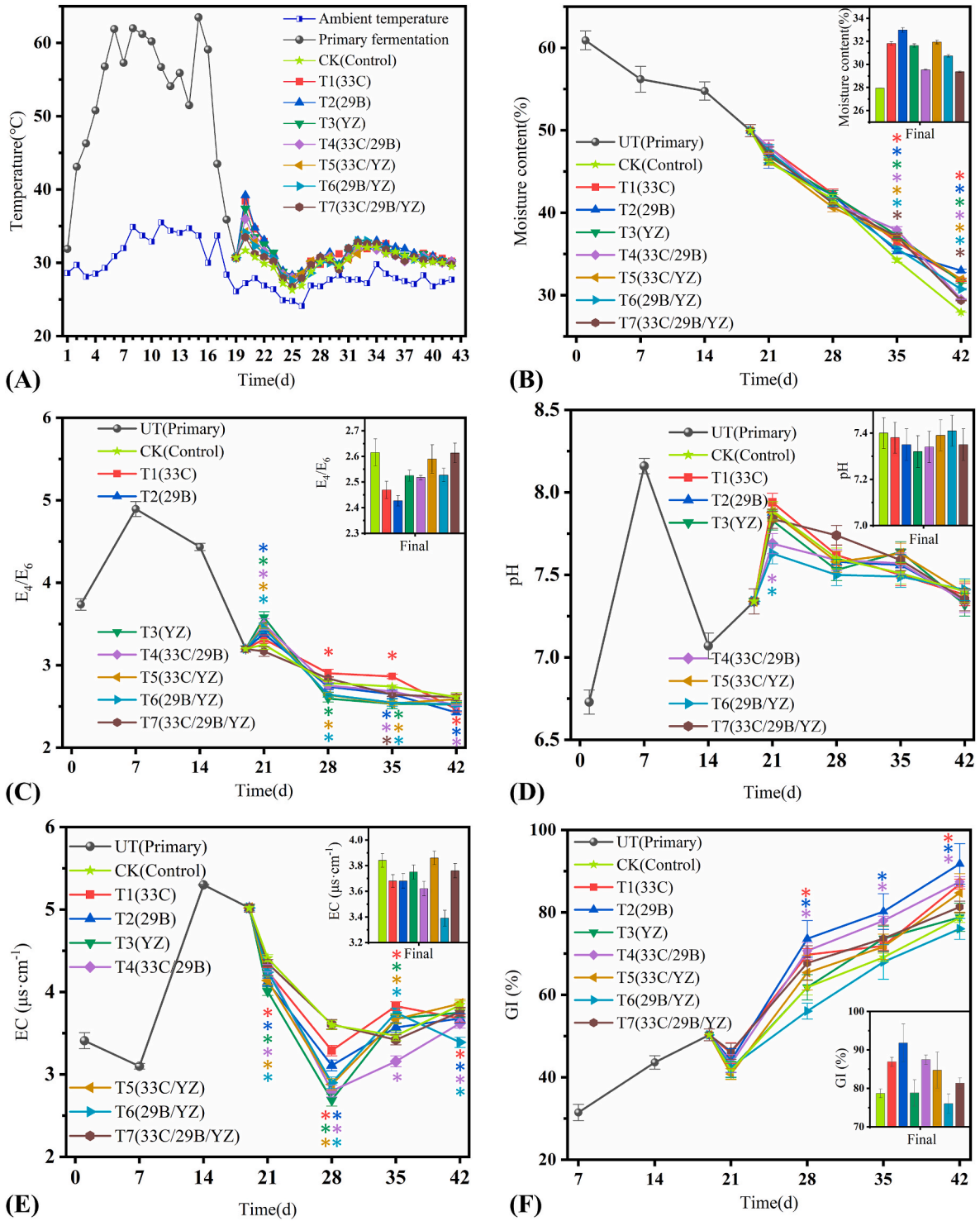

Fig. 3. Variation of compost temperature (A), moisture content (B), E_4/E_6 (C), pH (D), electrical conductivity (E) and gemination index (F) during the composting process. Inserted figures in (B) ~ (F) showing the differences of the final products at 42 d. Asterisks with different colors below or above the data points indicating that the corresponding treatment groups were significantly lower or higher than CK ($p < 0.05$, the Student-Newman-Keuls test). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

all groups began to decrease after the 20 d, the treatment groups still showed advantage at least for 7 d, suggesting higher microbial activities at the early stage of secondary fermentation. However, the highest temperature of all groups did not exceed 40 °C after the 20 d, suggesting the dominant role of mesophilic microorganisms in the mild conversion of organic matters during the secondary fermentation. Interestingly, the effects of three single strain inoculants on the temperatures were more significant than those of combined microbial inoculants. It suggested that although no antagonistic effect was observed in the previous test, competitive effects might still occur and decrease the microbial activities, which was commonly found during the application of combined microbial inoculants (Trabelsi and Mhamdi, 2013). Nevertheless, the stimulatory effects of microbial inoculants on the fermentation process were significant.

