Environmental Microbiome

RESEARCH

Open Access

# Rice phyllospheric *Pantoea* spp. suppress blast and bacterial blight diseases

Wenda Sun[1†], Qiao Liu[2†], Huilin Chen[1], Xiaofang Xie[1], Zhuan Zhang[1], Yu Zeng[1], Jianuan Zhou[1], Xiaofan Zhou[1], Xianya Jiang[3], Zhibin Liang[1*], Jian-Feng Li[2*] and Yizhen Deng[1*]

## Abstract

**Background** Rice is a major food crop in China as well as Asia, yet its production is threatened by microbial diseases including blast disease caused by fungal pathogen (*Magnaporthe oryzae*) and bacterial blight caused by several bacterial pathogens. To screen for bacterial microbiota associated with rice blast occurrence, and/or contributing to disease resistance, we performed microbiota analysis with rhizosphere soil, root, stem, and leaf samples of blast susceptible (CO39) and resistant (Y33R) rice grown in a blast disease nursery garden.

**Results** Our result showed no significant difference in microbiota of rhizosphere soil, root, or leaf between these two rice cultivars, but stem microbiota were significantly different. *Pantoea* spp. were enriched in stem of blast susceptible rice, suggesting that it may play a role after fungal infection. A total of 822 bacterial strains were isolated from the phyllospheric (including leaf and stem) samples of Y33R and CO39 rice. Based on 16S rRNA amplicon sequencing, and phylogenic analysis using 16S rRNA, *gyrB*, *leuS*, and *rpoB* gene sequences, the 3 isolated strains and 1 strain were identified as *P. ananatis* and *P. dispersa*, respectively. The strains A25-H1 and B10-A1 were selected for genome sequencing, and based on Average Nucleotide Identity (ANI) analysis, we confirmed that A25-H1 was *P. ananatis* and B10-A1 was *P. dispersa*. The *P. ananatis* consortium (A25-F1, A25-G1, and A25-H1 combination) A25-11 and *P. dispersa* strain B10-A1 displayed suppressive effect on blast disease when they were applied to the susceptible rice CO39. Although a *P. ananatis* strain SC7 has been reported to cause bacterial blight in rice, A25-11 or B10-A1 was non-pathogenic to rice under experimental conditions. Furthermore, they could also suppress bacterial blight caused by SC7 or *Xanthomonas oryzae* pv. *oryzae* strain Pxo99A. A25-11 and B10-A1 did not affect the growth of *M. oryzae* mycelia in confrontation culture analysis, but induced transcription of rice immunity genes and promoted ROS accumulation, suggesting that the biocontrol effect of A25-11 or B10-A1 may lie on immunity priming. We further showed that A25-11 and B10-A1 possessed growth promoting capacity including indole 3-acetic acid (IAA)

†Wenda Sun and Qiao Liu these authors contributed equally to this work.

*Correspondence:
Zhibin Liang
liangzb_gz@scau.edu.cn
Jian-Feng Li
lijfeng3@mail.sysu.edu.cn
Yizhen Deng
dengyz@scau.edu.cn

Full list of author information is available at the end of the article

production, phosphate solubilization, nitrogen fixation, and siderophore production. Under field condition, the consortium A25-11 and strain B10-A1 could effectively suppress leaf and panicle blast.

**Conclusions** Overall, this study established a microbiome method for identifying the rice bacterial communities of agricultural significance, with capacity of rice disease management and/or growth promotion.

**Keywords** *Pantoea* spp., Phyllospheric bacterial microbiota, Rice bacterial blight, Rice blast disease, Bio-control agents, Plant growth promoting (PGP)

## Background

Rice (*Oryza sativa* L.) is one of the most important food crops for people worldwide [1]. Stable rice production is constrained by various biotic stresses, including blast disease, sheath blight, false smut, bakanae disease, bacterial blight, bacterial leaf streak, and virus diseases [2]. Among these diseases, the rice blast caused by *Magnaporthe oryzae* is one of the most devastating diseases affecting rice crops worldwide, leading to 10% to 30% losses of the global rice yield every year [3–5]. Rice bacterial blight is one of the most devastating bacterial diseases, which caused by the pathogens include *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), *Enterobacter asburiae*, or *Pantoea ananatis* [2, 6]. The bacterial pathogens spread and propagate within the rice through leaf veins, and cause water-soaked spots on leaf tips and edges [7]. Chemical control and the development of resistant rice varieties can effectively control rice blast and bacterial blight diseases [2, 8]. However, chemical control is costly and harmful to the plant, environment and even consumers. Breeding of resistant rice cultivars (often based on a single gene) is time-consuming and normally not durable in the field, partially as a result of the high degree of pathogenic variability and fast evolution of the microbial pathogens [9–12]. Biological control is the most cost-effective, environment friendly and the most sustainable long-term solution for managing rice diseases.

In natural environment, plants can recruit a large number of microorganisms in their different parts, including the rhizosphere, phyllosphere, and endosphere [13]. These microbial communities are collectively coined as plant microbiomes or phytobiome [14, 15]. In recent years, the microbial communities in each rice compartment have been investigated to understand their composition, diversity, and possible function in rice growth and/or resistance to biotic stresses [16]. A previous work determined the difference in phyllosphere and rhizosphere bacterial composition, between healthy rice and the rice infected by the blast fungus *M. oryzae* [17, 18]. *P. agglomerans*, *Acidovorax wautersii*, and *Burkholderia pyrrocinia* with antagonistic effect on *M. oryzae* were showed to be enriched in the infected-rice [19]. It was also reported that the rice root-associated *Bacillus cereus* NJ01 enhances rice resistance against *Xanthomonas* infection [20]. Therefore, microbiota analysis provides an efficient way for identifying rice-associated biocontrol bacterial resources [21]. Interestingly, recent studies report that some *Pantoea* spp. are capable of causing the symptoms of leaf blight disease to crops including rice [22–24], while on the other hand, *Pantoea* spp. also include some beneficial strains [25–27]. The nature of association (beneficial or harmful) between *Pantoea* spp. and rice is not fully understood.

Understanding the fundamental mechanism of beneficial microbes will facilitate robust microbiome-based applications for agriculture. Beneficial microbes help improve plant performance by their growth-promoting capacity and/or immunity-priming [28, 29]. Salicylic acid (SA) and jasmonic acid (JA) represent two most important phytohormones in regulation of plant immune responses [30]. Resistance to biotrophic pathogens primarily involves SA-dependent pathway, whereas resistance to necrotrophic pathogens is often associated with the JA-dependent pathway [31]. *OsPR10*, *PBZ1*, and *NPR1* genes are involved systemic acquired resistance (SAR) in rice, mediated by SA pathway [32]. *Trichoderma* and endophytic fungi *Phomopsis liquidambaris* B3 were found to significantly induce the expression of *OsPR10*, thereby enhance rice resistance against bacterial and fungal pathogens [33, 34].

In this study, we compared the bacterial microbiota of rhizosphere soil, root, stem, and leaf samples between blast susceptible (CO39) and resistant (Y33R) rice grown in a blast disease nursery garden. Significant alterations of microbiome composition were detected in rice stem, suggesting that pathogen infection changed rice stem microbiome. Amplicon Sequence Variants (ASVs) belonging to *Pantoea* spp. were enriched in rice phyllospheric part, and 3 *P. ananatis* strains and 1 *P. disperas* strain exhibiting no antagonistic effect on *M. oryzae* growth were isolated. Application of a simplified synthetic community (SynCom) *P. ananatis* consortium A25-11 (combination of A25-F1, A25-G1, and A25-H1) or the *P. dispersa* strain B10-A1 on the susceptible rice CO39 promoted resistance to blast and bacterial blight diseases, which may mainly depend on plant immunity priming. More importantly, our field trials demonstrated that A25-H1 and B10-A1 could effectively control leaf and panicle blast, and thus of a great potential in translational outcomes. Overall, our study provides evidence

