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Reprogrammed endophytic microbial community in maize stalk induced by *Trichoderma asperellum* biocontrol agent against *Fusarium* diseases and mycotoxin accumulation

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Maize stalk rot and ear rot, caused by *Fusarium graminearum* and *Fusarium verticillioides*, respectively, are major diseases that threaten the sustainable production of maize. In this study, an artificial inoculation assay demonstrated that the control efficacy of maize stalk rot and ear rot by *Trichoderma asperellum* granules were 49.83 % and 39.63 %, respectively. By high-throughput sequencing of maize plants, a total of 76 196 ITS1 sequences and 887 226 V3–V4 16S rRNA sequences were analyzed and were grouped into 2934 fungal and 24 248 bacterial operational taxonomic units (OTUs), respectively. It revealed a significantly higher endophytic microbial abundance in the stem tissue of plants grown in *T. asperellum*-treated soil than in those grown in the control, with the largest increase observed in the basal stem section. In addition, the endophytic microbial diversity and corresponding control effects all gradually decreased from the basal to apical parts of the stem in plants grown in *Trichoderma*-treated soil, indicating that *Trichoderma* stimulated a more significant effect on the defense system in the basal section of the stalk than in the apical parts of plants. Furthermore, the accumulation of deoxynivalenol (DON) and fumonisin B1 (FB1) decreased in the stem and ear of maize grown in *T. asperellum*-treated soil.

1. Introduction

Maize is a main food crop globally (Bacon et al., 2001). In China, stalk rot and ear rot caused by *Fusarium* spp. have become the major diseases in maize-growing areas, leading to significant yield loss and *Fusarium* mycotoxin contamination of maize kernels (Logrieco et al., 2002; Audenaert et al., 2013; Ponts, 2015). In addition, maize stalk rot and ear rot usually occur in the late growth period, which makes the practices for controlling the two diseases laborious and costly. So far, the genetic resistance of the cultivars and the application of chemical seed coating agent and chemical fertilizers are the main measures to prevent maize stalk rot and ear rot, but it is not enough to effectively control the two diseases (Zhang et al., 2010). In addition, the long-term use of chemical seed coating agents and chemical fertilizers will destroy the ecological environment of the soil and will induce the resistance of the pathogen (Kandula et al., 2015). Therefore, it is of great ecological and economic benefit to development biological control methods to control *Fusarium*.

Trichoderma spp., antagonistic and beneficial fungi, are known to control crop soilborne diseases through a variety of mechanisms including competition, hyperparasitism and induction of plant resistance (Savazzini et al., 2009; Mukherjee et al., 2013; Verma et al., 2007). More importantly, the induced systemic resistance (ISR) primed by *Trichoderma* is viewed as a promising measure to achieve systemic control of whole-plant diseases, meaning that the control target of *Trichoderma* is not only the disease affecting underground parts of plants but also the disease affecting aboveground parts and that the control event occurs not only at the plant seedling stage but also at the adult stage. The outcome mainly depends on the level of mutual molecular interaction between the *Trichoderma* elicitor and the receptor localized in the plant root system and on the efficiency of signal transduction across plant organs and developmental stages (Verma et al., 2007).

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Previous research showed that *Trichoderma harzianum* T22 used to treat seeds of the maize inbred line Mo17 was able to induce leaf resistance to *Colletotrichum graminicola* (Harman et al., 2004). In addition, our previous study showed that cellulase and hydrophobin produced by *Trichoderma* within maize root endoderm could simultaneously control maize stalk rot and leaf spot disease caused by *Fusarium graminearum* and *Curvularia lunata*, respectively (Saravanakumar et al., 2016). Some studies have revealed the molecular mechanisms of systemic immune responses of maize plants induced by *Trichoderma* (Djonovic et al., 2007; Gaderer et al., 2015), but such studies usually focused on leaf defense response instead of the defense system of the whole plant, and most work is conducted at the molecular level instead of the microecological level. Thus, until now, few studies have been carried out regarding the effects of *Trichoderma* on the endogenous microbial population diversity and mycotoxin accumulation in all organs of maize plants. In consideration of the current threats to maize production posed by maize stalk rot and ear rot, this study was performed to understand the effect of *Trichoderma* biocontrol agent on the dynamic change in the endophytic microbial community and *Fusarium* mycotoxinaccumulation along stem tissue and the ear to reveal the microecological mechanism of *Trichoderma* against both diseases.

2. Materials and methods

2.1. Fungal strains

Trichoderma asperellum strain GDFS1009, a *F. graminearum* and a *Fusarium verticillioides* strain were used in this study. *T. asperellum* strain GDFS1009 was obtained from the Center for Culture Collection of *Trichoderma* (CCTC, http://www.china-cctc.org), Shanghai Jiao Tong University (SJTU). Through a series of *in vitro* and *in vivo* screening procedures, earlier studies have presented GDFS1009 as a good potential biocontrol microbe for use against soil-borne diseases in vegetables and maize. The *F. graminearum* and *F. verticillioides* strains were originally isolated from stalk rot-infected maize stem tissues and maize ears infected by ear rot, respectively. Both of the strains were maintained as spore suspensions in 30 % glycerol at −80 °C. Before the trials, the strains were grown on potato dextrose agar (PDA) medium at 28 °C for 5 d for activation.