MC, as another key factor affecting microbial activity, is highly correlated with substance transformation and functioning of microbial inoculants (Li et al., 2021). The MC was 49.97% at the end of primary fermentation (Fig. 3(B)), which guaranteed a favorable environment for microbial proliferation during the secondary fermentation. After the inoculation, MCs of all experimental groups kept dropping to around 30% finally, which met the standard of organic fertilizer. Nevertheless, MCs of all treatment groups after 35 d were significantly higher than that of CK ($p < 0.05$). It could be closely related with the higher microbial activity in the treatment groups, leading to higher yield of water and humus. Humus would cement the compost particles into aggregates, which increased the water-holding capacity of the composts (Senn and Kingman, 1973). Higher degree of condensation and aromatization of humus in the treatment groups was further confirmed by measuring E_4/E_6 ratio. As shown in Fig. 3 (C), the values of E_4/E_6 in all experimental groups peaked at the beginning of primary and secondary fermentation, and gradually decreased with time. At the end of fermentation, T1, T2 and T4 had significantly lower E_4/E_6 values than that of CK ($p < 0.05$). Due to lower value of E_4/E_6 indicating greater condensation and aromatization of humus, adding these three microbial inoculants could greatly promote the maturation and stabilization of composts (Moharana and Biswas, 2016).

The change of pH (Fig. 3 (D)) could also reflect the variation of microbial activities during the fermentation process. The sharp increase of pH during 1–7 d suggested the fast degradation of nitrogen-containing organics in the swine manure, producing large amount of ammonia. After that, the ammonia emission and the accumulation of small molecule organic acids would occur during the thermophilic phase (7–14 d), causing significant decrease of pH. The pH increased again during 14–21 d due to the gradual degradation of organic acids and the strengthening of denitrification under partially anaerobic environment. Finally, the pH slowly decreased and stabilized during the secondary fermentation (21–42 d). Although the differences were not significant ($p > 0.05$), the slightly lower pH of all treatment groups suggested adding microbial inoculants could enhance microbial activities, leading to the generation of more organic acids and CO_2. Nevertheless, the variation of pH kept in the range of 6.5–8.5, which guaranteed the metabolism of most microbes (Abdel-Rahman et al., 2016).

EC is an indicator of electrolyte contents in the compost. A high EC value usually means high toxicity to plant growth. The EC values in all experimental groups changed oppositely with the pH throughout the fermentation process (Fig. 3 (E)), proving its potential correlation with the variation of microbial activities. At the beginning of secondary fermentation (21 and 28 d), most of the treatment groups showed significantly lower EC values than CK ($p < 0.05$). However, except for T4, which was constantly lower than CK, adding microbial inoculants did not always benefit the decrease of EC at the later stage of fermentation (35 and 42 d). Nevertheless, all experimental groups showed relatively low EC values (~4 mS cm^{-1}), which all reached Chinese standard of organic fertilizer (China, 2012) and would not cause inhibition against plant growth.

GI is the most convincing indicator for evaluating the harmless and maturity of composting products. As shown in Fig. 3 (F), GIs of all experimental groups increased with fermentation time, except the slight decline at the 21 d, which might be related with the introduction of some extra undegraded organics during adding microbial inoculants. Throughout the whole secondary fermentation process, those inoculated with 33C and 29B (T1, T2 and T4) showed significantly higher GIs than CK ($p < 0.05$), suggesting higher maturity and quality of the composts after proper bioaugmentation (Zhong et al., 2021).

However, it should be noted that not all treatment groups showed significantly better compost quality than that of CK. Some studies showed that bioaugmentation might even lower the quality of the compost in some aspects, such as the GI index (Costa et al., 2021). It highlighted the importance of choosing the proper microbes for bioaugmentation during composting. Overall, microbial inoculants made with 33C and 29B (T1, T2 and T4) could significantly promote the composting process according to the E_4/E_6, EC and GI. As for why adding YZ could not improve the compost quality and would even eliminate the positive effects of 33C and 29B, the unique growth properties of YZ could be the key reasons. YZ had much higher growth rate and shorter lag phase than 33C and 29B (Fig. S2). It suggested YZ would outcompete 33C and 29B for nutrients, causing the demise of these two strains during the initial phase after inoculation. However, although *Rhodococcus* usually grew fast (Elsayed et al., 2017), its survival would be significantly stressed by the oxygen-limited condition (Fan et al., 2021). Therefore, without force ventilation, the hypoxia environment inside the compost pile during secondary fermentation would subsequently cause the demise of YZ. As a result, no functional bacteria would massively proliferate in the treatment groups inoculated with YZ, resulting in composting products similar to CK. Due to the negative impact of YZ and the superior performance of 33C and 29B, T1, T2 and T4 were further tested for IAA contents and pot experiments to judge their potential for promoting the growth of plants.