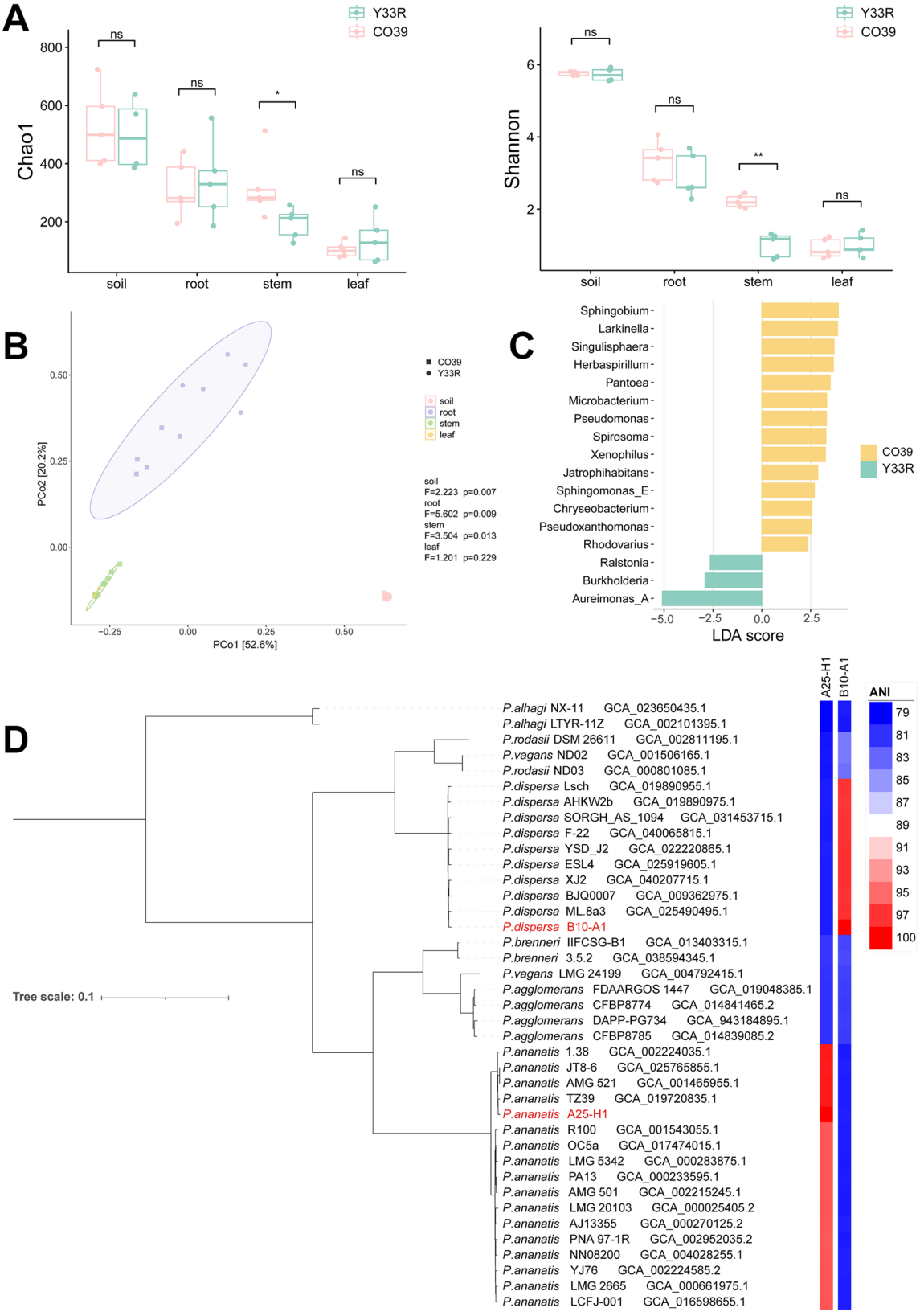


**Fig. 1** (See legend on next page.)

(See figure on previous page.)

**Fig. 1** Microbiota analysis of blast susceptible and resistant rice. **A** Bacterial α-diversity in soil, root, stem, and leaf samples from CO39 and Y33R rice, shown as Chao1 (left) and Shannon index (right). The box plots indicate the median (center line), the 25th and 75th percentiles (box), and the range of non-outlier values (whiskers). ns: no significance (Wilcoxon Rank Sum Test analysis). **B** Bacterial β-diversity between CO39 and Y33R in each compartment (soil, root, stem, and leaf), analyzed using PCoA. Statistical significance of the β-diversity variation was evaluated using PERMANOVA. **C** Differential abundance of the bacterial microbiome community between CO39 and Y33R rice stems at the genus level obtained by LEfSe analysis. **D** Phylogenetic analysis of A25-H1 and B10-A1 at genome level based on ANI analysis

that pathogen-infection led to recruitment of beneficial microbes rice, and offers a new strategy for the development of bio-control agents for maintaining rice productivity.

## Results

### Microbiota analysis and isolation of 4 *Pantoea* strains from rice phyllosphere

Following the work flow (Figure S1A-B), we sequenced the full-length 16S rRNA amplicons from the sampled rice roots, stems, leaves, and rhizosphere soil (details in Methods section). A total of 287,566 bacterial 16S rRNA amplicon sequences of high-quality were obtained, which could be assigned to 2,168 bacterial ASVs. Among them, the ASVs of rhizosphere soil, roots, stems, and leaves were 920, 1,186, 575, and 226, respectively. The alpha-diversity assessment based on Chao1 and Shannon index revealed that the bacterial diversity of Y33R stem was significantly lower than that of CO39 stem, and no significant difference was detected in bacterial microbiota of soil, root, or leaf between Y33R and CO39 (Fig. 1A). Unconstrained principal coordinate analysis (PCoA) and Permutational multivariate analysis of variance (PERMANOVA) analyses revealed significantly different bacterial compositions in soil, root, and stem samples (Figs. 1B). Top 10 most abundant genera in rice stems include *Aureimonas_A*, *Novosphingobium*, *Mucilaginibacter*, *Herbaspirillum*, and *Pantoea* (Figure S1C). To further determine which microbes were potentially associated with the health-disease status of rice, we conducted a Linear discriminant analysis Effect Size (LEfSe) analysis, and identified a total of 17 microbial biomarkers in the stems of CO39 and Y33R (Fig. 1C). Among these, significant difference was found in *Pantoea*, with *P. ananatis* showing an absolute abundance of 376 in CO39 versus 108 in Y33R, and *P. dispersa* showing an absolute abundance of 149 in CO39 but undetected in Y33R.

A total of 822 bacterial strains were isolated from the phyllospheric samples of Y33R and CO39 rice cultivars, among which 3 strains (A25-F1, A25-G1, and A25-H1) were identified as *P. ananatis* and 1 strain (B10-A1) as *P. dispersa* based on the 16S rRNA, *gyrB*, *leuS*, and *rpoB* gene sequences (Figure S2A). We selected A25-H1 and B10-A1 for genome sequencing. The Average Nucleotide Identity (ANI) analysis and the phylogenetic identification (Fig. 1D) of these 2 strains were consistent with the analysis result using 16S rRNA, *gyrB*, *leuS*, and *rpoB* gene sequences for species classification (Figure S2A). *P. ananatis* strain A25-F1, A25-G1, and A25-H1 and *P. dispersa* strain B10-A1 were cultured on TSB agar plates. The colonies of A25-F1, A25-G1, and A25-H1 were round, yellow, opaque, and sticky, with neat edges (Figure S2B). The *P. dispersa* strain B10-A1 appeared round, yellow, opaque, smooth and shiny, and sticky and slippery (Figure S2B).

### Suppressive effects of *Pantoea* strains on rice blast and rice bacterial blight diseases

Next, we tested the potential effect of *Pantoea* strains on rice blast. In the 1st test, we applied the *P. ananatis* consortium consisted of A25-F1, A25-G1, and A25-H1 strains (hereafter named A25-11), *P. dispersa* strain B10-A1, or the combination of the above *Pantoea* strains (A + B), to the soil and leaves of CO39 rice. At 24 h post *Pantoea* strains treatment, *M. oryzae* Guy 11 conidia were sprayed to the rice seedlings, and the leaf lesions were examined at 5 d post infection (dpi). We found that severe blast lesions were developed in the rice leaves pre-treated with LB medium (as a blank control), to 7–9 grade (susceptible) according to the established standard scale [35]. However, pre-treatment with A25-11, B10-A1, or A + B, significantly suppressed blast lesion development to around 4–6 grade (medium resistant) (Fig. 2A). We then removed the rice seedlings and kept the treated soils (A25-11, B10-A1, or A + B, respectively) for planting the rice seeds. When the rice seedlings grew to four-leaf stage, without soil drench or foliar application of *Pantoea* cultures, the *M. oryzae* conidia was directly sprayed on the rice seedlings and the lesion symptom was examined at 5 dpi. In the 2nd test, the rice seedlings grown in the LB pre-treated soil (from the 1st test) displayed severe leaf lesions (susceptible) and those grown in the *Pantoea* strain pre-treated soil (from the 1st test) were medium resistant to *M. oryzae* (Fig. 2A). We then detected the presence of *Pantoea* strains by PCR amplification of *PANsp_atpD* gene [36], using the rice leaves from the 2nd test. The result showed that the abundance of *Pantoea* spp. were significantly higher in the leaves of rice seedlings grown in *Pantoea* strain pre-treated soil, compared to those grown in LB pre-treated soil (Fig. 2B). Overall, these results indicated that *Pantoea* strains are able to persist in the soils and endowed the rice grown on such soil with better resistance to blast disease.