2.2. Preparation of T. asperellum *GDFS1009 granules and* Fusarium *inocula*

The 6 mm-diameter mycelial discs of *T. asperellum* GDFS1009 and *F. graminearum* strain were removed from the advancing edge of 5-d PDA culture and used to inoculate potato dextrose (PD) medium (4–5 discs per 200 ml PD). Cultures were incubated in a constant temperature shaker at 28 °C, 180 r/min for 3 d to generate primary fermentation broths of two strains.

To produce *T. asperellum* GDFS1009 granules, the primary fermentation broth (pH 4.17) of this strain was used to inoculate a 300 L fermenter containing chlamydospore-inducing medium in a 0.5 % (v/v) ratio and cultured at 28 °C, 126 r/min for 7 d. The concentration of chlamydospores in the final fermentation broth reached 10^8 CFU/ml. Next, the final *T. asperellum* fermentation broth was mixed with nutriment, which is produced by mixing diatomaceous earth and ground wheat bran (10:3, w/w), in a ratio of 13:10 (w/v). Then, the mixture was stirred with a mixer, squeezed with a granulator, and dried at 43–50 °C for 1–2 h to produce *T. asperellum* granules. The granules after drying were placed in a petri dish and an appropriate amount of water was added to culture at 28 °C. On the 3rd d, *T. asperellum* was observed to grow on the surface of the granules and the granule content was 2×10^8 CFU/g.

To prepare *F. graminearum* inocula, maize kernels were boiled and autoclaved at 121 °C for 60 min. Later, after cooling, the *F. graminearum* fermentation broth were transferred to the kernels at a proportion of 0.5 % (v/w) and incubated at 28 °C for 7 d until the *F. graminearum* mycelium covered the maize kernels.

F. verticillioides was cultured on potato dextrose agar (PDA) medium and incubated at 28 °C with a 12 h photoperiod for 7 d. The conidia were collected by scraping the medium surface with sterile water and mycelia disposed through filter paper. Finally, the spore suspension of *F. verticillioides* was prepared and adjusted into 10^8 CFU/ml.

2.3. Experimental design and sampling

The experiment was conducted in the test field of Shanghai Jiao Tong University. Before sowing, *T. asperellum* granules and compound fertilizers (15 % N, 15 % P_2O_5 and 15 % K_2O) were mixed and applied to the soil. The granules were applied at a dose of 75 kg per ha, and compound fertilizer applied alone was used as a control. Maize seeds were sown in the field in three replicates, with each replicate representing a block of 4 rows (5 m by 10 m). At the sowing stage, 15 g of *F. graminearum* inoculum was buried in the rhizosphere soil of each hole, into which 2 maize seeds were then planted. At the filling stage, 1 mL of spore suspension with 1.0×10^8 CFU/mL of *F. verticillioides* was injected into each ear. In the late stage of milk ripening, 20 maize plants were randomly selected for each treatment, and the occurrences of stalk rot and ear rot disease were investigated. Disease severity of maize stalk rot was visually assessed in each plant root according to a scale based on the percentage of the plant root surface area covered in lesions, where 0 = no symptoms, 1 = 1 %–10 %, 3 = 11 %–25 %, 5 = 26 %–50 %, and 7 = 51 %–100 %. Disease severity of maize ear rot grading standard (National industry standard for maize ear rot resistance identification NY/T 1248.8–2016): Grade 1, the ear lesion area accounts for 0 %–1 % of total area; Grade 3, the ear lesion area accounts for 2 %-10 % of total area; Grade 5, the ear lesion area accounts for 11%–25 % of the total area; Grade 7, the area of the disease accounts for 26 %–50 % of the total area; Grade 9, the area of the disease accounts for 51 %–100 % of the total area. The disease index (John et al., 2010) of maize stalk rot and ear rot and the control effect of *T. asperellum* GDFS1009 on the two diseases were calculated using the following formulas:

Disease index $= 100 \times \sum x_i / \mathrm{N} \times 4$, where x_i is the disease rating of ith replicate ($\mathrm{i} = 0–4$) and N is the total number of seedlings examined.

Control effect (%) $= (D_c - D_t)/D_c \times 100$, where D_c is disease index of the control and D_t is the disease index of the *Trichoderma* treatment.

At the end of the milk stage, 3 maize plants were randomly selected for each treatment and brought back to the laboratory. In the laboratory, cut 4–5 cm of the basal stem section (the first stem section above the ground), middle stem section (the fourth stem section above the ground) and the near-ear stem section (the stem section which the ear is located) for each plant using a sterile cutter, then washed the tissues with sterile water and stored them at −80 °C for subsequent DNA extraction and mycotoxin detection.

