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# Endophytic *Bacillus megaterium* triggers salicylic acid-dependent resistance and improves the rhizosphere bacterial community to mitigate rice spikelet rot disease

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ABSTRACT

Endophytes have been demonstrated to trigger salicylic acid-dependent resistance and alter rhizosphere bacterial communities. However, few comprehensive studies have been conducted to elucidate the interactions between the direct (systemic resistance) and indirect (rhizosphere microbiome) defenses triggered by endophytes. The present study aimed to illuminate the possible roles of endophytic *Bacillus megaterium* in rice resistance against rice spikelet rot disease (SRD), and to make inferences on the potential interactions between plant defenses and the rhizosphere microenvironment. An outdoor pot experiment was conducted to explore the degree to which endophytes mitigate disease in rice, where four combination treatments were designed (inoculation and non-inoculation with *B. megaterium*, and infection with and without *Fusarium proliferatum*). Rice plants and rhizosphere soil, at the heading stage, were sampled to measure the accumulation of defense-related enzymes, including *L*-phenylalanine ammonia-lyase (PAL), polyphenol oxidase (PPO), β-1,3-glucanase and chitinase, and to perform 16S rRNA Illumina sequencing. Defense-related enzyme activities in rice plants inoculated with *B. megaterium* showed local or systemic increases. Plant-beneficial bacteria were found to be enriched when rice plants were treated with the endophyte. A correlation between plant resistance and the rhizosphere microbiome was also observed. The present study highlights the significance of *B. megaterium* for plant defenses against SRD through dual mechanism — systemic resistance and rhizosphere microbiome. Inferences of this study elucidate correlations between plant defenses and the rhizosphere microenvironment, which were attributed to the presence of *B. megaterium*.

## 1. Introduction

Rice spikelet rot disease (SRD), caused by several fungi, such as *Fusarium proliferatum*, *Bipolaris australiensis*, *Curvularia lunata,* and *Alternaria tenuis* (Huang et al., 2011), leads to decreased yield in rice of 5–10%, with some yield losses up to 30% (Huang et al., 2012), and has received relatively less attention than other crop diseases worldwide. The pathogen first invades the rice plant at the heading stage, which results in grain discoloration, unfilled and malformed grains, and the accumulation of toxic compounds, such as deoxynivalenol (DON), that were reported to exhibit strong pathogenicity and carcinogenicity in mammals (Rheeder et al., 2002). Various disease management strategies have been employed to control disease development, but they were largely unsuccessful due to development of mutations in pathogenic races (George et al., 1997). Toxic residues are also concerning, which limits the application of antibiotics and chemicals (McManus et al., 2002). Biological control has become a popular research topic to sustainably manage pathogen infection through cost effective and environmentally friendly ways (Gnanamanickam, 2009).

Endophytes, which are defined as microbes living within plant tissues, have evolved to become beneficial plant-related microbes that may activate defense responses by enhancing the level of various phytohormones (Bastias et al., 2018). The salicylic acid (SA) and jasmonic acid (JA) are produced during the plant immune response and serve as central signal molecules that regulate plant defenses through sophisticated cross-talk (Doehlemann and Hemetsberger, 2013). Although beneficial microbes have been widely considered to trigger induced systemic resistance (ISR) which is JA-dependent (Martinez-Medina et al., 2017) and SA-independent (De Vleesschauwer et al., 2008), some plant growth promoting rhizobacteria (PGPR) were reported to trigger SA-dependent resistance that resembles pathogen induced systemic acquired resistance (SAR). For instance, PGPR

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*Paenibacillus Alvei* K165 (Tjamos, 2005) and *Pseudomonas fluorescens* SS101 (van de Mortel et al., 2012) were demonstrated to activate SA-dependent resistance of host plants and improve the expression of pathogenesis-related proteins (PRs) which function as prime markers in SA signaling pathway. It has also been reported that inoculation with *Gilmaniella* sp. may alleviate *Fusarium oxysporum*-mediated decline of SA accumulation in *Atractylodes lancea* (Ren et al., 2017), implying that endophytes may assist host plants in achieving slight advantages in the arms race against pathogens (Doehlemann and Hemetsberger, 2013).

Additionally, symbiotic microbes, including endophytes, may indirectly contribute to host plant resistance by facilitating nutrient absorption (Zhang et al., 2018) and shaping rhizosphere microbial community (Stringlis et al., 2018). *Arabidopsis thaliana* has been found to excrete scopoletin, upon colonization by beneficial rhizobacteia *P. simiae* WCS417 and *P. capeferrum* WCS358, to shape root microbiome assembly, leading to inhibition of the soil-borne fungal pathogens *Fusarium oxysporum* and *Verticillium dahliae* (Stringlis et al., 2018). Plant immunity signaling drives specific bacterial taxa to structure the root microbiome, where the foliar defense phytohormone SA is indispensable (Lebeis et al., 2015). According to a recent study, the composition and function of the geocaulosphere soil microbial community are closely associated with potato common scab severity in soil-root systems (Shi et al., 2019). A core microbiome containing a specific bacterial group among hosts of different resistance phenotypes has been verified to enable tomato plant resistance against *Ralstonia solanacearum,* which is the causal agent of bacterial wilt and can infect hundreds of plant species (Kwak et al., 2018). Evidence supports that plants protected by root-associated bacteria may achieve several advantages, such as the avoidance of growth retardation and energy costs, that may result from immune responses (Zhang et al., 2019a).

However, few comprehensive studies have elucidated the interactions between the direct (systemic resistance) and indirect (rhizosphere microbiome) defenses triggered by endophytes. *B. megaterium* acted as a kind of PGPR and has been reported to enhance host plants defenses against diverse pathogens (Kildea et al., 2008), while its mechanism of promoting rice resistance against SRD remains unclear. The situation may limite significance of exploiting microbial inoculants in agriculture. The present study aims to explore the possible roles of *B. megaterium* in plant resistance against SRD. It was hypothesized that rice resistance to SRD would be enhanced by *B. megaterium* inoculation through systemic resistance and microbial community alteration, and a correlation between plant immunity and the rhizosphere microbiome, driven by the endophyte, would be observed. This study illuminates the interactions between plant defenses and the rhizosphere microenvironment, which were attributed to the symbiotic relationship between *B. megaterium* and the rice plant.

## 2. Materials and methods

### 2.1. Fungal and bacterial strains

As described by He et al. (2013), the pathogenic fungus *F. proliferatum* strain E01A was isolated from diseased panicles of Wuyunjing 23 (a japonica subspecies of *Oryza sativa* L.) at the mature stage from experimental rice fields at Nanjing Normal University (32°6.318′ N, 118°54.88′ E) in Jiangsu Province, China. Infection of *F. proliferatum* was the primary cause of SRD in the experimental region among the four pathogens reported by Huang et al. (2011), *F. proliferatum, Bipolaris australiensis*, *Curvularia lunata,* and *Alternaria tenuis*. An re-inoculation experiment on healthy plants was used to confirm the pathogenicity of this isolate (Supplementary text 1).

The bacterial strains *B. megaterium* N8 was isolated from the paddy soil at the same site as previously described (He et al., 2013). The pathogen and the bacterium were identified on the basis of their 18S rDNA gene and 16S rDNA sequence, respectively (primers were listed in Table S1). The conserved gene sequencing of the strains was submitted to the GenBank database and accession numbers were allocated (*F. proliferatum* strain E01A, MK828253; *B. megaterium* strain N8: MK828351).

The fungus was stored at 4 °C on PDA (200 g $L^{-1}$ potato extract, 20 g $L^{-1}$ glucose and 20 g $L^{-1}$ agar, pH 7.0). To be applied in pot experiment, *F. proliferatum* was activated in PDB medium (200 g $L^{-1}$ potato extract and 20 g $L^{-1}$ glucose, pH 7.0) for 72 h at 28 °C in a shaker at 180 rpm (Ren et al., 2017). Spores were separated from the mycelia by passing through a sterile 0.22-μm pore size filter. Spore suspension was diluted to working concentration by $1 \times 10^5$ spores $mL^{-1}$.