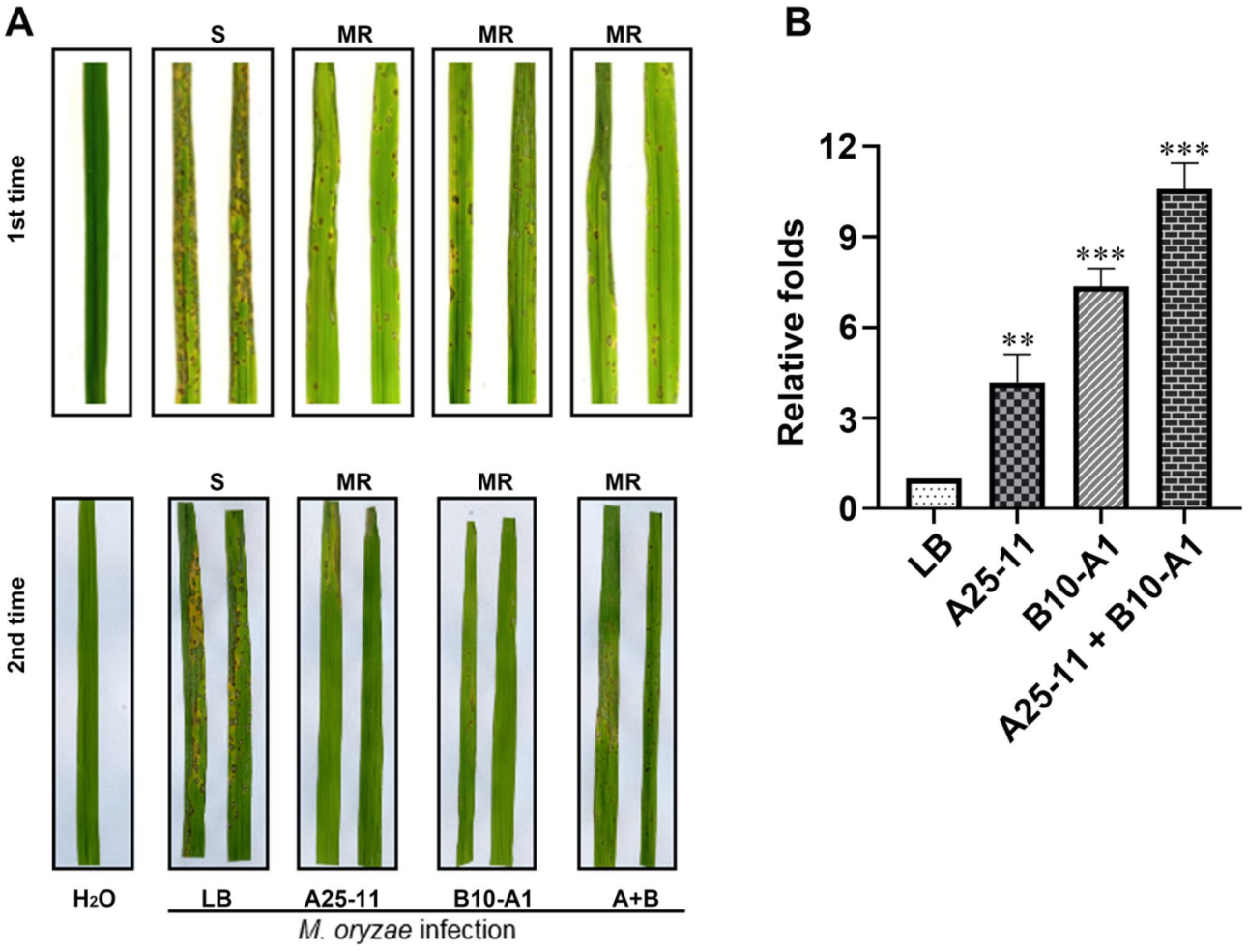


**Fig. 2** *Pantoea* strains suppressed rice blast disease. **A** Pathogenicity assay was performed by spraying the *M. oryzae* conidia ($10^5$/ml; 10 ml for each pot containing > 5 seedlings) to the CO39 rice seedlings. For the 1st test, A25-11 or B10-A1 ($O.D._{600}$ = 1.0), or combination (A + B) was respectively drenched to the soil (20 ml/pot) and sprayed to the rice leaves (10 ml/pot) at 1 d before *M. oryzae* infection. For the 2nd test, the germinated rice seeds were sowed in the pot containing the soils pre-treated with A25-11, B10-A1, or A + B from the 1st test, and spraying infected by *M. oryzae* conidia at 14th d of growth. For both 1st and 2nd tests, three biological repeats containing > 10 seedlings for each instance were performed, and disease symptoms were examined at 5 d post infection. S: susceptible; MR: medium resistant. **B** qPCR for quantitatively analyzing the presence of *Pantoea* strains in phyllosphere of CO39 rice seedlings in the 2nd test. Mean ± *S.D.* was derived from three biological repeats. ** or ***: $p < 0.01$ or $p < 0.001$ vs LB (Student's *t*-test)

Given that *Pantoea* spp. include many strains reported as plant pathogens, e.g., SC7 strain that was reported to cause rice bacterial blight [6], we then tested virulence of A25-11 and B10-A1 towards rice, using *Xoo* strain Pxo99A and *P. ananatis* strain SC7 as positive controls. The result showed that, unlike the pathogenic strain Pxo99A or SC7, which caused yellowish brown lesions along the midvein of the infected rice leaves, neither A25-11 nor B10-A caused lesion on rice leaves, under 28 °C (Fig. 3) or 22 °C (Figure S2C). Furthermore, application of A25-11 or B10-A1 suppressed the blight lesion caused by Pxo99A or SC7 (Fig. 3A-B).

Taken together, the *Pantoea* strains isolated in this study are non-pathogenic to rice under experimental conditions. Moreover, they showed great potential as bio-control agents against blast and bacterial blight diseases of rice.

### *Pantoea* strains activated rice immunity

To investigate the biocontrol mechanism of these *Pantoea* strains, we determined whether A25-11 and B10-A1 inhibited the mycelial growth of *M. oryzae* strain Guy11 and Zhong1, respectively. Neither A25-11 nor B10-A1 was capable of suppressing mycelial growth of fungal strain Guy11/Zhong1 (Figure S2D). Also, they were unable to suppress the growth of pathogenic bacterial strain SC7/Pxo99A in confrontation culture (Figure S2E), indicating that their biocontrol effects were not due to direct suppression on the fungal or bacterial pathogens.

Furthermore, we noticed that when inoculated on the detached rice (CO39 or Y33R cultivar) leaves, A25-11 or

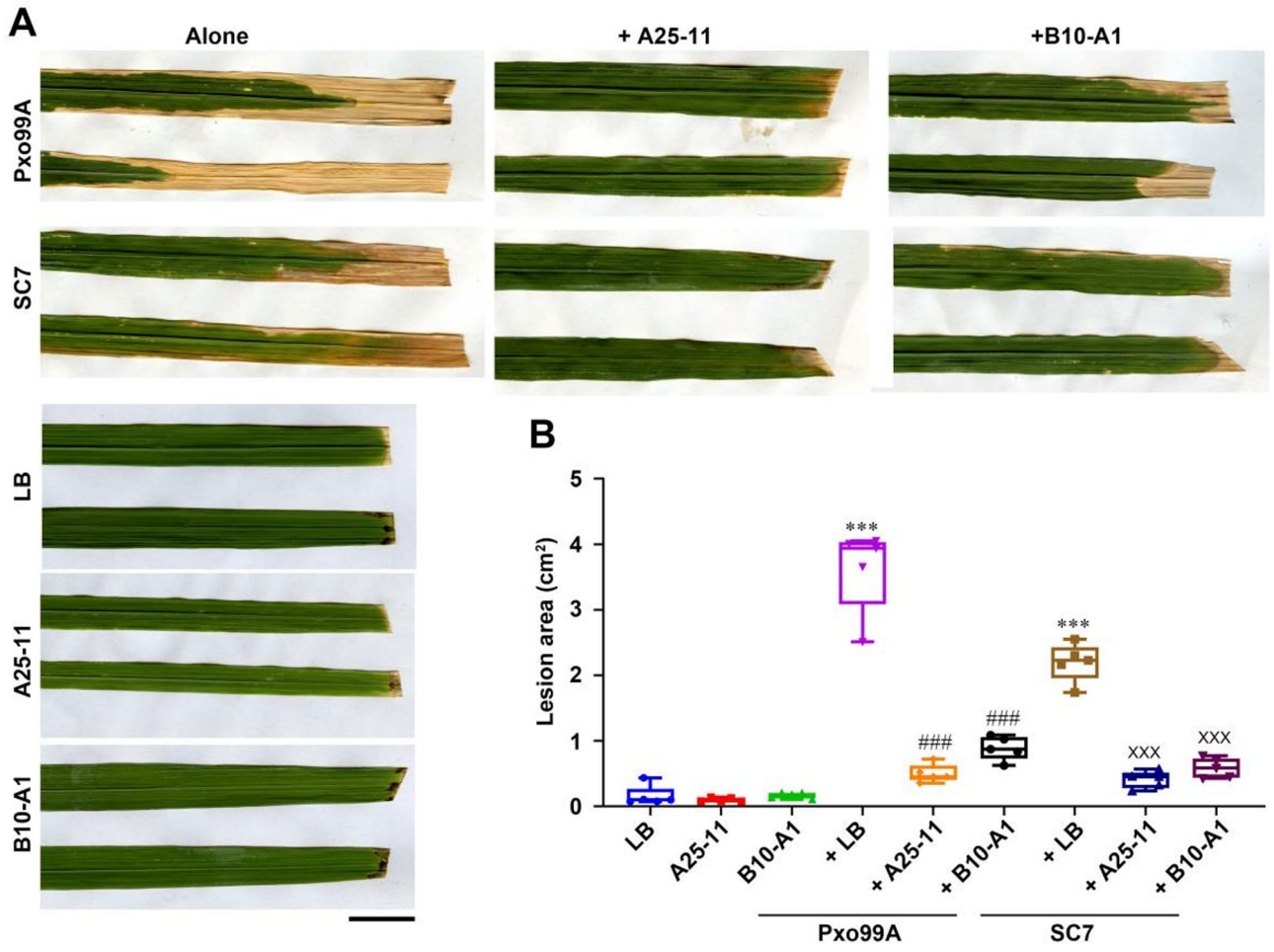


**Fig. 3** *Pantoea* strains suppressed rice bacterial blight. **A** Pathogenicity assay for A25-11 and B10-A1 strains at 28 °C, following the established leaf clipping inoculation method [37]. The known pathogenic strain Pxo99A and SC7 served as positive controls and LB as a blank control. Co-inoculation of Pxo99A + A25-11, Pxo99A + B10-A1, SC7 + A25-11, and SC7 + B10-A1, were used to test the bio-control ability of A25-11 and B10-A1 strains against rice bacterial blight. The inoculated leaves were cut from the seedlings and documented for blight symptoms at 14 dpi. Scale bar, 1 cm. **B** Quantitative analysis of blight lesion based on the results of (A) ($n = 5$). Mean ± *S.D.* was derived from three biological repeats. ***: $p < 0.001$ vs LB; ###: $p < 0.001$ vs Mock (Pxo99A); × × ×: $p < 0.001$ vs Mock (SC7) (Student's *t*-test)

B10-A1 caused lesion mimic, which did not expand as the blast lesions caused by *M. oryzae* conidia inoculation on the susceptible rice CO39 (Fig. 4A; arrowheads). Furthermore, the *Pantoea* strains could also cause such lesion mimic on Nipponbare (NPB) and Zhonghua11 (ZH11) (Figure S2F). The lesion mimic was similar as the non-expanded lesion caused by the fungal strain Guy11 or Zhong1 on the resistant rice Y33R (Fig. 4A; arrowheads). We infer that like incompatible interaction between *M. oryzae* and rice, inoculation of *Pantoea* strains may elicit rice immunity that restrict spread of the pathogen within the host cells.