2.4. Genomic DNA extraction and high-throughput sequencing

To remove the surface microorganisms, the samples were disinfected with 75 % (v/v) ethanol for 2 min and 2.5 % sodium hypochlorite for 5 min individually and then cleaned with sterile water three times (Xia et al., 2014). Then the myeloid portion of each tissue was subjected to high-throughput sequencing to detect the intratumoral microorganism group. Following the instructions of the Fast DNA SPIN extraction kit (MP Biomedicals, Santa Ana, CA, USA), total genomic DNA was extracted; then, the DNA quantity and quality were measured using a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) and agarose gel electrophoresis, respectively.

The V3–V4 region of the 16S rRNA gene was amplified using the primer set 338F (5′-ACTCCTACGGGAGGCAGCA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) for bacterial community analysis. For fungal community analysis, the ITS sequence was amplified with primer set ITS 5F (5′-GGAAGTAAAAGTCGTAACAAGG-′) and ITS2 (5′- GCTGCGTTCTTCATCGATGC-3′) (Lievens et al., 2005). Sample-specific 7-bp barcodes were added to the primers for multiplex sequencing. The PCR components contained 5 μL of Q5 reaction buffer (5 ×), 5 μL of Q5 High-Fidelity GC buffer (5 ×), 0.25 μL of Q5 High-Fidelity DNA Polymerase (5 U/μL), 2 μL (2.5 mM) of dNTPs, 1 μL (10 μM) of each forward and reverse primer, 2 μL of template DNA, and 8.75 μL of ddH_2O. The PCR protocol consisted of initial denaturation at 98 °C for 2 min; 25 cycles including denaturation at 98 °C for 15 s, annealing at 55 °C for 30 s and extension at 72 °C for 30 s; and a final extension of 5 min at 72 °C. Then, PCR amplicons were purified with a DNA gel extraction kit (Axygen, Union City, CA, USA) and quantified using a PicoGreen dsDNA Assay Kit (Invitrogen, Carlsbad, CA, USA). The PCR amplicon sequencing was performed using an Illumina MiSeq platform with a MiSeq Reagent Kit v3 at Shanghai Personal Biotechnology Co., Ltd (Shanghai, China).

2.5. *Sequence analysis*

As previously described, the Quantitative Insights Into Microbial Ecology (QIIME, v1.8.0) pipeline (Caporaso et al., 2010) was used to process the sequencing data. Briefly, raw sequencing reads with exact matches to the barcodes were assigned to respective samples and identified as valid sequences. The low-quality sequences were filtered out if they met the following criteria (Chen and Jiang, 2014): sequences with a length of <150 bp, average Phred scores of <20, ambiguous bases, and mononucleotide repeats of >8 bp. Paired-end reads were assembled using FLASH (Magoc and Salzberg, 2011). After chimera detection, the remaining high-quality sequences were clustered into OTUs at 97 % sequence identity by UCLUST (Edgar, 2010). A representative sequence was selected from each OTU using default parameters. For different types of sequences, each specific database is used as the template sequence for OTU classification status identification: the 16S rRNA gene database of bacteria and archaea was Silva database (Release115, http://www.arb-silva.de), The database of the ITS fungi sequence was the UNITE database (Release 5.0, https://unite.ut.ee/). An OTU table was further generated to record the abundance of each OTU in each sample and the taxonomy of these OTUs. OTUs containing less than 0.001 % of total sequences across all samples were discarded. To minimize the difference in sequencing depth across samples, an averaged, rounded rarefied OTU table was generated by averaging 100 evenly resampled OTU subsets under a 90 % minimum sequencing depth for further analysis.

2.6. Fusarium *mycotoxin extraction*

Fusarium mycotoxin was extracted according to a published method, with minor modifications (Varga et al., 2012). Briefly, ground and homogenized maize samples (5.00 ± 0.01 g) were added into a 50-mL centrifuge tube. Then, 20 mL of acetonitrile/water/formic acid (80:19.9:0.1, v/v/v) extraction solution was added. After shaking for 60 min at room temperature, the tubes were centrifuged at 3500 rpm for 5 min. Then, the raw extract was transferred into a new 50-mL centrifuge tube, and the residue was extracted with 20 mL of extraction solvent consisting of acetonitrile/water/formic acid (20:79.9:0.1, v/v/v). Afterwards, the samples were shaken again for 30 min at room temperature and centrifuged at 3500 rpm for 5 min. Then, the supernatant combined with the first extract was centrifuged at 3500 rpm for 5 min, and the supernatant was moved into a new tube and evaporated to dryness using a nitrogen gas stream at 55 °C. Finally, the residue was redissolved with 500 μL of water containing 5 mM ammonium acetate, passed through a 0.22-μm filter, and then injected into an LC-MS/MS device for analysis.