*B. megaterium* N8 isolated from paddy soil at the site described above was stored in LB medium (10 g $L^{-1}$ tryptone, 5 g $L^{-1}$ yeast extract, 10 g $L^{-1}$ NaCl) mixed with 20% (*v*/v) glycerol at −80 °C. As described by Han et al. (2019) with some modifications, *B. megaterium* was activated in LB medium for 24 h at 37 °C in an orbital shaker at 180 rpm. Cells were harvested by centrifugation at 5000 rpm for 10 min at 4 °C and washed twice and then resuspended with sterile distilled water.

### 2.2. Colonization ability of B. megaterium

Rice seeds (*Oryza sativa* L., “Wuyunjing 23”, obtained from Jiangsu academy of agricultural sciences) were thoroughly surface disinfected after being dehulled as described by Hu et al. (2018) and subsequently transferred to 150 mL Erlenmeyer flasks, which contained 40 mL of 1/2 MS medium (1 L MS medium contains: $NH_4NO_3$ 1650 mg, $KNO_3$ 1900 mg, $CaCl_2{\cdot}2H_2O$ 440 mg, $MgSO_4{\cdot}7H_2O$ 370 mg, $KH_2PO_4$ 170 mg, KI 0.83 mg, $H_3BO_3$ 6.2 mg, $MnSO_4{\cdot}4H_2O$ 22.3 mg, $ZnSO_4{\cdot}7H_2O$ 8.6 mg, $Na_2MoO_4{\cdot}2H_2O$ 0.25 mg, $CuSO_4{\cdot}5H_2O$ 0.025 mg, $CoCl_2{\cdot}6H_2O$ 0.025 mg, $FeSO_4{\cdot}7H_2O$ 27.8 mg, $Na_2$-EDTA·$2H_2O$ 37.3 mg, cyclohexanehexol 100 mg, niacin acid 0.5 mg, pyridoxine hydrochloride 0.5 mg, aneurine hydrochloride 0.1 mg, Glycine 2.0 mg). After 7 days of growth, 50 μL of the inoculation fluid of *B. megaterium* ($OD_{600}$ = 0.8, $2.35 \times 10^7$ CFU $mL^{-1}$) was added to each flask, while sterile distilled water was applied to the control. After 2 weeks, the roots of seedlings were collected and rinsed with a 0.1 M phosphoric acid buffer solution (pH 7.0). A 4% (*v*/v) glutaraldehyde solution was used to fix the plant tissues, and scanning electron microscopy (JSM-5610LV, JEOL corporation, Japan) was performed to observe a cross section of the roots (Mansvelt and Hattingh, 1987).

### 2.3. Outdoor pot experimental design and plant cultivation

The experiment had the following treatments with seven replications: i) Field condition: non-inoculated with *B. megaterium* and non-infected by *F. proliferatum*; ii) *B. meg*: inoculated with *B. megaterium* and non-infected by *F. proliferatum*; iii) *F. pro*: non-inoculated with *B. megaterium* and infected by *F. proliferatum*; and iv) *B. meg* + *F. pro*: inoculated with *B. megaterium* and infected by *F. proliferatum*. A completely randomized block design with factorial arrangement was performed to prevent cross infection (Zhang et al., 2019c).

Wuyunjing 23, which is widely cultivated in the south-eastern region of China was used in this experiment. Surface disinfected seeds were germinated and grown in Petri dishes in a growth chamber (29 °C days and 25 °C nights, 80% relative moisture, 16 h light /8 h dark photoperiod at 250 μmol $m^{-2}$ $s^{-1}$). Rice seedlings that were 14-days-old were randomly divided into two groups and transferred to pots (volume 500 mL; 5 seedlings per pot) that were filled with sterilized vermiculite. The inoculated group was treated with 10 mL of *B. megaterium* inoculation fluid ($OD_{600}$ = 0.8, $2.35 \times 10^7$ CFU $mL^{-1}$) by directly adding into the sterilized vermiculite (size: 3–6 mm, pH 7.0) (Yasuda et al., 2014). The non-inoculated group was treated with 10 mL of sterile distilled water, which was added to each pot as a control. After 21 days of growth, seedlings of similar developmental stages were transplanted into plastic pots (21 cm in diameter, 27 cm in height; 5

hills per pot; 7 pots per treatment), which contained about 15 kg of paddy soil, and were grown under outdoor field conditions with natural light.

The inoculated and non-inoculated groups were further divided into two groups each. The *F. proliferatum* infected treatments, which contained *B. megaterium* inoculated and non-inoculated groups, were sprayed with 100 mL of a spore suspension ($1 \times 10^5$ spores $mL^{-1}$) per pot, on their panicles at 7 days after entering the heading stage. For the *F. proliferatum* non-infected treatments, 100 mL of sterile distilled water was applied as a control. The *F. proliferatum* infected and non-infected treatment groups were separately placed to avoid cross contamination in the experimental process. Paddy soil (soil texture: loamy sand; fineness of sand: 77%, fineness of clay: 5%, fineness of silt: 18%) was applied in pots with the following nutrient parameters (detected by Jiangsu academy of agricultural sciences): pH 6.603, the organic matter content 1.22%, total N 0.93 g $kg^{-1}$, total P 0.39 g $kg^{-1}$, total K 0.73 g $kg^{-1}$, available N 43.757 mg $kg^{-1}$, available P 24.85 mg $kg^{-1}$ and available K 76.92 mg $kg^{-1}$. Microbial population of the soil was calculated: bacteria, $1.09 \pm 0.05 \times 10^7$ CFU $g^{-1}$; fungi, $3.3 \pm 0.4 \times 10^4$ CFU $g^{-1}$. The fertilization strategy is described in the Supplementary text 1.

The experiment lasted about seven months (April 7—October 27), covering the whole growth period of rice, which included the seedling stage (S1), the tillering stage (S2), the heading stage (S3), and the maturing stage (S4). The rice was irrigated every other day until 45 days before harvesting. Every pot was filled with water when irrigating.

### 2.4. Sample collection and preparation

Plant sample of each stage were collected from different pots at 5.00 pm and separated into roots and shoots and then washed. Parts of the samples were dried for analyses of biomass and the others were immediately frozen in liquid nitrogen and stored at −80 °C until required. The grain yield was measured after harvesting all plants in pots at maturing stage.

Rice root was roughly washed and soaked with 150 mL 0.1 M phosphoric acid buffer solution (pH 7.0) in a 500 mL Erlenmeyer flasks (5 g root per flask) for 2.5 h at 28 °C in a shaker at 180 rpm, followed by bath sonication at 50 Hz for 30 s. Roots were removed and turbid solution was centrifuged at 8000 rpm for 20 min at 4 °C. Sediment was stored at −80 °C after vacuum freeze-drying and supernate was stored at 4 °C for further use.

### 2.5. Degree of disease, deoxynivalenol (DON) content, and F. proliferatum abundance in rice grains

The methods of quantitatively calculating the incidence and index of spikelet rot disease (SRD) are described in the Supplementary text 1, which refers to Huang et al. (2011).

Ten g milled grains were homogenized with 20 mL 70% (*v*/v) methanol and subjected to bath sonication at 50 Hz for 3 min, and the supernatant was obtained by centrifuging at 12,000 *g* for 20 min at 4 °C. A Vomitoxin ELISA Quantitative Kit (MB-4662A, Jiangsu Mei Biao Biological Technology Co., Ltd., China) was used to determine vomitoxin concentrations in the grain according to the manufacturer's instructions.

A EZUP Column Type Plant Genome DNA Extraction Kit (B518261, Sangon Biotech (Shanghai) Co., Ltd., China) was used to extract the total DNA of milled grains. The relative amounts of *F. proliferatum* in all samples were determined with three replicates using a Real-Time PCR System (Applied Biosystems), according to the methods of Yang et al. (2015), with some modifications. A pair of *F. proliferatum* specific primers (SPF-F: 5'-CAACGGTTTCATTTCTGC-3′ and SPF-R: 5'-TCTAATCT CAAGCAACCCAC-3′) that spanned about 145 bp of a *F. proliferatum* specific gene were used in this assay. A plasmid standard containing the target region was generated using a pEASY®-Blunt Cloning Kit (Beijing TransGen Biotech Co., Ltd., China) and was cloned into *Escherichia coli* DH5α. The copy numbers of the *F. proliferatum* specific gene were calculated based on the standard curves. *F. proliferatum* abundance were expressed as copies per ng gDNA (copies $ng^{-1}$ gDNA).