To verify such, we measured transcriptional levels of immunity genes *OsPR10* and *PBZ1*, in CO39 or Y33R leaves, inoculated with or without A25-11 or B10-A1. The result showed that A25-11 and B10-A1 inoculation caused a significant increase in *OsPR10* and *PBZ1* transcription in CO39 (Fig. 4B), which may account for the enhanced resistance of CO39 to blast fungus. For Y33R, the basal levels of *OsPR10* and *PBZ1* transcription were significantly higher than CO39 (Fig. 4B), which may explain the higher level of blast resistance of Y33R compared to CO39, even without *Pantoea* treatment. Furthermore, we stained the *Pantoea* inoculated site of the CO39 or Y33R leaves with 3,3-diaminobenzidine (DAB) to visualize ROS accumulation. Heavy DAB stain appeared on the pre-treated sites of rice leaves inoculated with A25-11 or B10-A1 (Fig. 4C). Treatment with A25-11 or B10-A1 triggered the ROS burst in rice (Fig. 4D). These results support that rice immunity is indeed induced by the *Pantoea* strains, likely contributing to rice resistance to blast disease.

Given that both *OsPR10* and *PBZ1* genes respond to salicylic acid (SA) signaling pathway upon pathogen

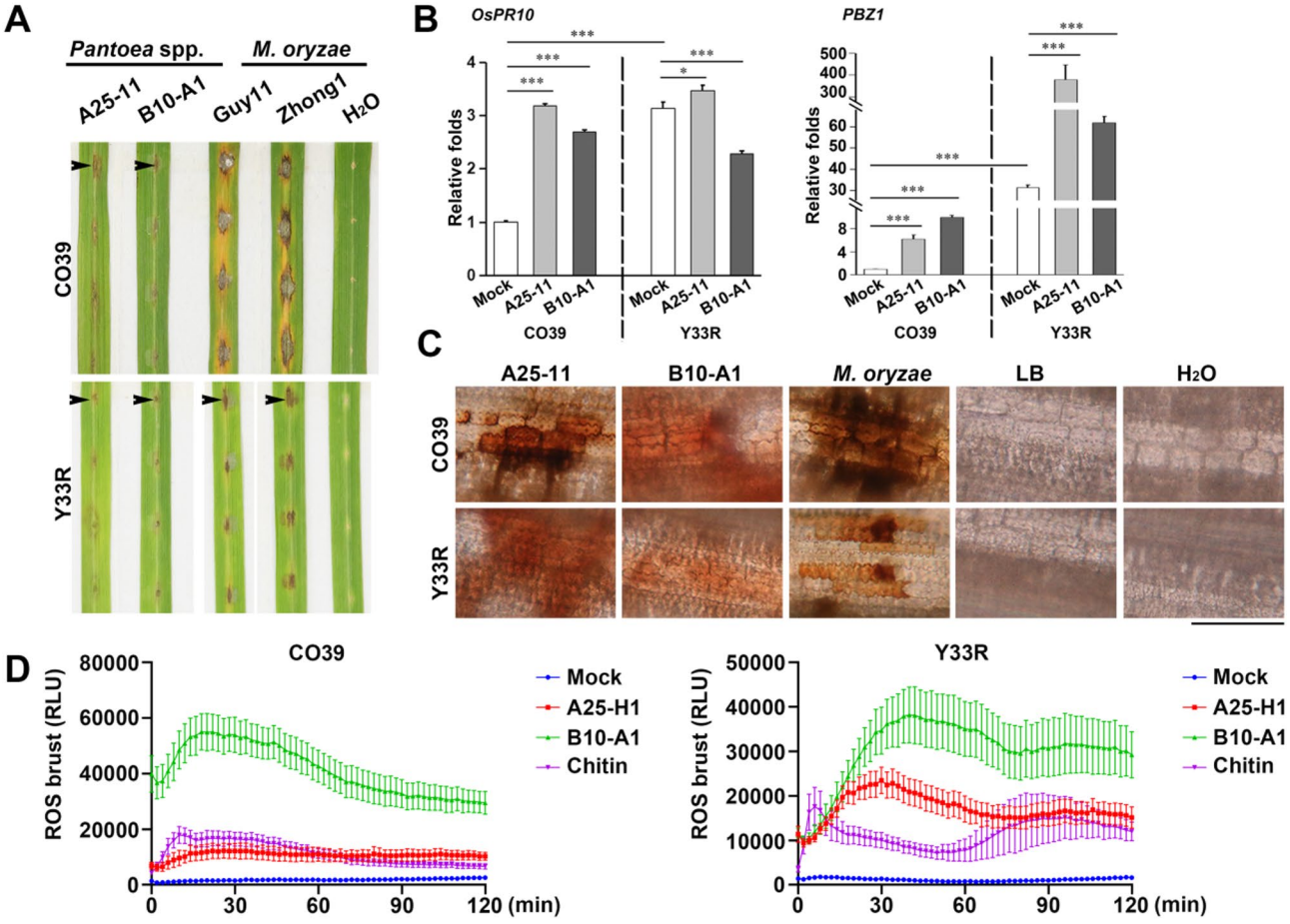


**Fig. 4** *Pantoea* strains promoted rice immunity. **A** Inoculation with A25-11, B10-A1, Guy11, or Zhong1, individually, on the detached rice leaves of CO39 (susceptible to *M. oryzae* strain Guy11 or Zhong1) or Y33R (resistant to the 2 tested *M. oryzae* strains). Photographs were taken at 5 dpi. Arrowheads denote to the examples of lesion mimics that were not expanded. **B** RT-qPCR (Reverse transcription-quantitative PCR) for expression profiling of rice immunity genes *OsPR10* and *PBZ1* in the leaves inoculated with A25-11 or B10-A1. Relative expression was calculated using $2^{-\Delta\Delta CT}$ method, with *OsACTIN* as an internal control. Gene expression level in CO39 mock (rice leaves without inoculation of *Pantoea* strain) was set as "1". Mean ± *S.D.* was based on three independent biological repeats. *, ***: $p < 0.05$, $p < 0.001$ respectively (Student's *t* test). **C** DAB straining with the inoculated rice leaves. Scale bar = 100 μm. **D** ROS burst induced after treatment of A25-11 or B10-A1. Chitin (500 μg/mL) was used as a positive control. Mock: without treatment

infection, to trigger programmed cell death of plant [33], we inferred that such immunity priming triggered by *Pantoea* strains may rely on SA pathway. To investigate such, we applied the rice leaves with aminoindanphosphonic acid hydrochloride (AIP), the inhibitor of the phenylalanine ammonia-lyase (PAL) enzymes critical for phenylpropanoid and SA biosynthesis and thus the induction of plant defenses [38]. Application of AIP suppressed the A25-11-based induction of *OsPR10*, *PBZ1*, and *NPR1* genes, all of which are involved in SA-mediated immunity pathway (Fig. 5A). Correspondingly, suppression of leaf blast by A25-11 was compromised by AIP treatment (Fig. 5B), indicating that the immune priming function of A25-11 depends on SA signaling pathway in rice.

### Growth promoting capacity of the *Pantoea* strains

To further rule out the possibility that the isolated *Pantoea* strains were pathogenic to plant, we then tested the enzyme activity for Peh (polygalacturonase), Cel (cellulase), and Prt (protease), which are common bacterial virulence factors required for plant cell wall degradation, with the known pathogen *Dickeya oryzae* EC1 as a positive control and LB medium as a negative control. We found that A25-11 or B10-A1 did not display any enzymatic activity in these aspects, as no clear zones were evident at the margin of the holes inoculated with the cell culture of A25-11 or B10-A1 (Fig. 6A). Our results confirmed that these *Pantoea* strains are not pathogenic to rice, and could be used as biocontrol agents for protecting rice from blast disease.

Furthermore, we found that A25-11 and B10-A1 were able to promote germination of rice seeds, as the rice shoots were obviously longer under treatment with