2.7. Fusarium *mycotoxin determination by LC-MS/MS*

Chromatographic separation was performed on an Acquity BEH-C18 column (50 mm × 2.1 mm, 1.7 μm) at 25 °C using an Acquity UPLC System. The mobile phase consisted of water containing 5 mM ammonium formate and 0.1 % formic acid (A) and 95 % acetonitrile containing 5 mM ammonium formate and 0.1 % formic acid (B), and the gradient elution program was as follows: 0 min of 10 % B, 0.5 min of 10 % B, 1.0 min of 50 % B, 2.5 min of 80 % B, 3.5 min of 95 % B, 5.0 min of 95 % B, and 5.2 min of 10 % B. The injection volume was 10 μL, and the total flow rate was 0.4000 mL/min.

MS/MS analysis of the target mycotoxins was performed using a SCIEX SelexION Triple Quad 5500 system in positive electrospray ionization (ESI+) multiple reaction monitoring (MRM) mode. The ionization source parameters were set as follows: curtain gas (CUR), 35.0 psi; collision gas (CAD), 8; ion spray voltage (IS), 5500.0 V; temperature (TEM), 500.0 °C; ion source gas 1 (GS1), 50.0 psi; and ion source gas 2 (GS2), 50.0 psi. The MS/MS parameters of detected mycotoxins were summarized in a table (Table S1).

2.8. *Data analyses*

Mean differences among the treatments were evaluated by ANOVA and Duncan's new multiple range-test at 5 % significance.

3. Results

3.1. *The effect of* T. asperellum *granules on the incidence of maize stalk rot and ear rot*

At the maturity stage, the control of maize stalk rot and ear rot by *T. asperellum* was investigated. The results showed that the disease index of maize stem rot and ear rot in the soil treatment with *T. asperellum* was significantly lower than that in the treatment with inoculated pathogens only (Fig. 1), with a decrease in severity of 49.83 % and 39.63 %, respectively. This indicated that *T. asperellum* was able to control both diseases at the same time.

3.2. *Effect of* T. asperellum *on the richness and diversity of the endophytic microbial community of the maize stem*

To study the systemic effect of *T. asperellum* on the endophytic microbial community of maize plants, high-throughput sequencing technology was used to determine the endophytic fungal and bacterial richness and diversity within maize stem tissue from the bottom to the top. A total of 76 196 ITS1 sequences and 887226V3–V4 16S rRNA sequences were analyzed, and the sequences were grouped into 2934 fungal and 24 248 bacterial OTUs, respectively. The Chao 1 and abundance-based coverage estimator (ACE) indexes of both fungi and bacteria in the stem samples from the *T. asperellum* treatment (inoculated *Fusarium* pathogens, T+F+) were higher than those from the control treatment without

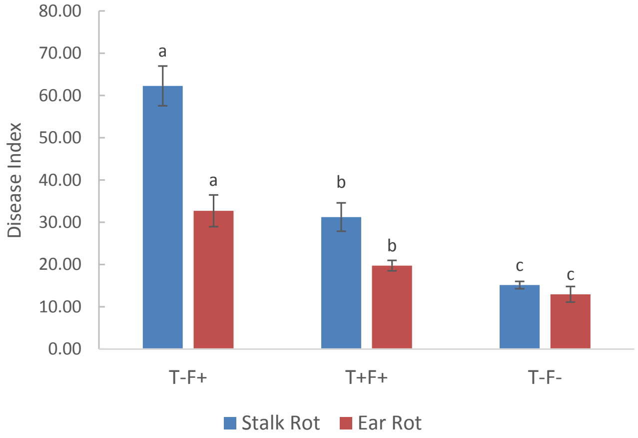

Fig. 1. The disease index of maize stalk rot and ear rot. T−F+: *Fusarium*; T+F+: *Trichoderma* granules and *Fusarium*; T−F−: Blank Control. Different letters above the bars indicate significant difference at $P < 0.05$ by Duncan's new multiple range test.

Trichoderma (inoculated *Fusarium* pathogens, T−F+) (Table S2). Furthermore, the increase in the Chao 1index gradually weakened along the stem from the bottom to the top (Fig. 2). Similarly, the increase in the diversity of endophytes measured as the Shannon index or Simpson's index caused by *T. asperellum* treatment was most significant in the basal stem section, followed by the middle stem section (Table S2). However, the change in the Simpson index of the endophytic microbial community in the near-ear stem tissues did not follow the trend mentioned above (Fig. 2), indicating that *T. asperellum* was able to promote the richness and diversity of the endophytic microbial community of the maize stem, especially that of the stem tissue near the soil.