### 2.6. Analysis of the rhizosphere bacterial community

The structure of the rhizosphere bacterial community among plants with diverse disease degrees was analyzed based on 16S rRNA Illumina sequencing. Total DNA was extracted from 0.5 g samples of soil using an UltraClean Soil DNA isolation Kit (MoBio Loker Ave West, Carlsbad, CA, USA). The V3–V4 region of the 16S rRNA genes were amplified with barcoded universal primers (338F: 5′-ACTCCTACGGGAGGCAG CAG-3′ and 806R: 5′-GGACTACHVGGGTWTCTAAT-3′) that spanned about 468 bp of the 16S rRNA gene (Nan et al., 2016). The amplified PCR products were further processed for purification and pyrosequencing at the Shanghai Majorbio Bio-pharm Technology Co., Ltd. in China. Illumina sequencing was performed with three replications, using samples from three independent experiments. Data analyses were performed using the online platform of Majorbio I-Sanger Cloud Platform (cloud.majorbio.com). Algorithm parameters used in these analyses were shown in figure captions.

Bacterial strains were isolated to perform in vitro and in vivo experiments and their ability to promote plant-growth and antagonize fungus were assessed. The methods of rhizosphere bacterial isolations, pathogen antagonism tests, and growth promotion trials are described in the Supplementary text 1. Strains showing ability of either pathogen antagonism or growth promotion were identified by 16S rDNA gene sequencing (TSINGKE Biological Technology Co., Ltd., Beijing, China) and a phylogenetic analysis (MEGA 5.05).

### 2.7. Measurement of the defense related enzymes

The defense-related enzyme activities and related gene expression in rice plants at the heading stage were evaluated to assess the systemic defenses triggered by *B. megaterium*. Fresh tissues (0.5 g) collected from rice root and rice leave at heading stage were grinded in liquid nitrogen to break open the cells, and were immediately homogenized with 2.5 mL chilled 100 mM sodium borate buffer (pH 8.8), supplemented with 1 mM EDTA, 5 mM β-mercaptoethanol, and 5% (*w*/*v*) polyvinyl pyrrolidone. The crude enzyme solution was obtained by centrifuging at 12,000 *g* for 20 min at 4 °C. Phenylalanine Ammonia-Lyase (PAL) activity was measured by monitoring the reaction product, trans-cinnamate (Hahlbrock and Ragg, 1975). Controls were conducted by incubating the tissue extract without *L*-phenylalanine. One unit of PAL activity was defined as the amount that caused an increase of 0.01 in A290 per hour. PAL activity was expressed as delta-OD per hour per gram fresh weight ($\triangle$OD $h^{-1}$ $g^{-1}$ FW).

The Polyphenol Oxidase (PPO) activity was measured using a Polyphenol Oxidase Colorimetric Assay Kit (A136, Nanjing Jiancheng Bioengineering Institute Co., Ltd., China). Chitinase activity was measured using a Chitinase Colorimetric Assay Kit (A139, Nanjing Jiancheng Bioengineering Institute Co., Ltd., China). The same previously described extraction of the crude enzyme solution was used here. PPO and chitinase activities were expressed as a unit per gram fresh weight (U $g^{-1}$ FW).

Grinded fresh tissues (1 g) were homogenized with 5 mL 0.05 M sodium acetate buffer (pH 5.0). The extraction of the crude enzyme solution was the same as previously described. The β-1,3-glucanase activity was measured by monitoring the formation of free reducing groups (sugars) (Stieglitz and Stern, 1973). Controls were conducted by incubating the laminarin alone and by incubating the tissue extract without laminarin. One unit of β-1,3-glucanase activity was defined as the amount that caused an increase of 0.01 in A540 per min. The β-1,3-glucanase activity was expressed as delta-OD per minute per gram fresh

weight ($\triangle$OD $min^{-1}$ $g^{-1}$ FW).

### 2.8. RNA isolation and real-time quantitative PCR

To determine the expression levels of defense related genes (*OsPAL1, OsPPO, OsPR1ai,* and *OsPR2*), a TRIZOL reagent was used to extract total RNA and an EasyScript RT reagent kit with gDNA removal (TransGen Biotech) was used to synthesize the first-strand complementary DNA (cDNA). The qRT-PCR was conducted using the StepOne Real-time PCR system (Applied Biosystems). The reaction mixture (20 mL) contained 10 mL of SYBR green probe (AceQ qPCR SYBR Green Master Mix (High ROX Premixed), Vazyme), 2 mL of cDNA, and 0.4 mL of each primer (Ji et al., 2014; Liu et al., 2017) (Sequences of primers used can be found in Table S1). The *ubq5* gene was used as the internal control, where the relative mRNA abundancies of the defense related genes were normalized to the levels of *ubq5*. Three replicates were completed for each sample. The relative expression of the target genes was calculated using the $\triangle$Ct method (Livak and Schmittgen, 2001).

### 2.9. Statistical analyses

The means ± SE from at least three biological replicates were used to present the experimental data. Data were subjected to analysis of variance via Tukey's multiple comparison method at 5% level or Student's *t*-test using SPSS software (SPSS Inc., Chicago, IL, USA) to determine the significant differences. Correlations were identified by Pearson correlation analysis. A two-way ANOVA was performed when groups were compared (Zhang et al., 2019b).

## 3. Results

### 3.1. Scanning electron microscopy of B. megaterium in rice roots

The presence of *B. megaterium* in rice roots was observed by scanning electron microscopy. In roots, the bacteria were visible in the interior of cortical cells and biofilms were observed to fill the cortical gap (Fig. 1a). Bacteria and biofilms were not present in the control samples that were cultivated in sterile conditions (Fig. 1b).

### 3.2. Effects of B. megaterium on the degree of disease and yield parameters

Apparently empty grains and browning of grains were observed in the Field condition and the *F. pro* treatment, which were significantly reduced following *B. megaterium* inoculation (Fig. 2a). The disease incidence in the *F. pro* treatment was increased by 45.82% compared to the Field condition (Fig. 2a). The disease incidence and the disease index in the *B. meg* treatment were decreased by 24.10% and 55.59% compared to the Field condition, respectively (Table 1). These parameters were decreased by 8.47% and 56.73%, respectively, in the *B. meg* + *F. pro* treatment when compared to the *F. pro* treatment (Table 1).

The DON content of grains was significantly lower in the treatments with the endophyte inoculation than in the non-inoculation treatments (Field condition, 553.97 ± 80.46 ng $kg^{-1}$ vs. *B. meg*, 39.92 ± 25.48 ng $kg^{-1}$; *F. pro*, 541.86 ± 47.62 ng $kg^{-1}$ vs. *B. meg* + *F. pro*, 18.17 ± 7.78 ng $kg^{-1}$) (Fig. 2b). For the *F. proliteratum* abundancies in grains, the treatments with *F. proliteratum* had higher values than the treatments without the fungus (Field condition, $2.28 \times 10^4$ copies $ng^{-1}$ gDNA vs. *B. meg*, $1.80 \times 10^4$ copies $ng^{-1}$ gDNA; *F. pro*, $9.00 \times 10^4$ copies $ng^{-1}$ gDNA vs. *B. meg* + *F. pro*, $6.06 \times 10^4$ copies $ng^{-1}$ gDNA) (Fig. 2c). The statistical analysis showed significant differences ($P < 0.01$) between the *F. proliferatum* infected and non-infected groups.

It was observed that the dry weight of the rice shoot, the number of panicles per plant, the panicle weight per plant, and the seed setting rate of the *B. meg* + *F. pro* treatment were increased by 26.54%, 21.83%, 47.84%, and 8.14%, respectively, at the maturing stage compared to the *F. pro* treatment (Table S3). No apparent differences were observed between the Field condition and *B. meg* treatment (Table S3). Notably, the thousand grain weight of the *B. meg* treatment showed a significant increase when compared to the Field condition, where this measurement for the *B. meg* + *F. pro* treatment showed a decrease when compared to *F. pro* treatment (Table S3).