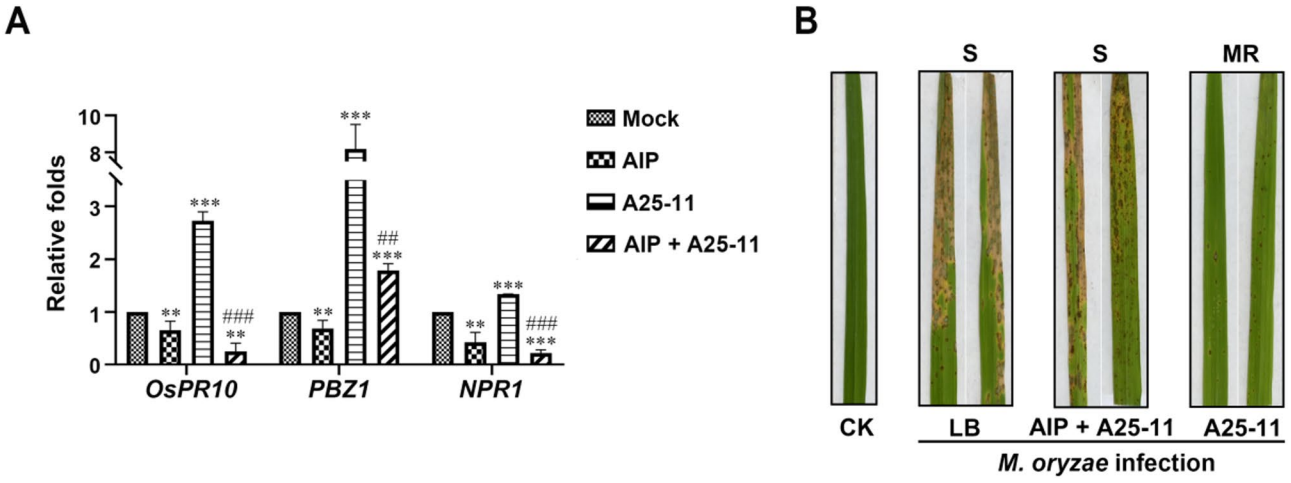


**Fig. 5** Salicyclic acid (SA) pathway mediates *Pantoea* strain based rice immunity priming. **A** RT-qPCR for expression profiling of rice immunity genes *OsPR10*, *PBZ1* or *NPR1* in the leaves under treatment with AIP, A25-11 or AIP + A25-11. Relative expression was calculated using $2^{-\Delta\Delta CT}$ method, with *OsUBQ* as an internal control. Gene expression level in mock (rice leaves without treatment) was set as "1". Mean ± *S.D.* was based on three independent biological repeats. **, ***: $p < 0.01$, $p < 0.001$ vs Mock respectively; ##, ###: $p < 0.01$, $p < 0.001$ vs A25-11 respectively (Student's *t* test). **B** The CO39 rice seedlings were treated with AIP (200 μM) for 24 h, then by LB (blank control) or A25-11 treatment. *M. oryzae* Guy11 conidia ($10^5$/ml; 10 ml for each pot containing > 5 seedlings) were sprayed onto rice seedlings at 24 h post treatment with A25-11, and disease symptoms were examined at 5 dpi. Three biological repeats containing > 10 seedlings for each instance were performed, and representative images were displayed. S: susceptible; MR: medium resistant

liquid-cultured strains ($O.D._{600} = 1.0$), compared to those treated by TSB medium or $H_2O$, at 3 d post germination (Fig. 6B; Table 1). At 7 d post germination, we found the rice roots were significantly longer under treatment of A25-11 or B10-A1, compared to un-treated seeds (Fig. 6C; Table 1). These results support a growth promoting capacity of A25-11 and B10-A1 on rice.

A25-H1 and B10-A1 were capable of phosphate solubilization, nitrogen fixation, siderophore production (Figure S3A) and indole-3-acetic acid (IAA) production (Figure S3B). The intracellular and extracellular levels of IAA from A25-11 and B10-A1 were further quantitatively analyzed by using an established LC–MS method [39]. The peak with the *m*/*z* ratio of 129.8 and molecular weight of 175.18, at a retention time (RT) of 4.6 min, corresponds to IAA (Figure S3C). A standard curve was generated with serial diluted IAA solutions (Fig. 6D) and the intracellular and extracellular IAA contents of A25-H1 and B10-A1 were calculated (Fig. 6E).

We further examined transcriptional levels of *Expansin* (*EXP*) family genes, encoding cell wall-loosening proteins in response to elevated levels of IAA [40, 41], upon A25-11 or B10-A1 treatment. *OsEXPA7*, *OsEXPB3*, and *OsEXPB7* were significantly up-regulated by A25-11 or B10-A1, to an extent comparable or even higher than IAA (100 μM) treatment (Figure S3D). This further confirmed that A25-11 and B10-A1 were able to produce and secrete IAA, which could be perceived by rice.

Overall, our findings indicated that the *Pantoea* strains isolated in this study possessed growth promoting ability.

### Application of *Pantoea* consortium/strain under field conditions

Given that application of A25-11 and B10-A1 effectively suppressed leaf blast in laboratory settings (Fig. 2), we investigated the use of these strain/consortium under field conditions. Seeds of the susceptible rice CO39 were soaked in culture of A25-11, B10-A1, or A + B (high concentration: $O.D._{600} = 1.0$, low concentration: $O.D._{600} = 0.5$) before sowing and then transplanted to a blast nursery at the Yangjiang Baisha Experimental Station. When the seedlings were transplanted to the experimental plots, foliar spray of A25-11, B10-A1, or A + B was performed using high ($O.D._{600} = 1.0$) or low ($O.D._{600} = 0.5$) concentrations. The well-known fungicide Tricyclazole was applied in parallel to compare its efficacy to that of A25-11 and B10-A1. At 21 d post transplanting, severe leaf blast symptoms developed on the LB-treated rice seedlings. However, application of A25-11 and B10-A1 at low concentration ($O.D._{600} = 0.5$) significantly suppressed rice blast incidence, to an efficiency comparable to Tricyclazole treatment (Fig. 7A-C).

During the early booting stage, the A25-11 consortium or B10-A1 strain ($O.D._{600} = 0.5$) was applied to the rice plants, and the severity of panicle blast was assessed at 21 d post treatment. Tricyclazole was applied in parallel. Severe panicle blast symptoms (majority > 5 grade) were observed in the panicles of LB-treated rice, whereas the treatment with A25-11 or B10-A1 significantly reduced disease incidence (majority < 5 grade), exhibiting comparable disease control efficacy as Tricyclazole (Fig. 7D-F).

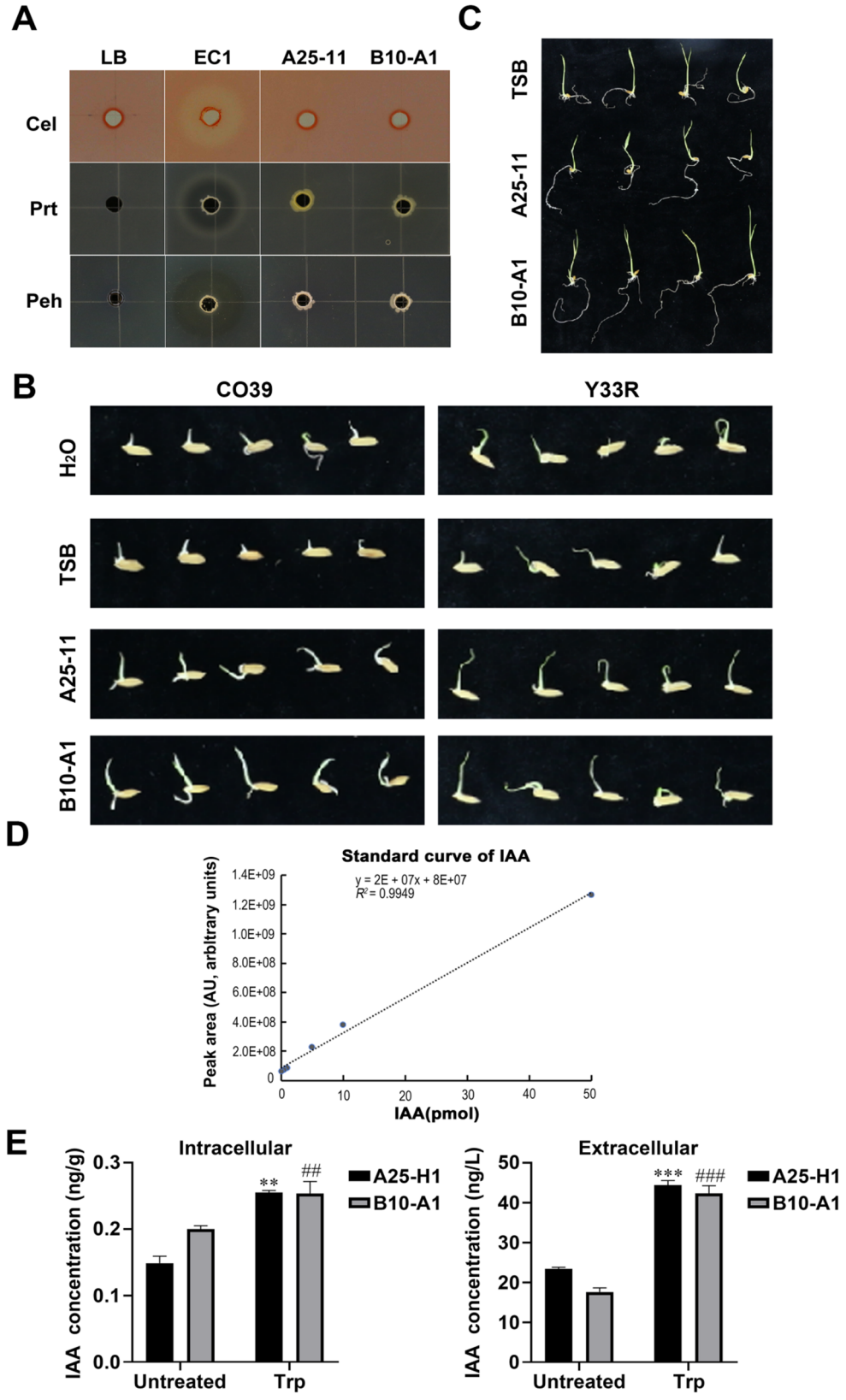


**Fig. 6** (See legend on next page.)

(See figure on previous page.)

**Fig. 6** Assessment of virulence factors and growth promoting capacity of *Pantoea* strains. **A** Detection of virulence factors Cel, Prt, and Peh. Fresh cultures ($O.D._{600}$ = 1.0) of A25-11, B10-A1, or EC1 (positive control) was introduced to the wells in the assay plates, with LB medium as the negative control. All the experiments were repeated three times. Promotion of rice shoots and roots **B** and **C** by treatment with A25-11 or B10-A1. Photographs were taken at 3 d (**B**) or 7 d (**C**) post germination, respectively. $H_2O$ and TSB medium served as blank controls. **D** Standard curve generated by using serial diluted IAA solutions (respectively containing 0.1, 0.5, 1, 5, 10, and 50 pmol IAA). **E** Quantification of IAA produced by A25-H1 and B10-A1. Mean ± *S.E.* was derived from three biological repeats. **, ***: $p < 0.01$, $p < 0.001$ vs Untreated A25-H1 sample respectively; ##, ###: $p < 0.01$, $p < 0.001$ vs Untreated B10-A1 sample respectively (Student's *t* test)

### Comparative genome analysis with A25-H1 and selected *P. ananatis* strains

As *P. ananatis* include both pathogenic and beneficial strains, we wonder whether the genomic character could reflect this divergence. Therefore we performed a phylogenomic analysis using OrthoFinder (v3.0.1) with A25-H1 genome and 18 *P. ananatis* genomes retrieved from the NCBI genome database. Single-copy orthologous protein sequences were extracted and aligned with MAFFT (v7.525). A maximum likelihood (ML) phylogenetic tree was subsequently constructed using IQ-TREE (v2.4.0) under the Q.PLANTFIR3 substitution model. A25-H1 is clustered with four rice endophytes and two rice pathogens (Figure S4A), suggesting that the closeness of genomic sequences is not determined by pathogenic or non-pathogenic nature of the strains.