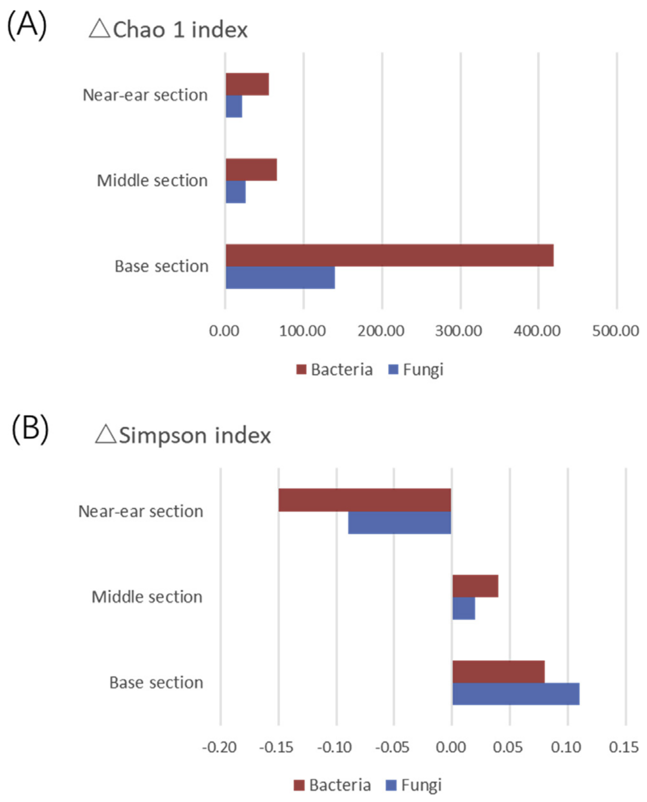

Fig. 2. The effect of *T. asperellum* GDFS1009 on the diversity of endophytic bacteria and fungi of different maize stem sections. (A) △Chao 1 index: the growth extent of Chao 1 index in different maize stem sections after rhizosphere soil treated with *T. asperellum* GDFS1009; (B) △Simpson index: the growth extent in Simpson index of different maize stem sections after rhizosphere soil treated with *T. asperellum* GDFS1009.

3.3. *Effect of* T. asperellum *biocontrol agent on endophytic fungi and bacteria in the basal stem section*

In the basal stem section, fungal sequences were mainly from the phyla *Basidiomycota* and *Ascomycota*, and the two phyla accounted for 99.35 % of the total fungal sequences (Fig. 3) (Table S3). *Ascomycota* and *Rozellomycota* were more abundant in *T. asperellum*-treated basal stem tissue, whereas *Basidiomycota* showed the opposite trend. Bacterial sequences were classified into a total of 25 different phyla, with the most dominant phyla being *Cyanobacteria* (74.99 %), *Proteobacteria* (17.55 %), *Firmicutes* (5.92 %), *Actinobacteria* (0.64 %), *Bacteroidetes* (0.46 %) and *Deinococcus-Thermus* (0.20 %). Moreover, *Firmicutes*, *Actinobacteria*, *Bacteroidetes*, *Acidobacteria*, *Deinococcus-Thermus*, *Verrucomicrobia*, *Armatimonadetes*, *Saccharibacteria* and *Gemmatimonadetes* were more abundant in the basal stem tissue of plants grown in *Trichoderma*-treated soil than in the control, whereas *Cyanobacteria* was more abundant in the control.

To analyze the difference between the *Trichoderma* treatment and the control, a classification tree was built using the Galaxy web application (http://huttenhower.sph.harvard.edu/galaxy/) (Fig. 4). The fungi in *Ascomycota*, *Hypocreales*, and *Eurotiomycetes* were more abundant in the *Trichoderma*-treated basal stem issue than in the control, and *Ascomycota* was hyperdominant in *Trichoderma*-treated basal stem issue, accounting for 6.22 % of the total fungal sequences (only 2.17 % in the control). Many more bacterial orders than fungal taxa showed a greater abundance in *Trichoderma*-treated basal stem tissue than in the control, such as *Firmicutes*, *Bacillales*, *Lactobacillales*, *Betaproteobacteria*, *Burkholderiales* and *Rhizobiales*.

3.4. *Effect of* T. asperellum *on the accumulation of DON and FB1 in maize plants*

To explore the effect of *T. asperellum* applied to soil on *Fusarium* mycotoxin accumulation inside the whole maize plant, the concentrations of deoxynivalenol (DON) and fumonisin B1 (FB1) in different parts of the stem and kernel were measured by liquid chromatography tandem mass spectrometry (LC-MS/MS). The DON and FB1 concentrations in either maize stem tissue or the kernel were significantly lower in the *T. asperellum* treatment than in the control (Table 1). In the basal stem, middle stem, near-ear stem and kernels that received the *T. asperellum* granule treatment, DON was 96.64 %, 98.75 %, 99.76 % and 100 % lower and FB1 was 61.65 %, 55.41 %, 51.80 % and 92.30 % lower than that in the control, respectively. This indicated that *T. asperellum* granules applied to the soil were able to highly inhibit mycotoxin accumulation throughout the maize plant.