### 3.3. Structure of the rice rhizosphere bacterial community

A total of 388,373 validated bacterial sequences were obtained after quality filtering. Sequence read numbers per sample ranged from 27,703 to 41,250 (mean ± SE = 31,673 ± 2261 in the Field condition; 31,447 ± 1346 in the *B. meg*; 30,408 ± 1363 in the *F. pro*; 35,930 ± 2774 in the *B. meg* + *F. pro*). According to the principal coordinate analysis (PCoA), clustering of the bacterial communities showed distinct differences among the four treatments, with the exception of a few stands (Fig. 3a). The Bray-Curtis PCoA explained 76.3% (PC1 and PC2) of the total variation within the rhizosphere bacterial community. Clear separations based on the partial least-squares discriminant analysis were observed among the four treatments (Fig. 3b). Although bacterial community structure in the *B. meg* treatment and in the *B. meg* + *F. pro* treatment showed no difference in COMP1, these communities formed distinct groups compared to those of the Field condition and the *F. pro* treatment, and separation between the *B. meg* and *B. meg* + *F. pro* treatments could be found on COMP2.

Among the 32 bacterial phyla identified, the most frequently detected phyla in all treatments were *Proteobacteria* (28.63% - 65.96% in all treatments), *Chloroflexi* (7.65% - 23.98%), *Acidobacteria* (5.84% - 22.24%), and *Actinobacteria* (5.51% - 22.94%), which accounted for 76.38–89.71% of the total identified bacteria (Fig. 4a, Fig. S1a). *Proteobacteria,* which was the dominant phylum in all four treatments, showed lower relative abundancies in the *F. pro* treatment (30.92%) than in the *B. meg* + *F. pro* treatment (40.40%), with similar results observed for *Actinobacteria* (9.18% in the *F. pro* treatment and 16.40%

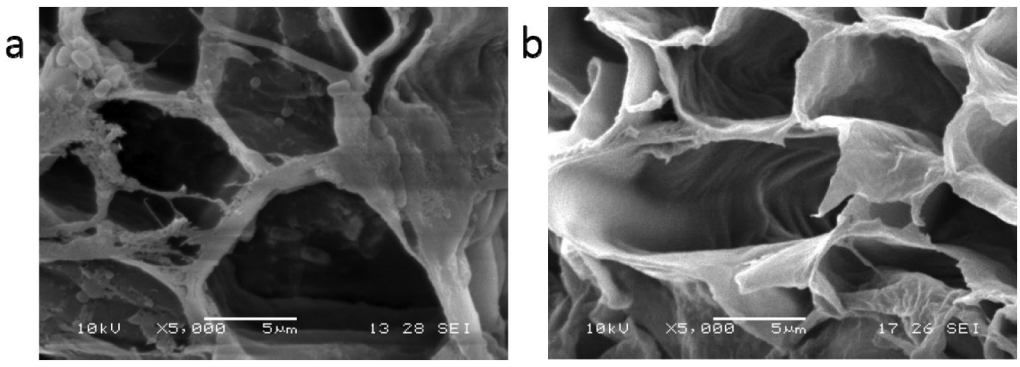


**Fig. 1.** Scanning electron microscopy analysis of bacterial endophyte *Bacillus megaterium*-colonized rice seedlings. a Bacterial cells in rice seedling root grown inoculated with *B. megaterium*. 7-days-old rice plant was inoculated with *B. megaterium* and then cultivated for 2 weeks. b No bacterium in rice seedling root grown with non-inoculation. 7-days-old rice plant was treated with sterile distilled water and then cultivated for 2 weeks.

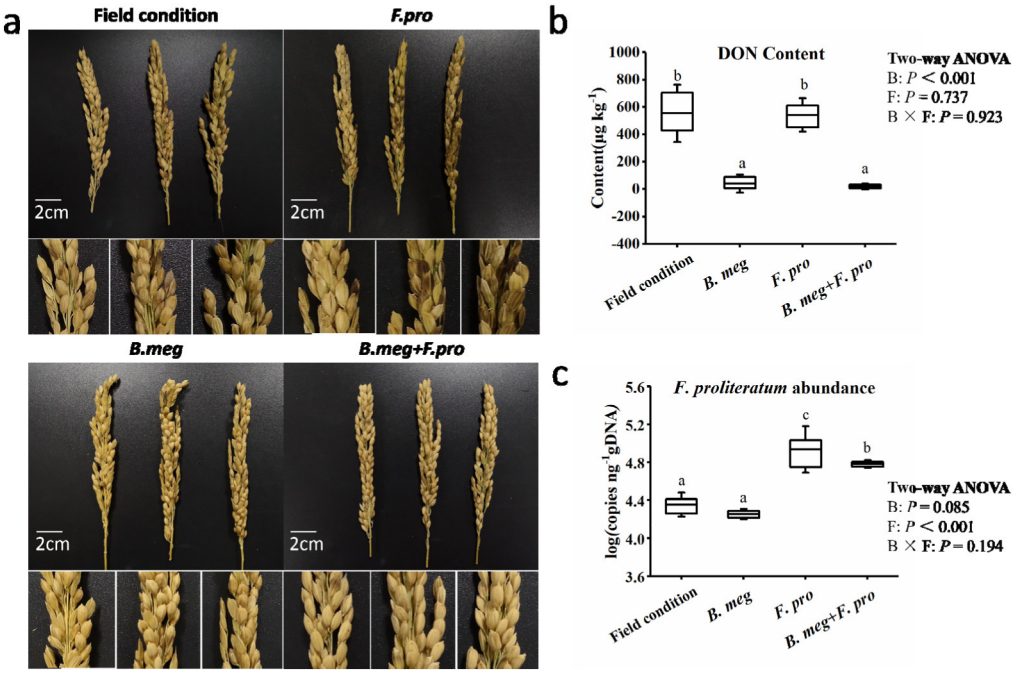


**Fig. 2.** Disease degree of rice grains at the ripening stage. **a** Disease characteristics of rice panicles among four treatment. **b** DON Content of rice grains. **c** Pathogen abundance of rice grains. Values are the means ± SE from three biological replicates ($P < 0.05$, one-way analysis of variance (ANOVA) with Tukey's test). Two-way ANOVA, F means *F. pro* treatment level, B means *B. meg* treatment level. Field condition, non-treated; *B. meg*, treated with *B. megaterium*; *F. pro*, treated by *F. proliferatum*; and *B. meg* + *F. pro*, treated with both *B. megaterium* and *F. proliferatum*.

**Table 1**
SRD Degree of rice panicles at maturing stage: Disease Incidence and Disease Index.

| | Disease incidence % | Disease index |
|---|---|---|
| Field condition | 91.78 ± 0.83% bc | 27.549 ± 1.14 b |
| *B. meg* | 69.66 ± 2.98% a | 12.234 ± 1.26 a |
| *F. pro* | 96.92 ± 1.27% c | 40.171 ± 3.07 c |
| *B. meg* + *F. pro* | 88.71 ± 1.74% a | 17.383 ± 1.60 a |

Data are the means ± SE from three biological replicates. Field condition, non-treated; *B. meg*, treated with *B. megaterium*; *F. pro*, treated by *F. proliferatum*; and *B. meg* + *F. pro*, treated with both *B. megaterium* and *F. proliferatum*. Different letters means significantly difference ($P < 0.05$).

in the *B. meg* + *F. pro* treatment) and *Bacteroidetes* (4.20% in the *F. pro* treatment and 5.77% in the *B. meg* + *F. pro* treatment). *Chloroflexi*, *Acidobacteria,* and *Gemmatimonadetes* accounted for 21.20%, 19.60%, and 4.86% of the identified bacteria in the *F. pro* treatment, respectively, which were higher relative abundancies than those observed in the *B. meg* + *F. pro* treatment (13.53%, 14.17%, and 2.90%, respectively).