We further compared the genomes of four pathogens and four plant-growth-promoting (PGP) strains by generating a gene presence/absence matrix. The *MCP II* gene, encoding a Methyl-accepting chemotaxis protein II, was found to be ubiquitous present in all four PGP strains but absent in the four phytopathogenic strains (Table S1). We further detected and compare the presence of this *MCP II* gene in the three *P. ananatis* strains (A25-F1, G1, H1) obtained in this study, and the established pathogenic strain SC7 [6], by PCR amplification. *MCP II* gene was present in A25-F1, G1 and H1 strains but not in SC7 (Figure S4B), suggesting that this gene may potentially implicate functional divergence between PGP and pathogenic *P. ananatis* strains.

## Discussion

Plant-associated microbial communities exert profound impacts on soil health, plant vitality, and ecosystem stability. Driven by recent breakthroughs in multi-omics technologies, microbiome engineering tailored for agricultural crops is rapidly evolving into a transformative frontier shaping the future of sustainable agriculture [42]. Plants can recruit beneficial rhizosphere microorganisms when attacked by pathogens, a process known as the "cry for help" response [43, 44]. For example, *Arabidopsis thaliana* promotes or enriches the growth of specific beneficial microorganisms in the rhizosphere following exposure to different plant pathogens [45–47]. After the pathogenic fungus *Rhizoctonia solani* infects sugar beets, *Chitinophaga*, and *Flavobacterium* are enriched in the roots to suppress the disease [48]. A significant enrichment of the dominant bacterium *Stenotrophomonas rhizome* (SR80) was observed in *Fusarium pseudograminearum*-infected wheat. Interestingly, the study also showed that SR80 not only promoted wheat growth but also boosted wheat defenses, which occurred in the presence of the pathogen [49]. Current research has predominantly focused on the rhizosphere or root-associated microbiomes, while studies on phyllosphere (leaf-associated) microbial communities remain relatively limited. The phyllosphere serves as a critical habitat for diverse microbial communities, including pathogens and the beneficial taxa that actively modulate host plant fitness and growth resilience to abiotic or biotic stresses [50–54].

Rice is a major food crop for a significant population of the world, yet its production is threatening by blast disease caused by the fungal pathogen *M. oryzae* [55, 56]. In this study, we attempted to investigate the function of microbial communities associated with rice in the occurrence of rice blast, based on microbiota analysis with rhizosphere soil, root, stem, and leaf of rice cultivars CO39 and Y33R. Our results showed that no significant difference between susceptible (CO39) and resistant (Y33R) rice cultivar in bacterial microbiota associated with rhizosphere soil, root, or leaf, while difference exists in rice stem (Fig. 1A-B). Our findings suggest that the underground microbial communities may have less pronounced impact on rice health and disease than aboveground communities, which is consistent with what has been reported [57, 58]

*Pantoea* spp. are widely distributed in soil and may colonize in different plant hosts including rice [6, 26, 59]. *P. ananatis* contain beneficial strains with growth promoting capacity [26, 60], by producing amino acid and useful secondary metabolites [61], as well as pathogenic strains causing plant diseases [62] including rice bacterial blight [6]. In this study, *Pantoea* spp. were found to be significantly enriched in CO39 rice phyllosphere (Fig. 1C). Simultaneously, we isolated several *Pantoea* strains from rice phyllosphere, including 3 strains of *P. ananatis* and 1 strain of *P. dispersa* (Fig. 1D, S2A-B). The *P. ananatis* consortium A25-11 and *P. dispersa* strain B10-A1 displayed suppressive effect of rice blast disease (Fig. 2) and rice bacterial blight (Fig. 3), but not pathogenic to rice (Fig. 3B and S2C). Furthermore, A25-11 and B10-A1 were able to promote rice seed germination and root growth (Fig. 6B-C; Table 1), likely due to IAA production

**Table 1** Growth promoting capacity of *Pantoea* consortium/strain

| Treatment | Rice shoot length[a] (cm) | Rice root length[b] (cm) |
|---|---|---|
| $H_2O$ | CO39: 0.78 ± 0.19 [B]<br>Y33R: 0.96 ± 0.05 [B] | – |
| TSB | CO39; 0.62 ± 0.06 [B]<br>Y33R: 0.90 ± 0.11 [B] | 5.60 ± 0.12 [C] |
| A25-11 | CO39: 1.44 ± 0.09 [A]<br>Y33R: 1.37 ± 0.04 [A] | 7.25 ± 0.43 [B] |
| B10-A1 | CO39: 1.75 ± 0.06 [A]<br>CO39: 1.45 ± 0.03 [A] | 10.52 ± 0.19 [A] |

[a] Rice shoot length was measured at 3 d post germination

[b] Rice root length was measured at 7 d post germination

For a-b, mean ± *S.D.* was derived from three biological repeats, and different capitalized letters denote statistically significant difference ($p < 0.05$) in each category

by these strains (Fig. 6E, S3B-C). Expansins are cell-wall loosening proteins to modulate plant cell wall flexibility during growth, abiotic or biotic stresses (including pathogen attack) [63]. Some members of *EXP* family genes are induced by elevated IAA level [41], which plays a role in plant resistance or susceptibility to pathogens. It has been reported that phytol activates *EXP-A20* that mediates maize defense responses against the pest *Ostrinia furnacalis* [64]. Exogenous application of IAA facilitates mycorrhizal symbiosis through regulation of *EXP* family gene expression [65]. Therefore, IAA may play a dual role in regulating plant growth and microbe-plant interaction. In this study, we found that both the A25-11 consortium and B10-A1 strain were able to significantly induce the expression of *OsEXPA7*, *OsEXPB3*, and *OsEXPB7* in rice, likely contributing to colonization of the biocontrol bacteria and/or promoting disease resistance.

Our result further demonstrated that the biocontrol effect of A25-11 and B10-A1 were not based on suppression of the growth of fungal or bacterial pathogens (Figure S2D-E), but may depend on immunity priming, as *OsPR10* and *PBZ1* genes in rice were significantly upregulated and ROS accumulated after treatment with A25-11 or B10-A1 (Fig. 4B-D). *OsPR10* and *PBZ1* genes are involved in SA-mediated systemic acquired resistance (SAR) to protect rice from diseases [32]. As an inhibitor of phenylalanine ammonia-lyase (PAL), AIP effectively suppresses SA biosynthesis, ROS generation, and pathogenesis-related (PR) gene expression in *Gossypium hirsutum* [66] and in rice [38]. Our results demonstrated that AIP application counteracted the A25-11 based immunity priming against blast fungus (Fig. 5), suggesting that A25-11 activates rice defense response likely through SA signaling pathway.

Although both the plant and soil associated microbiome have been studied over the decades, the efficiency of translating the laboratory and greenhouse findings to the field is largely dependent on the ability of the beneficial microorganisms to colonize the soil and maintain stability in the ecosystem [67]. Our study specifically targets foliar diseases as the primary focus of pathological investigation. We proved that the A25-11 and B10-A1 inoculated soil could be used for growing the next batch of rice, and endowed the newly planted rice with resistant to blast disease without treatment of fresh A25-11 and B10-A1 cultures (Fig. 2). Furthermore, A25-11 consortium and B10-A1 strain provided substantial protection against blast disease (both leaf and panicle blast) in the fields, under the natural climatic conditions as air temperature ranging from 21 to 33 °C and mean relative humidity ≥ 80%, and slightly acidic soils in the disease plots (pH = 5.5–7.0). We conclude that the *Pantoea* strains obtained in this study could exert ideal biocontrol effect under such climatic and soil conditions, while it remains to be determined for the suitability of these strains in other types of soils and climatic conditions.

## Conclusions

In summary, this study unveiled that the diseased rice recruited more beneficial phyllosphere microbes, among which the *Pantoea* strains (A25-F1, A25-G1, A25-H1, and B10-A1) can be used as new resources for controlling both rice fungal and bacterial diseases, depending on their significant effect in triggering rice immunity responses rather than direct antagonism against the microbial pathogens. The immunity-priming capacity of the *Pantoea* strains may lie on induction of SA-mediated systemic resistance. Overall, this study advances our mechanistic insight into how microbes impact plant health and productivity.

## Methods

### Sample collection and 16S rRNA amplicon analysis

The blast susceptible (CO39) and blast resistant (Y33R) rice were grown in a rice blast nursery of Yangjiang Institute of Agricultural Sciences (Baisha) Experimental Base (111°50′E, 21°52′N) (Figure S1A). Analysis of bacterial microbiota was performed on rhizosphere soil, root, stem, and leaf of CO39 and Y33R using full-length 16S rRNA amplicon sequencing. Meanwhile, bacterial strains were isolated and identified from these samples (the working flow scheme shown in Figure S1B).