4. Discussion

4.1. *The systemic control of maize stalk rot and ear rot by* T. asperellum

Stalk rot and ear rot caused by *Fusarium* commonly occur at the adult stage, requiring that the biocontrol agent be applied at the sowing stage to promote a highly continuous root colonization ability lasting until the late period of plant growth and to maintain lasting induced resistance to both diseases. In recent years, some beneficial *Trichoderma* spp., such as *T. harzianum* Th22 (Saravanakumar et al., 2018), *T. asperellum* ZJSX5003 (Li

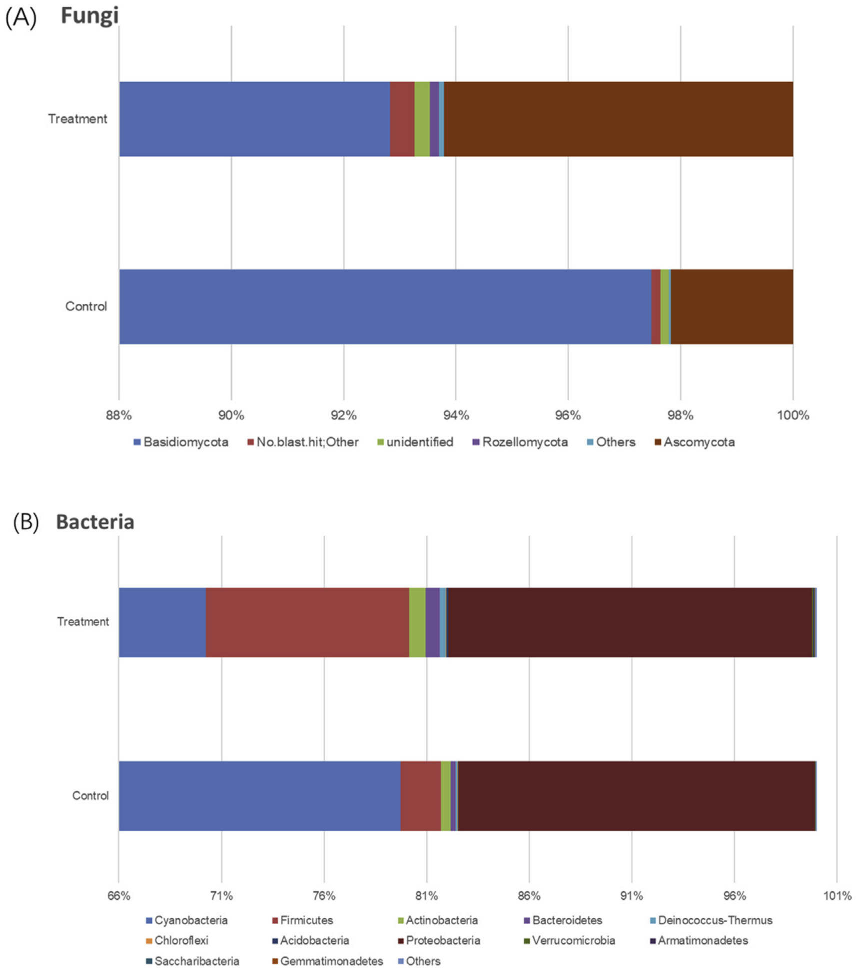

Fig. 3. Effect of *T. asperellum* GDFS1009 on average relative abundances of endophytic microorganism phyla of maize basal stem section. (A) Fungi (B) Bacteria. Note: “Others” includes phyla below 0.1 % of relative abundance and the unclassified phyla Treatment: rhizosphere soil treated with *T. asperellum* GDFS1009 and inoculated with *Fusarium* (*F. graminearum* and *F. verticillioides*); Control: inoculated with *Fusarium* (*F. graminearum* and *F. verticillioides*).

et al., 2016), *Trichoderma gamsii* 6085 and 6317, and *Trichoderma velutinum* 4837 (Matarese et al., 2012) have been shown to possess good *in vitro* antagonistic activities against *F. graminearum*. *Trichoderma* has already been demonstrated as useful for stalk rot control; however, few studies has shown that *Trichoderma* biocontrol agent is able to control ear rot through soil treatment that effectively controls stalk rot. This study demonstrated that *T. asperellum* biocontrol agent applied to soil at the sowing stage could significantly control ear rot. In 2016, our naturally infected field trail in Qujing, Yunnan province found that *T. asperellum* biocontrol agent had significant efficacy on maize ear rot, and in 2017, we found *T. asperellum* applied to soil at the sowing stage was capable of controlling maize leaf spot in Xinzhou, Shanxi province (not published). Our previous study found that the strain of *T. harzianum* overexpressing cellulase elicitor (*ThPH1*/*ThPh2*) and *hydrophobin1* used in seed treatment could induce upregulated expression of brassinolide (BR) and jasmonic acid/ethylene (JA/ET) in maize blades. The same study also found that the increase in resistance against leaf spot disease caused by coating maize seeds with (Saravanakumar et al., 2016). ISR-inducing SM1, produced by *Trichoderma virens* strain GV29-8, could downregulate the expression of *ZmLox3*, a negative regulator of ISR, in maize roots and promote ISR signal transduction from root to leaf sheath, enhancing resistance against *C. graminicola* (Constantino et al., 2013). The systemic resistance signal of maize induced by *T. asperellum* might be vertically transferred from the root to the apical parts of plants, eventually resulting in ear rot control.