The bacterial abundancies at the genus level shown in Circos and LEfSe were analyzed to identify the specific bacterial differences between the non-inoculation and inoculation treatments of *B. megaterium* (Fig. 4b, Fig. S1b, Table S4). The relatively abundant genera in the *F. pro* treatment included *Acidobacteria*, *Anaerolineaceae*, *KD4–96*, *Nitrosomonadaceae*, *Sphingomonas*, *RB41*, *Azoarcus*, *Bryobacter,* and *Gemmatimonadetes* (Fig. S1b, Table S4). However, some these genera could be cultured in vitro and some were unclassified at genus level in the Silva database. Several genera were also found to be more abundant in the *B. meg* + *F. pro* treatment compared to the *F. pro* treatment, including *Pseudarthrobacter*, *Aeromonas*, *Pseudomonas*, *Tolumonas*, *Vogesella*, *Enterobacter*, *Rheinheimera*, *Chryseobacterium,* and *Acinetobacter* (Fig. S1b, Table S4). It is remarkable that *Bacillus* was less abundant in the Field condition than in any other treatment, but the genus was not one of the dominant species in all four treatments (Fig. S1b, Table S4). Additionally, the rhizosphere soil was shown to have a higher species richness in the *B. meg* + *F. pro* treatment than in the *F. pro* treatment, according to the Simpson, ACE, and Chao indices (Table S5). The raw Illumina sequencing data was submitted to NCBI (SRA accession: PRJNA534166).

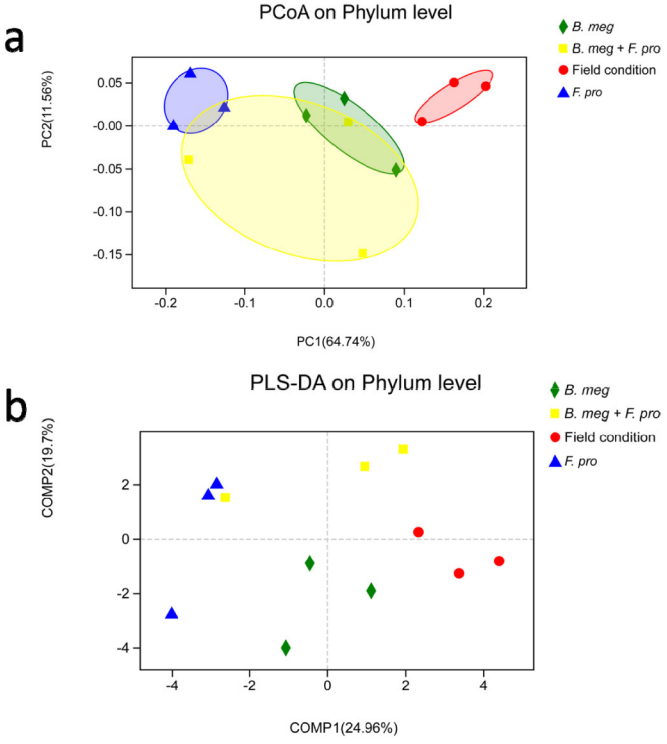


**Fig. 3.** Comparative analysis of samples among different treatment. a Principal co-ordinates analysis (PCoA) based on Bray-Curtis dissimilarity distance measures. b Partial least squares discrimination analysis on Phylum level. Field condition, non-treated; *B. meg*, treated with *B. megaterium*; *F. pro*, treated by *F. proliferatum*; and *B. meg* + *F. pro*, treated with both *B. megaterium* and *F. proliferatum*.

### *3.4. Ability of rhizosphere bacteria associated with B. megaterium strain N8 to antagonize the pathogen and promote plant growth*

A total of 108 bacterial strains were isolated from rhizosphere soil,

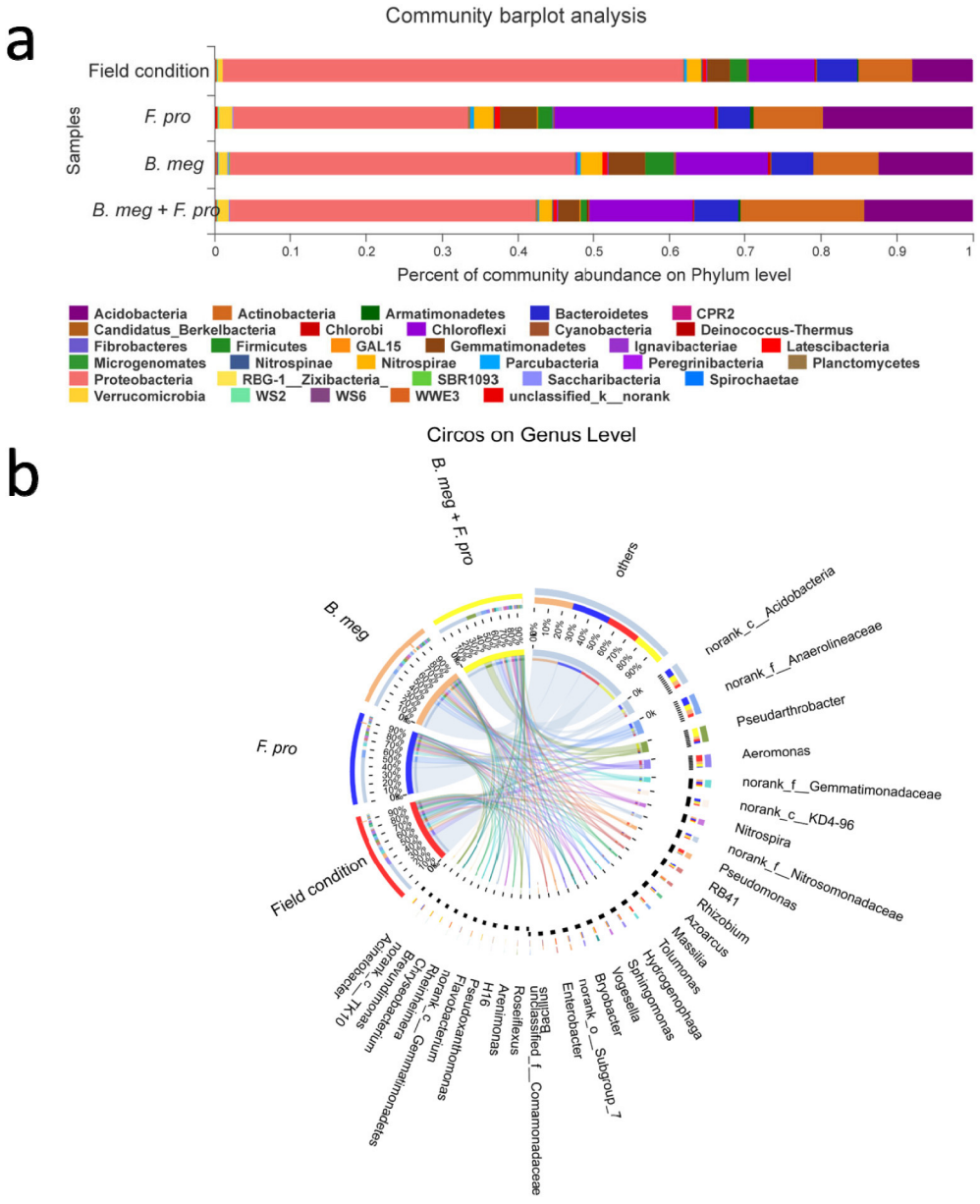


**Fig. 4.** Comparison of the rhizosphere bacterial community structures in the mesocosm experiment based on illumina quencing of 16S rDNA amplicons. **a** Community Barplot analysis on Phylum level. Grouped samples are calculated by means value. Species whose abundance ratio is less than 0.0001 in all samples are classified as 'other'. **b** Samples and Species Co-occurrence Diagram (Circos) at Genus level. The left half circle represents the species composition in the samples; the right half circle represents the distribution proportion of species in different samples at the taxonomic level. The corresponding data is listed in Table S3. Field condition, non-treated; *B. meg*, treated with *B. megaterium*; *F. pro*, treated by *F. proliferatum*; and *B. meg* + *F. pro*, treated with both *B. megaterium* and *F. proliferatum*.

of which 32 strains showed growth inhibition against *F. proliferatum* in vitro (12 strains in Field condition, 6 strains in the *B. meg* treatment, 3 strains in the *F. pro* treatment, and 11 strains in the *B. meg* + *F. pro* treatment) (Table S6). Three strains isolated from the rhizosphere soil of the Field condition showed growth promotion of seedling shoots and 3 strains showed growth inhibition on seedling roots, while only one strain isolated from the rhizosphere soil of the *B. meg* treatment showed growth inhibition on the seedling roots and all strains showed promotion on the shoots (Fig. 5, Table S7). For bacteria isolated from the rhizosphere soil of the *F. pro* treatment, 4 and 3 strains were found to inhibit the growth of shoots and roots, respectively. In contrast, all strains isolated from the rhizosphere soil of the *B. meg* + *F. pro* treatment promoted the growth of shoots and 2 strains inhibited the growth of roots (Fig. 5, Table S7).