To amplify the full-length sequence of the 16S rRNA gene, the primers 27F (5'-AGRGTTYGATYMTGGCTCAG-3') and 1492R (5'-YGRTACCTTGTTACGACTT-3') were used to perform PCR amplification [68]. The $ddH_2O$ was used as extraction blank control during DNA extraction and no template control during PCR amplification. The amplified products were then used for library construction and sequenced by the PacBio Sequel I high-throughput sequencing platform to obtain high-quality single-end full-length 16S rRNA sequence data. The raw sequencing data underwent preliminary

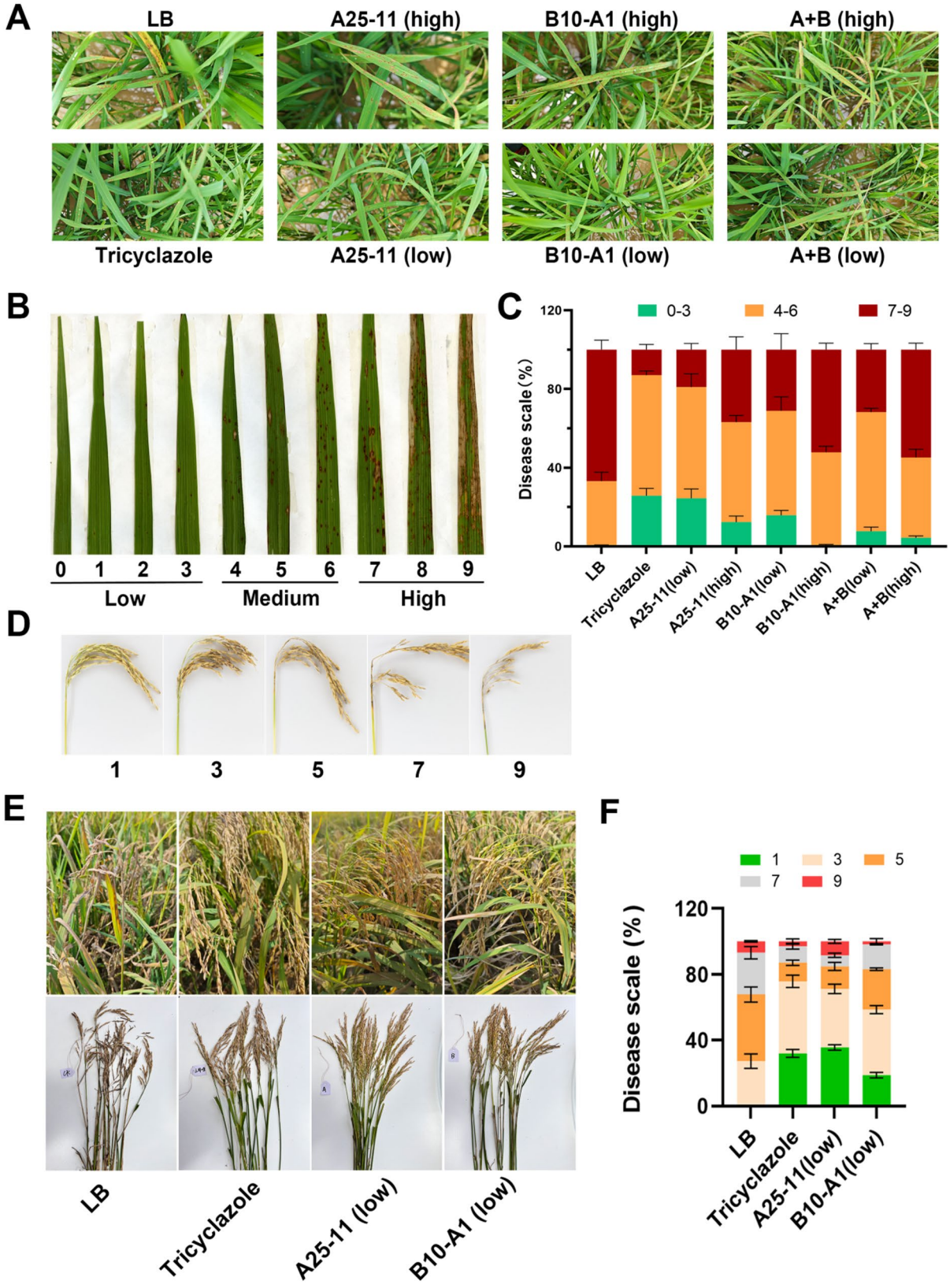


**Fig. 7** (See legend on next page.)

(See figure on previous page.)

**Fig. 7** Control of leaf and panicle blast by *Pantoea* strains in field condition. **A** Treatment with *Pantoea* strains/consortium (A25-11, B10-A1, or A + B) in experiment plots. Fresh cultures of A25-11, B10-A1, or A + B, of high ($O.D._{600}$ = 1.0) or low ($O.D._{600}$ = 0.5) concentration were sprayed uniformly onto rice seedlings. Tricyclazole served as the positive control and LB medium as the negative control. Leaf blast symptoms were photographed at 21 d post treatment. **B** Leaf blast scale for different susceptibility levels (low: 0–3 grade; medium: 4–6 grade; high: 7–9 grade) following the established standard [35]. **C** Statistical analysis of leaf blast scale for the tested rice seedlings in experiment plots. Percentage (%) represents number of leaves out of 300 leaf blades assessed for each instance. **D** Panicle blast scale for different susceptibility levels following the established standard [35]. **E** Treatment with *Pantoea* strains/consortium (A25-11, B10-A1, or A + B) in experiment plots. Fresh cultures of A25-11, B10-A1, or A + B, of $O.D._{600}$ = 0.5, were sprayed uniformly onto rice at the booting stage. Tricyclazole served as the positive control and LB medium as the negative control. Leaf blast symptoms were photograghed at 21 d post treatment. **F** Statistical analysis of panicle blast scale for the tested rice in experiment plots. Percentage (%) represents number of panicles ($n$ = 150) for each instance

quality control through fastplong (v0.2.2, with default settings) to evaluate the quality of the sequences and remove low-quality data. Subsequently, cutadapt (v4.5) software was used to accurately remove primers at both ends of the sequence to obtain filtered high-quality effective sequences. For the preprocessed effective sequences, DADA2 plugin with the denoise-ccs method in QIIME 2 (v2023.9.1) was used to denoise and remove chimeras and redundancy. Finally, a high-resolution ASV table and representative sequences were generated. Taxonomic analysis of ASVs was performed using the DADA2 assignTaxonomy function with three reference databases (SILVA v138.1, GTDB r207, and RefSeq + RDP). The Naive Bayes classifier was employed to assign taxonomy, and the most reliable annotation was selected for each ASV. The R package microeco (v1.8.0) was used to calculate and visualize alpha and beta diversity. The composition of microbial communities was analyzed at the genus level and the results were visualized using ggplot2 (v3.5.1). Lefse method [69] was used to identify microbial genera with significant differences between groups.

#### Plant materials, fungal and bacterial strains, media, and growth conditions

Rice cultivars used in this study included CO39 (susceptible to Guy11) and Y33R (resistant to Guy11). *M. oryzae* strain Guy11 was used for pathogenicity assay. *M. oryzae* strains were grown on prune agar medium (PA, 40 mL/liter prune, 2.5 g/liter sucrose, 2.5 g/liter lactose, 1 g/liter yeast extract, 1.5% *wt*/*vol* agar, final pH 6.5 ± 0.2). *Pantoea* strains A25-F1, A25-G1, A25-H1, and B10-A1 were isolated in this study. *P. ananatis* strain SC7 [6], *Xoo* strain Pxo99A [6], and *D. oryzae* EC1 [70] were lab collections.

Isolation of the bacteria strains from rice phyllosphere was carried out using an established method previously described [28, 71, 72]. Rice leaves were washed with sterile water to remove dirt, and then cut into small pieces (0.5 to 1 $cm^2$) and subjected to surface disinfection with 70% ethanol solution for 30 s, 5% sodium hypochlorite for 1 min, and sterile water wash three times. The cutted tissues were placed onto Tryptic Soy Broth (TSB, 17 g/liter tryptone, 3 g/liter soya peptone, 5 g/liter sodium chloride, 2.5 g/liter dipotassium hydrogen phosphate anhydrous, 2.5 g/liter dextrose, final pH 7.0 ± 0.2) for incubation at 28 °C for 24 h. Bacterial colonies from the plates were respectively streaked onto fresh plates for purification and grown in TSB medium with shaking at 200 rpm overnight for preservation.

Luria–Bertani (LB, containing 10 g/liter typtone, 5 g/liter yeast extract, and 10 g/liter NaCl) agar (1.5% *wt*/*vol*) was used for culturing *Pantoea* strains, at 28 °C, 200 rpm for 12–16 h, for rice bacterial blight disease assay and bacteria confrontation culture.

For detection of virulence factor Peh, Cel, and Prt, the following assay media were used: Peh assay medium: 0.5% polygalacturonic acid, 0.2% sucrose, 0.2% ammonium sulfate, pH 5.5, 1.5% agarose. Cel assay medium: 0.1% carboxymethyl cellulose, 0.38% sodium phosphate, pH 7.0, 0.8% agarose. Prt assay medium: 1% non-fat milk, 0.5% yeast extract, 1% typtone, 1% sodium chloride, pH 7.0, 1.5% agar.

The rice seeds were disinfected with 75% alcohol, washed three times with $ddH_2O$, resuscitated in a shaking platform at 28 °C for 3–4 h, and incubated under humid conditions for 2–3 days. The germinated seeds were planted in pond mud accompanied by matrix soil, and cultured under alternate dark: light cycle (12 h:12 h) in rice room at 28 °C for 2–3 weeks.

The wild-type strain of *M. oryzae*, Guy11, and Zhong1, were cultivated on rice flour (20 g/liter rice flour, 2 g/liter yeast extract) agar (1.5% *wt*/*vol*) medium at 25 °C for 3 days in the dark, followed by growth under a 12 h light / 12 h dark photoperiod for 7 days to induce conidiation. The conidia suspension was adjusted to $10^5$ conidia/mL before inoculation to rice leaves or seedlings.