4.2. T. asperellum *had a significant effect on the reprograming of the endogenous microbiome of the whole plant*

High-throughput sequencing technology was used to characterize the endophytic microbial community from the bottom to the top of the maize plant stem. For the same part of the stem, the richness of endophytic fungi and bacteria of *Trichoderma*-treated maize plants (inoculated *Fusarium* pathogens, T+F+)was higher than that of the control without *Trichoderma* (inoculated *Fusarium* pathogens, T-F+). Moreover, the degree of the increase in richness or diversity of endophytic fungi and bacteria was gradually lessened along the length of the stem, indicating that the origin of induced stem resistance was the *Trichoderma*-treated rhizosphere soil. The isolation and quantification of *Trichoderma* were not done, but the reads of *Trichoderma* genus in T+F+ treatment were more than that in T−F+ treatment (Table S3). Unfortunately, we did not perform high-throughput sequencing of the treatment of *T. asperellum* alone (T+F−) and the blank control (T−F−). However, we have recently carried out a more complete test in greenhouse pot assay. In pot assay (Fig. S1), we found the number of OTUs (operational taxonomic units) of the endophytic bacteria in maize

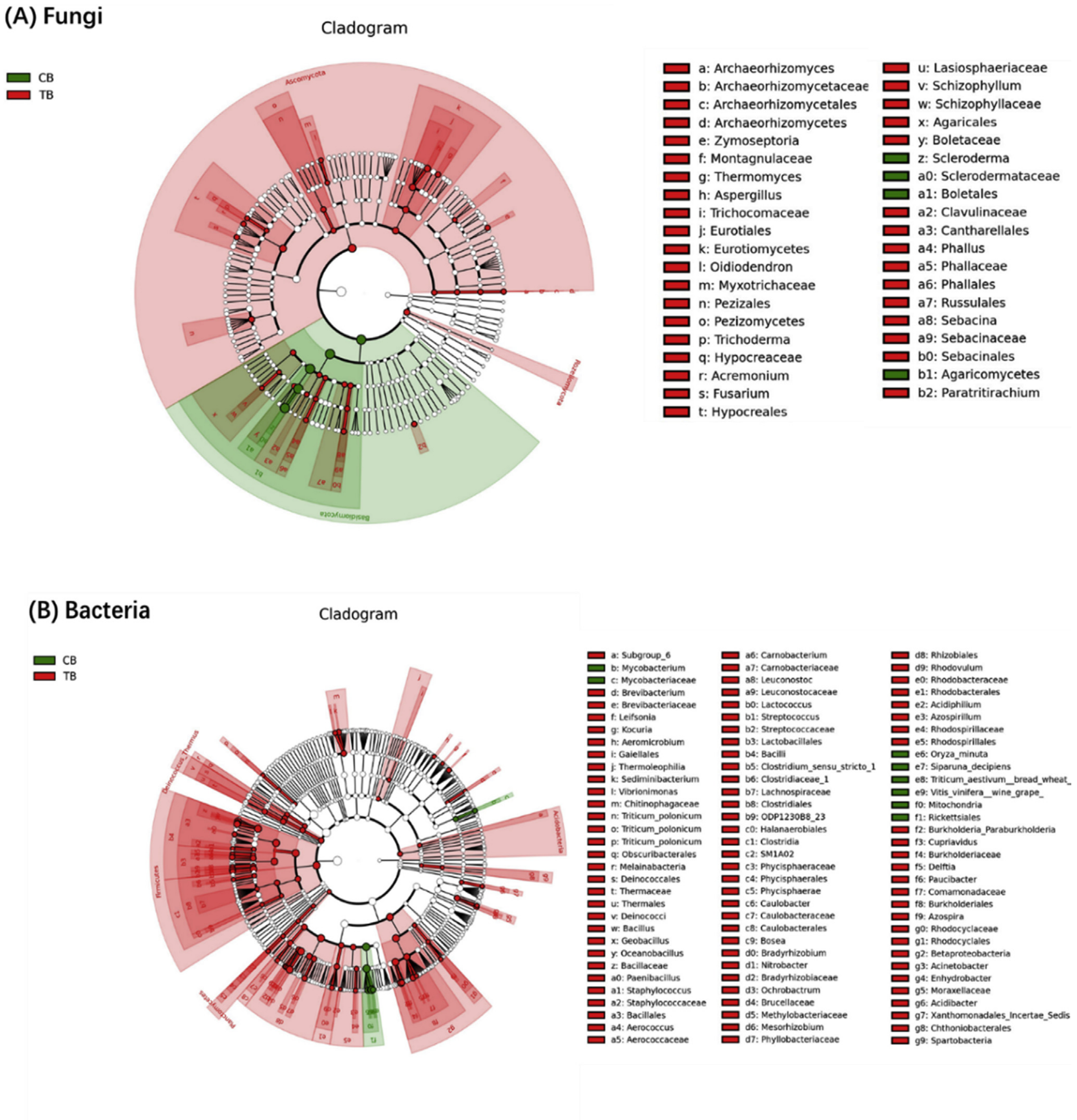

Fig. 4. The sample classification tree of endophytic microorganism of maize basal stem section. (A) Fungi; (B) Bacteria. TB: Maize basal stem section treated with *T. asperellum* GDFS1009 and *Fusarium* (T+F+); CB: Maize basal stem section treated with *Fusarium* (T−F+).