Thirty-seven strains that showed the pathogen antagonism ability or growth promotion ability were identified by 16S rDNA gene sequencing and a phylogenetic tree was constructed to demonstrate their evolutionary classifications (Fig. S2). Sequences that were more than 95% similar within each single strain were downloaded from the NCBI database to use as references in the phylogenetic tree. Of the 37 sequences shown, 7 sequences aligned with *Pseudomonas* spp., which consisted of strains isolated from the Field condition and the *F. pro* treatment. Seven sequences aligned with *Agrobacterium* spp., which mostly consisted of strains isolated from the *B. meg* + *F. pro* treatment. Five sequences aligned with *Pseudarthrobacter* spp., which consisted of strains isolated from all four treatments. Four sequences aligned with *Bacillus* spp., which mostly consisted of strains isolated from the *B. meg* and *B. meg* + *F. pro* treatments. Other sequences were found to align with *Acinetobacter* spp., *Enterobacter* spp., *Leclercia* spp., *Pantoea* spp., *Kluyvera* spp., *Pseudomonas* spp., *Sphingomonas* spp., *Micrococcus* spp. etc. The 37 sequences were submitted to NCBI (accession numbers are listed in Table S7).

### 3.5. Local and systemic defense enzyme activities and related gene expressions

For rice shoots at the heading stage, the PAL activity was shown to be significantly higher in the *B. meg* treatment and the *B. meg* + *F. pro* treatment when compared to that in the Field condition, but no significant differences were observed among the *B. meg*, *F. pro,* and *B.*

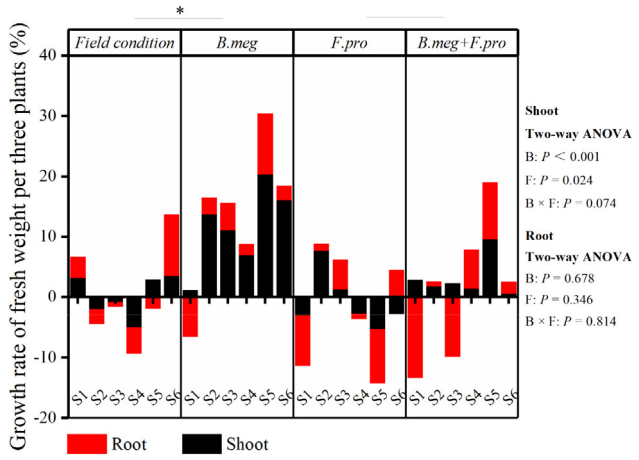


**Fig. 5.** Growth rate of fresh weight per three plants treated by rhizosphere bacteria after 14 days relative to control. Abscissa label means serial number of strains isolated from rhizosphere soil of four treatments, every columns represent different strains which were all listed in Table S4. " * " means significantly difference ($P < 0.05$). Two-way ANOVA, "F" means *F. pro* treatment level, "B" means *B. meg* treatment level. Field condition, non-treated; *B. meg*, treated with *B. megaterium*; *F. pro*, treated by *F. proliferatum*; and *B. meg + F. pro*, treated with both *B. megaterium* and *F. proliferatum*.

*meg + F. pro* treatments (Fig. 6a). The PAL activity of rice roots in the *F. pro* treatment was observed to be significantly higher than that of any other treatment (Fig. 6a). The relative expression levels of *OsPAL1* in all treatments supported the PAL activity results (Fig. 6e, i).

The PPO activity in rice shoots in the *F. pro* treatment was significantly higher ($P < 0.05$) than that in the *B. meg + F. pro* treatment and in the Field condition. No apparent differences in PPO activity were observed among the four treatments for rice root tissues (Fig. 6b). The relative expression levels of *OsPPO* were consistent with the PPO activity results (Fig. 6f, j).

Chitinase activity in rice shoots in the *B. meg + F. pro* treatment were shown to be significantly higher ($P < 0.05$) than that of any other treatment, while no apparent differences were observed among the four treatments in rice root tissues (Fig. 6c), which was verified by the relative expression levels *OsPR1a* in all treatments (Fig. 6g, k).

For rice shoots at the heading stage, the β-1,3-glucanase activity was significantly higher ($P < 0.05$) in the *B. meg*, *F. pro,* and *B. meg + F. pro* treatments compared to the Field condition, while no significant differences were observed among these three treatments (Fig. 6d). For the rice root tissues, the β-1,3-glucanase activity of treatments with the endophyte inoculation were significantly higher than those with no inoculation, regardless of whether they were infected *F. proliferatum* or not (Fig. 6d). The β-1,3-glucanase activity of rice root tissues was significantly higher in the *F. pro* treatment than that in the field condition. The relative expression levels of *OsPR2* were mostly consistent with the β-1,3-glucanase activity results (Fig. 6h, l).

### 3.6. Correlation between the induction system resistance and the bacterial community

According to RDA analysis on the genus level, the relative abundancies in the *F. pro* and the *B. meg + F. pro* treatments correlated positively with the activities of all four enzymes in rice shoot, while the Field condition and *B. meg* treatment showed negative correlations (Fig. 7a). The RDA and Spearman correlation heatmap showed that *Acidobacteria*, *Pseudarthrobacter,* and *RB41* had significantly positive correlations with the activities of all four enzymes, while *Aeromonas* and *Rhizobium* had negative correlations (Fig. 7a, Fig. S3a). For root enzyme activities, the relative abundancies in the *F. pro* and *B. meg + F.*