3.5. Assessment of growth-promoting effects of composting products inoculated with IAA-producing bacteria

There were two purposes of inoculating IAA-producing bacteria during secondary fermentation: promoting the fermentation process, and increasing IAA content for endowing composting products with growth-promoting property. As shown in Table 3, the CK showed relatively low content of IAA even it was accumulated during the secondary fermentation (1.73–3.16 ng g^{-1}). By comparison, the inoculation of IAA-producing bacteria greatly increased the IAA content by 2.9–5.2 times, which indicated the proliferation of IAA-producing bacteria in the composts. Previous studies have also shown that the application of microbial inoculants benefited the accumulation of IAA. Kavitha and Subramanian (2007) reported that adding *Azobacter*, *Pseudomonas* and Phosphobacteria into the compost of municipal solid waste could increase the IAA content by 1.14–3.71 times. Kumar and Shweta (2013) found out that inoculating *Trichoderma viride* alone or in combination with either *Azotobacter chroococcum* or *Pseudomonas striata* into the timber solid waste vermicompost would increase the IAA content by 38–42%. However, these studies applied microbial inoculants at the very beginning of fermentation, which might lead to negative impact on IAA biosynthesis considering the inhibitory effect of high temperature during primary fermentation (Molina et al., 2018). Therefore, judging from the degree of IAA content enhancement, our study exhibited the superiority of applying microbial inoculants during the secondary fermentation which highly benefited the accumulation of IAA in the composting products.

Pot experiments were then carried out to determine the correlation between IAA content and growth-promoting effect. As shown in Fig. 4, the plant heights in all treatment groups were taller than BL and CK. Among these three treatment groups, the growth-promoting effect of T2 was significantly greater than those of T1 and T4 at 10 d, which showed highly positive correlation with IAA contents. However, the difference of plant heights among these three treatment groups almost disappeared at 20 and 30 d. Judging from the median and mean values of the plant heights and the dry weight (Fig. S3), the T4 was slightly better than the T1 and T2, indicating the advantage of combined microbial inoculant on

Table 3
IAA contents in composting products of different experimental groups.

Sample IDs	IAA content (ng·g^{-1})	Percentage compared to CK
CK (14 d)	1.73	/
CK	3.16	/
T1	8.93	285.53%
T2	16.40	518.43%
T4	9.11	287.98%

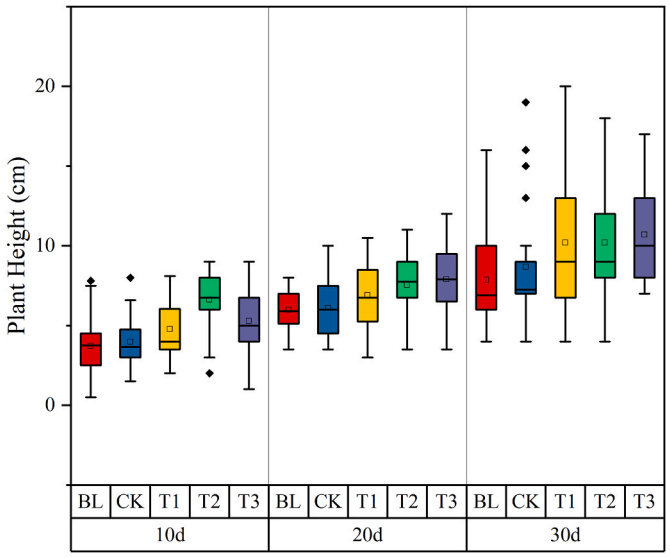

Fig. 4. Plant height of tomatoes in different experimental groups at 10, 20 and 30 d.

long-term fertilizer efficiency. It was highly possible that the application of combined microbial inoculant would benefit the increase of microbial diversity in the soil (Alori et al., 2017), which was proved to be a key driver for plant growth (Saleem et al., 2019). As for why the positive correlation between IAA content and growth-promoting effects only occurred in the first 10 days, it was known that IAA could promote the germination and vegetative growth of plants, however, the enhancement effect was not permanent (El-Mergawi and Abd El-Wahed, 2020). Besides the assimilation by plants, IAA-degrading microbes are also wide-spread in the soil (Spaepen et al., 2007), leading to a fast depletion of IAA. Therefore, the growth-promoting effect of indole acetic acid from the composts was more significant in the germination and early vegetative growth stages of plants. Meanwhile, the overall improvement of the compost quality and the microbiome biodiversity after amending microbial inoculants during the secondary fermentation stage might be the key factors endowing composts with long-term growth-promoting properties.

4. Conclusion

In this study, two bacterial strains with high IAA-producing capability were isolated from the compost of manure. According to the morphology, physiological and biochemical properties and 16s rRNA gene sequences, they were identified as *Bacillus safensis* 33C and *Rhodococcus rhodochrous* YZ, respectively. Together with another previously isolated strain 29B, their effects on enhancing the secondary fermentation of the composts were verified. The results showed that only the microbial inoculants made with 33C and 29B (single and combined inoculants) significantly improved the quality of the composts. The high compost quality of these treatment groups was further verified by the high IAA content and great growth-promoting effect. This was the first report on improving compost quality via inoculating IAA-producing bacteria during the secondary fermentation. The present study supplied an efficient composting strategy for upgrading the quality of organic fertilizer.

Credit author statement

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Declaration of competing interest

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.

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Appendix A. Supplementary data

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