seedling basal stem section was significantly higher than that in other treatments when treated with *T. asperellum* and inoculated with *F. graminearum* (T+F+). And the endophytic bacteria OTUs number of the treatment with *T. asperellum* granules (not inoculated with *F. graminearum*, T+F−) was higher than that of the treatment only inoculated with *Fusarium* (T−F+) and blank control (T−F−), but there was no significant difference. Studies have shown that healthy plants have more microbial diversity than plants infected by pathogens, and endophytic microorganisms can colonize plant roots and move to different organs of plants through the vascular system (Gaiero et al., 2013; Laforest-Lapointe et al., 2017). Under sterile conditions, the *Arabidopsis* cuticle mutant *bdg* (BODYGUARD) lost most of its resistance to *Botrytis cinerea*, however, when the leaf microbes of nonsterile plants were washed off and reintroduced onto sterile leaf surfaces, *bdg* became significantly more resistant to *B. cinerea* (Ritpitakphong et al., 2016). It suggests that microbial diversity may be closely related to plant disease resistance.

Table 1
The accumulation of DON and FB1 in different parts of maize plants. B: Basal stem section, the first stem section above the ground); M: Middle stem section, the fourth stem section above the ground; N: Near-ear stem section, the stem section which the ear is located; K: Maize kernels.

Samples	FB1 (ng/g)		DON (ng/g)	
	T−F+	T + F+	T−F+	T + F+
B	1195.00 ± 1.67	458.33 ± 12.27	240.50 ± 3.50	8.09 ± 1.15
M	457.78 ± 24.37	204.11 ± 49.80	10.43 ± 1.59	0.13 ± 0.02
N	376.22 ± 30.69	181.33 ± 33.67	20.79 ± 4.15	0.05 ± 0.01
K	4286.67 ± 497.64	330.00 ± 10.00	0.59 ± 0.02	0.00 ± 0.00

T + F+: treated with *T. asperellum* GDFS1009 biocontrol agent and inoculated with *F. graminearum* and *F. verticillioides*; T-F+: inoculated with *F. graminearum* and *F. verticillioides*.

4.3. *The abundance and community composition changes in the endogenous microbial community induced by* T. asperellum *were important for whole-plant resistance*

This study found that maize plant endogenous microbial groups became diversified due to soil treatment involving *T. asperellum*. And *Basidiomycota* were more abundant in *Fusarium*-inoculated basal stem tissue (T−F+), and *Ascomycota* were more abundant in *T. asperellum*-treated basal stem tissue when inoculated *Fusarium* (T+F+). It is very likely that *T. asperellum* inhibits the growth and development of *Basidiomycota*. *Trichoderma* could control wood decay *Basidiomycetes* because of its wide arsenal of antagonistic mechanisms (Schmidt, 2007). And we speculated that the stimulation of *Trichoderma* and *Fusarium* and the decrease in the abundance of *Basidiomycota* increased the abundance of *Ascomycota*. Endophytic bacteria have been studied in almost every plant (Ryan et al., 2008). On review of 17 different plants, three major endophytic bacteria phyla *Actinobacteria*, *Proteobacteria*, and *Firmicutes* were most predominan (Suman et al., 2016). Based on the results of our study, the main groups of bacteria in maize basal stem section were *Cyanobacteria*, *Proteobacteria*, *Firmicutes*, *Actinobacteria*, and *Bacteroidetes*. *Firmicutes*, *Actinobacteria*, *Bacteroidetes*, and *Acidobacteria* were more abundant in the basal stem tissue of plants grown in *Trichoderma*-treated soil than in the control. *Proteobacteria* includes a wide variety of pathogens (Xia et al., 2016). *Acidobacteria* distributes in nature widely and plays an important role in various ecosystems (Zhou et al., 2004). The *Trichoderma*-primed plant microbiome led to recovery of the pathogen-damaged ecological system. Further analysis found that the abundance of the classical biocontrol microorganisms such as *Bacillus* (Sziderics et al., 2007) and *Burkholderia* (Compant et al., 2007) was markedly increased, as was predicted, which meant that the changes in one or more beneficial microbes also played a very important role in inducing plant resistance. Thus, the induced resistance against *Fusarium* diseases by *T. asperellum* may be the result of comprehensive induction resulting from diversity in the rhizosphere microflora and plant endogenous microbiota.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.funbio.2019.03.003.

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