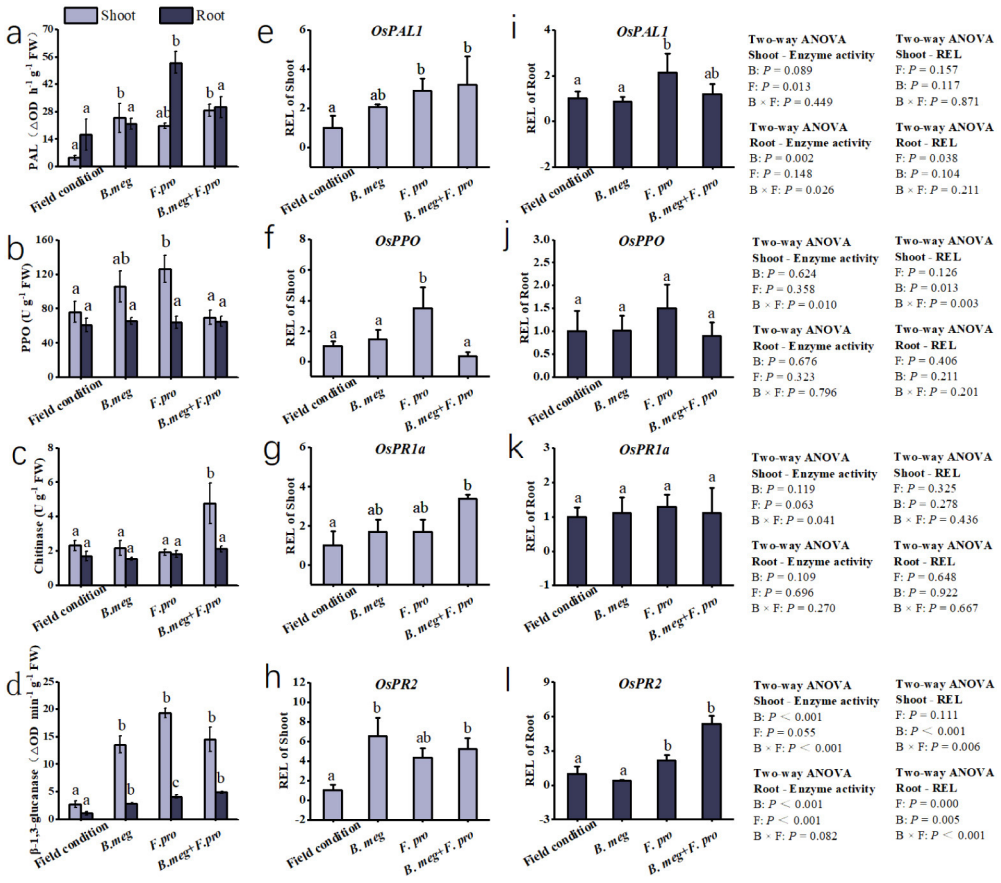


**Fig. 6.** Defense-related enzymes activity and relative expression at Transcriptional level of rice shoot and root at the ripening stage. **a-d** PAL, PPO, Chitinase and β-1,3-glucanase activity of rice shoot and root. Grey violet means enzymes activity of rice shoot and dark violet means that of rice root. **e-h** *OsPAL1*, *OsPPO*, *OsPR1a* and *OsPR2* relative expression level of rice shoot. **i-l** *OsPAL1*, *OsPPO*, *OsPR1a* and *OsPR2* relative expression level of rice root. Values are the means ± SE from four biological replicates ($P < 0.05$, one-way analysis of variance (ANOVA) with Tukey's test). Two-way ANOVA, "F" means *F. pro* treatment level, "B" means *B. meg* treatment level. Field condition, non-treated; *B. meg*, treated with *B. megaterium*; *F. pro*, treated by *F. proliferatum*; and *B. meg + F. pro*, treated with both *B. megaterium* and *F. proliferatum*.

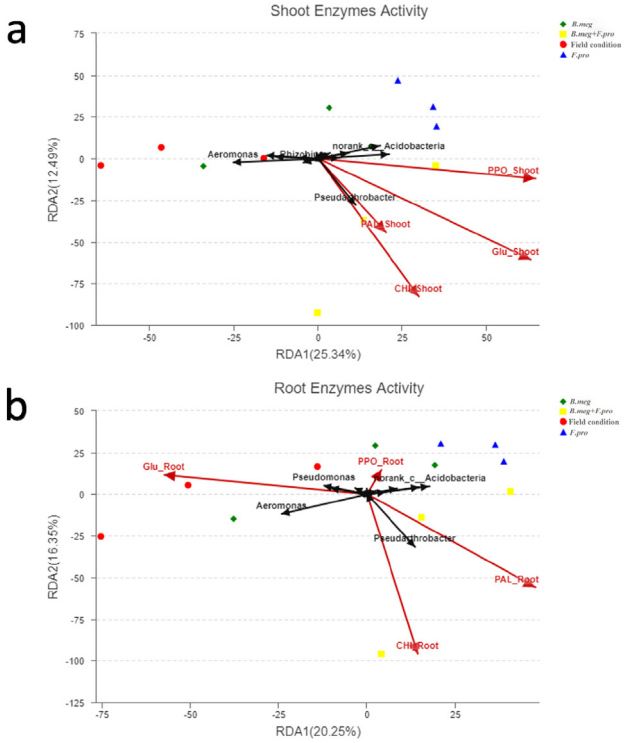


**Fig. 7.** Redundancy analysis (RDA) of relative abundances in bacterial communities accessed at genus level. **a** Shoot enzymes activity as environmental factors. **b** Root enzymes activity as environmental factors. PAL: Phenylalanine Ammonia-Lyase enzymes activity; PPO: Polyphenol Oxidase enzymes activity; GLU: β-1,3-glucanase enzymes activity; CHI: Chitinase enzymes activity. Black arrows indicate species; Red arrows indicate quantitative environmental factors. Field condition, non-treated; *B. meg*, treated with *B. megaterium*; *F. pro*, treated by *F. proliferatum*; and *B. meg + F. pro*, treated with both *B. megaterium* and *F. proliferatum*.

*pro* treatments positively correlated with PAL and chitinase activities, and negatively correlated with β-1,3-glucanase activity, which was found to have better correlations with that in the Field condition. PPO had a weaker effect (shorter arrow) on the bacterial community composition and was positively correlated with the relative abundancies in the *F. pro* treatment (Fig. 7b). *Acidobacteria* and *Pseudarthrobacter* had significantly positive correlations with PAL, chitinase, and PPO activities, while *Aeromonas* and *Pseudomonas* had negative correlations with these enzymes, where the latter was more correlated with β-1,3-glucanase activity (Fig. 7b, Fig. S3b).

## 4. Discussion

Endophytes have generally been reported to serve as biocontrol agents against plant diseases (Yasuda et al., 2014). *B. megaterium*, a typical bacterium used for biocontrol, was used in this study to support its disease control ability. *B. megaterium* acted as a kind of PGPR and has been reported to enhance host plants defenses against diverse pathogens (Kildea et al., 2008). Root colonization of free-living PGPR were considered as one of the early signaling events to coordinate the production and release of compounds related to plant growth promotion, nutrition, and ISR (Pieterse et al., 2014). It was reported that population of the endophyte was approximately $1 \times 10^6$ CFU $g^{-1}$ 4 days after inoculation of *Bacillus* spp. (Reva et al., 2004). The *B. megaterium* strain N8 used in this study was originally isolated from paddy soil and it was investigated as an facultative endophyte when considering its ability of colonization in rice confirmed by scanning electron microscopy (Fig. 1).

Experimental data showed that inoculation with *B. megaterium* led to a reduced rice SRD incidence and index, as well as reduced DON content (Table 1, Fig. 2). High values of DON content and disease incidence in the Field condition group were caused by the complex field environment in the summer of 2018, during which time cloudy conditions, high relative humidity and temperature favor the germination of fungal spores (Huang et al., 2011). Disease-suppression by endophytes in a host plant has been previously reported in many cases (Clarke et al., 2006; Ren et al., 2017). *Bacillus* strains isolated from tomato plants were reported to control fusarium wilt disease and stimulate plant development in fungi-infested soil (Rocha et al., 2017). It was notable that infection of *F. proliferatum* resulted in higher disease index when compared to Field condition group (Table 1), while DON content shows no significant correlation with infection of *F. proliferatum* (Fig. 2). Besides *F. proliferatum*, these results may be attributed to other SDR related pathogens, such as *Bipolaris australiensis*, *Curvularia lunata,* and *Alternaria tenuis* (Huang et al., 2011). Due to the variety of pathogens that may contribute to this disease, SRD has become an especially intractable problem that differs from other major crop diseases, such as rice blast, sheath blight, and wheat scab, which are caused by single pathogens. Despite the effects of *F. proliferatum* on disease degree, *B. megaterium* is the focus of this research. *B. megaterium* strain N8 showed activity of lipase and β-1,3-glucanase which can degrade cell wall molecule (Colville and Smirnoff, 2008; Lugtenberg and Kamilova, 2009), and exhibited plant growth-promoting traits (Diaz Herrera et al., 2016), such as IAA, siderophores and ACC deaminase production and phosphate-solubilization activities (Table S2). The results of this study, based on field conditions, demonstrated that inoculation with the strain N8 could mitigate SRD to a certain degree though *F. proliferatum* abundance was not changed. *B. megaterium* had positive effects on the dry weight of rice shoots, number of panicles per plant, panicle weight per plant, and the seed setting rate, but had negative effects on the thousand grain weight. The latter result was attributed to the energy consumption of SA-dependent resistance due to inoculation with the endophyte (Bezemer and van Dam, 2005), which was worth the cost when considering the decrease in the degree of disease. Furthermore, according to the results of this study, the increase in the number of panicles per plant and the higher rate of seed setting after endophyte inoculation were considered to be the primary contributing factor in regards to the increase in the grain output.

It is widely believed that plants rely greatly on their root microbiome for the uptake of nutrients and protection against environmental stressors (Bakker et al., 2018). The microbiome-involved protection of a host plant against infectious diseases has been intensively studied, especially regarding bacterial microbiomes (Kwak et al., 2018). It has been reported that endophytes can promote host plant growth and alleviate disease damage in which alteration of rhizosphere microbial community was the key factor (Buyer et al., 2010; Han et al., 2019). The relatively abundant genera among the different inoculation strategies are shown in Fig. 4b and Fig. S1b. The potential functions of these genera in symbiotic relationships with plants were analyzed based on previous studies (Doehlemann and Hemetsberger, 2013). It has been proposed that the biocontrol agents *Acinetobacter*, *Bacillus* (Ricci et al., 2019), *Chryseobacterium*, *Pseudomonas* (Domenech et al., 2006), and *Pantoea* (Trotel-Aziz et al., 2008) exhibit the ability to promote growth and biological control in major crops. Some genera may have both pathogenicity (Doehlemann and Hemetsberger, 2013) and biocontrol effects (Khan et al., 2017) in plants, such as *Sphingomonas*, which was more abundant in the *F. pro* treatment. The antimicrobial activity and beneficial effects on plants by *Rheinheimera* (Chen et al., 2010; Presta et al., 2017), *Acinetobacter* (Xue et al., 2009), and *Enterobacter* (Nie et al., 2002) have also been explored in previous reports. In summary, the rhizosphere bacterial community of the group inoculated with *B. megaterium* contained relatively more beneficial bacteria that may have positive effects on plant growth, stress resistance, and pathogen defenses.

In addition, bacterial strains isolated from the rhizosphere soil of rice at the heading stage tended to show the ability of plant growth

promoting and pathogen antagonizing when the host plant was treated with *B. megaterium* (Fig. 5, Table S7). Experimental verification on growth promoting bacteria and antagonistic bacteria became an supplement for the inference of microbiome functions based on 16S rRNA Illumina sequencing. According to another study, *Arabidopsis thaliana* was able to specifically recruit a group of disease resistance-inducing and growth-promoting beneficial microbes upon pathogen infection (Berendsen et al., 2018). Several groups of bacteria that may partially represent the plant-beneficial microbiome were screened for different phenotypes caused by plant diseases. It could be inferred that the development of an efficient biocontrol agent may be sourced from these bacterial groups. This is not the first proposal of the concept of using a 'core microbiome' to address phytopathogens when confronted with highly complex microbial communities (Niu et al., 2017).

Local and systemic resistance induced by the facultative endophytes primes the whole plant for enhanced defense against diverse pathogens (Pieterse et al., 2014), where defense-related enzymes have been shown to have increased expression and transcription levels following pathogen attack (Wang et al., 2012). According to the results on the defense enzyme activities and related gene expression, inoculation with *B. megaterium* promoted PAL, chitinase, and β-1,3-glucanase activities in rice shoots at the heading stage, where chitinase activity was only improved when treated with both *B. megaterium* and *F. proliferatum* (Fig. 6a, c, d). Similarly, the fungal endophyte *Gilmaniella* sp. and its elicitors were shown to enhance the activities of these defense enzymes and improve the total volatile oil content in *Atractylodes lancea* plantlets (Wang et al., 2012). PAL is the key enzyme in the SA synthesis pathway, which plays a central role in plant resistance against biotrophic pathogens (Rekhter et al., 2019). These results implied that PAL activity could be increased by pathogen infection, but *B. megaterium* may further promote this activity to some degree. Considering that this promotion was mainly found in the above-ground tissues of rice, the effects of the strain N8 may be indirect and systemic. It could be inferred from the results that inoculation with *B. megaterium* promotes chitinase activity, which mainly exists in fungal cell walls, and the promotion may be fungal infection-dependent. The chitinase-related protein PR-1 and the β-1,3-glucanase protein PR-2 hydrolyze conserved components of fungal and bacterial cell walls, respectively (Colville and Smirnoff, 2008). *OsPR1a* and *OsPR2* are widely considered to be prime markers in the SA signaling pathway (Van Loon et al., 2006), where they signal the induction of local or systemic resistance in rice. Jung et al. (2012) considered that the activation of plant local and systemic resistance enhanced by symbiotic relationships lead to the establishment of a primed state in the plant, which is mild, but also effective. The endophyte sensitizes the plant immune system for enhanced defenses without directly activating more costly defenses (Pieterse et al., 2014), which may explain the pathogen-dependence on chitinase activity promotion by bacterial endophytes. Correspondingly, β-1,3-glucanase activity promotion, independent of the pathogen, may be attributed to the type of the inoculated strain. PPO, along with PAL and POD, was involved in production of melanin and lignin to strengthen cell walls, which contributes to the first level of the plant defense system (Bastias et al., 2018). Localized to chloroplasts, PPO shows no activity, but is activated when pathogen induced cell damage occurs (Thipyapong et al., 2004), where it is directly involved with ROS detoxification by oxidation of flavonoids and phenolic acids (Spanic et al., 2017). In the present study, PPO activity was more likely to be stimulated by pathogen infection, however, this stimulation was notably suppressed after inoculation with *B. megaterium* (Fig. 6b, f). It was speculated that cell injury caused by *F. proliferatum* was alleviated by inoculation of *B. megaterium*.

It is interesting that correlations were found between plant defenses and the rhizosphere microenvironment (Fig. 7). Berendsen et al. (2018) reported that infection of aboveground plant tissues result in the recruitment of beneficial root-associated microbes that have the potential to protect plant offspring growing in the same soil against downy mildew infection. Symbiotic microorganisms may be one of the driving forces which alter the rhizosphere microbiome composition to improve host adaptability, though few reports on this subject have been conducted (Di Salvo et al., 2018). It is not yet clear how these driving forces shape the rhizosphere bacterial community. A well-recognized hypothesis is that the root exudate profiles shaped by a microorganism, often by a pathogen, selectively enrich the plant-protective microbes in the soil (Zhalnina et al., 2018), which may be SA-dependent (Lebeis et al., 2015), and lead to a 'soil memory' to induce resistance in subsequent plant generations (Bakker et al., 2018). As a response , ISR triggered by beneficial rhizosphere microbes primes plant tissues for enhanced defense against a broad spectrum of pathogens (Lugtenberg and Kamilova, 2009). Correlation analyses (Fig. 7) have provided evidence supporting the relationships between defense enzyme activities and the structure of the bacterial community. It is reasonable to believe that *B. megaterium* may indirectly mediate the assembly of the rhizosphere microbiome by altering root exudate profiles, where defense phytohormones may also be involved.

Taken together, this study revealed that *B. megaterium* improved plant defenses against SRD via enhanced systemic resistance and shifted rhizosphere microbiome. The endophyte's ability to induce plant systemic resistance (Bastias et al., 2018) and alter the rhizosphere bacterial community (Rout et al., 2013) has been well demonstrated in previous studies. Our results further illuminated the close correlationship between plant defenses and the rhizosphere microenvironment. It is considered that the microbiome is part of the plant defense strategy, but definite evidence is needed to demonstrate the concrete and complete construction of the internal and external defense systems of plants. An endophyte-mediated benign and sustainable cycle consists of the endophyte-plant interaction, plant systemic resistance, and the rhizosphere microbiome, which demonstrates a systemic perspective on disease management. An endophyte may be used as a key to activate the cycle when of its influence on the microbiome assembly and its beneficial effects on the microbiome of the host plant have been well studied.

## 5. Conclusion

Inoculation with *B. megaterium* decreased the rice SRD incidence and index, and decreased the accumulation of DON. Further evidence demonstrated that SA-dependent resistance and the assembly of the rhizosphere bacterial community triggered by endophytes may be responsible for the host defenses against phytopathogens. It is the first time to directly confirm the correlationship between systemic resistance and rhizosphere microbiome. The present study highlighted the significance of the endophyte for plant defenses against fungal pathogens by both systemic resistance and rhizosphere microbiome. The inferences based on this experimental data updated the recognition of plant immunity strategies, and redefined the research and application value of biocontrol agents.

## Declaration of competing interest

We declare that we do not have any commercial or associative interest that represents a conflict of interest in connection with the work submitted.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.apsoil.2020.103710.

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