#### Phylogenetic analyses of *Pantoea* strains

The 16S rRNA, *gyrB*, *leuS*, and *rpoB* gene sequences of A25-F1, A25-G1, A25-H1, and B10-A1 strains were amplified by PCR using Green Taq Mix (Vazyme, Nanjing, China) and the primers listed in Table 2, with the following amplification conditions: 1 cycle at 95 °C for 3 min, 30 cycles at 95 °C for 15 s, 62 °C for 15 s, and 72 °C for 60 s/kb, followed by 1 cycle at 72 °C for 5 min. The amplified DNA fragments were sequenced by Sangon Biotech. BLAST was performed to analyze sequence identity between the sequenced fragments and the sequences retrieved from GenBank database. MEGA

**Table 2** Primers used in this study

| Gene name | Primer sequence (5′-3′) |
|---|---|
| 16S rRNA | F: AGAGTTTGATCATGGCTCAG |
| | R: AAGGAGGTGATCCAACCGC |
| *gyrB* | F:GAAGTCATCATGACCGTTCTGCAYGCNGGNGGNAARTTYGA |
| | R: AGCAGGGTACGGATGTGCGAGCCRTCNACRTCNGCRT-CNGTCAT |
| *leuS* | F: CAGTGGTTTATCAAAATCACC |
| | R: GGATAACCGGAAGCTGATCTT |
| *rpoB* | F: GGCTGAAAACCAGTTCCGCGT |
| | R: CCAGAGAGACACACGGCATCT |
| *PANsp_atpD* | F: GATGAACGAGCCGCCGGGTA |
| | R: GCCAGCGTTGGCTGATAACC |
| *OsACTIN* | F: GTGGTCGCCCCTCCTGAAAG |
| | R: GGCTTAGCATTCTTGGGTCCG |
| *OsUBQ* | F: AAGAAGCTGAAGCATCCAGC |
| | R: CCAGGACAAGATGATCTGCC |
| *OsPR10* | F: CCTCAGCCATGCCATTCAG |
| | R: CTTGTCCACGTCCAGGAACTC |
| *PBZ1* | F: GCTACAGGCATCAGTGGTCA |
| | R: GACTCAAACGCCACGAGAAT |
| *NPR1* | F: TGGCAGGTGAGAGTCTACGA |
| | R: AGGTGGATTTGCACCAGAAC |
| *OsEXPA7* | F: TGCCCATGTTCCTCCACATC |
| | R: CGTGATCAGCACCAGGTTGA |
| *OsEXPB3* | F: GTCTACCGTTCTTTCGTCCAGT |
| | R: CCATACAATACCGAGGGAGAGC |
| *OsEXPB7* | F: CTCCATCGTCCAGTTCGACTAG |
| | R: GCATATACGCACTGAAACGGAC |
| *MCP II* | F: CACCAAATCCGATAACCTGA |
| | R: GCTACCTGCCGAAACTATCA |

**Table 3** Gene sequences used for phylogenetic analysis

| Species | 16S rRNA | *gyrB* | *leuS* | *rpoB* |
|---|---|---|---|---|
| *P. ananatis* | JF756690.1 | MW981331.1 | MW981336.1 | MW436597.1 |
| *P. agglomerans* | AY741162.1 | OR544063.1 | KU168122.1 | LC722493.1 |
| *P. allii* | OL911051.1 | MZ299242.1 | MN065823.1 | GU458429.1 |
| *P. stewartii* | OR616746.1 | MH300621.1 | EU010051.1 | MH479188.1 |
| *P. dispersa* | MT826239.1 | OM863796.1 | OR881882.1 | OM792196.1 |
| *P. calida* | KF555071.1 | KF554595.1 | KF482640.1 | GQ892190.1 |
| *P. vagans* | PQ740424.1 | OP272647.1 | KF482636.1 | OP272662.1 |
| *E. coli* | AY776276.1 | KJ868400.1 | EU010053.1 | JX471606.1 |

5.1 was used for comparison analysis, and the 4 gene sequences were integrated for constructing the phylogenetic tree using NJ method, with bootstrap set as 1000 times. The reference species and gene accession numbers are listed in Table 3.

Genome of A25-H1 and B10-A1 strains were sequenced by the second-generation paired-end Illumina sequencing platform, and MaSuRCA (v4.1.0) was used for genome assembly. The proteomes of 37 *Pantoea* spp. were downloaded from the NCBI database, and used for phylogenetic analysis with A25-H1 and B10-A1. Single-copy orthologous genes were identified using Orthofinder (v3.0.1b1), and MAFFT (v7.520) was used to perform multiple sequence alignments for each single-copy orthologous gene. The multiple sequence alignments were trimmed using Trimal (v1.5.0) and concatenated. A phylogenetic tree was then constructed with IQ-TREE2 (v2.3.1) to infer the evolutionary relationships among the genomes. Meanwhile, in order to quantitatively evaluate the similarity of A25-H1 and B10-A1 with other genomes, we used FastANI (v1.34) to calculate their ANI (Average Nucleotide Identity) values. Finally, the visual analysis of the phylogenetic tree and ANI values was completed through iTOL (https://itol.embl.de/) for visual display and further explanation of the results. KEGG map of tryptophan metabolism made with KOALA (https://www.kegg.jp/, version 3.1, with default settings).

Phylogenetic analysis based on the whole genome sequences was conducted using OrthoFinder (version 3.0.1). Single-copy orthologous protein sequences were extracted and aligned using MAFFT (version 7.525). A maximum likelihood (ML) phylogenetic tree was then constructed using IQ-TREE (version 2.4.0) with the Q.PLANTFIR3 substitution model and 1,000 bootstrap replicates. Eight selected genomes were annotated using Prokka (version 1.14.6) and subsequently analyzed by using Roary (version 3.13.0) under a strict gene clustering mode to identify homologous gene clusters among strains and to generate a gene presence/absence matrix. This matrix was used to compare gene content variation among different strains.

### Disease assays

For blast disease assay, rice seedlings grown in the pots up to the four-leaf stage, and *M. oryzae* conidia suspension ($10^5$/mL; 80 mL/pot) was sprayed to the seedlings, according to the established method [73, 74]. Three biological repeats each with no less than 5 rice seedlings was performed. The sprayed seedlings were incubated in a dark dew chamber at 25 °C and 95% relative humidity for 24 h, and subsequently transferred to a moist greenhouse with a cycle of 12 h light / 12 h dark at 25 °C to 28 °C. The disease symptoms were observed and photographed on the 7th day. For inoculation using the detached rice leaves, the 2-week-old rice leaves were cut and placed in clean and square petri dishes. *M. oryzae* conidia suspension ($10^5$/mL; 20 µL/droplet) [75] or cultures of A25-11, B10-A1 strain ($O.D._{600}$ = 1.0; 20 µL/droplet) were inoculated onto the detached leaves, incubated at 25 °C under dark for 24 h and then under dark:light cycle (12 h:12 h) for 5–6 days before photographing.

For bacterial blight disease assay, fully expanded leaves of rice plants (6 weeks old) were inoculated using a

leaf-tip clipping method [37]. The bacterial cells (A25-11, B10-A1, SC7, and Pxo99A) were streaked out on TSB plates and incubated at 28 °C for 2–4 d. The cells were harvested from the plates and re-suspended in sterilized PBS solution ($O.D._{600}$ = 1.0). Scissor blades were immersed in bacterial cell suspension and used to clip about 2 cm from the leaf tip. All the inoculated plants maintained under plant growth chamber (28 °C; 80 to 85% relative humidity). The lesion lengths were measured 14 d after inoculation [37]. Three replications with about ten leaves from two to five plants per replicate were inoculated per strain.

Rice CO39 seeds were soaked with A25-11, B10-A1, or A + B co-culture ($O.D._{600}$ = 0.5 or 1.0) for 24 h, then the gernimated seeds were planted into the blast nursery of Yangjiang Baisha Experimental Station. At the time of transplanting rice seedlings to the experimental plots, foliar application of high-concentration ($O.D._{600}$ = 1.0) or low-concentration ($O.D._{600}$ = 0.5) suspensions of A25-11, B10-A1, or A + B were performed. Leaf blast symptom was documented and photographed at 21 d post treatment. During the early booting stage, A25-11 consortium or B10-A1 strain ($O.D._{600}$ = 0.5) was applied to the rice plants, and the severity of panicle blast was assessed at 21 d post treatment. Tricyclazole fungicide and LB were employed as the positive and negative control treatments, respectively, in the experiment.

#### Confrontation culture

Confrontations between *Pantoea* strains and *M. oryzae* strains were performed on PA medium. A disc of agar from the edge of the *M. oryzae* strains colony was cut and placed in the center of the Petri dish. An aliquot of 1.0 µL of *Pantoea* A25-11 or B10-A1 ($O.D._{600}$ = 0.6) was spotted evenly around the periphery of *M. oryzae.* The Petri dish was then incubated in darkness at 28 °C for 7 days before photograph. Confrontations between *Pantoea* strains and leaf blight strain SC7 or Pxo99A were performed on LB agar medium. An aliquot of 1.0 µL of bacterial suspension ($O.D._{600}$ = 0.6) was spotted equidistantly on the Petri dish with LB agar medium. The Petri dish was then incubated in darkness at 28 °C for 3–4 days before photograph.

#### ROS determination

Two-week old rice leaves were excised to four 2-mm-diameter leaf discs and placed in a pre-filled 96-well microplate (black plate with white wells) containing $ddH_2O$ (200 µl/well). The plate was incubated statically in the dark at 28 °C for 8–14 h to dissipate ROS generated by wounding. After removing $ddH_2O$ from the microplate, a 200 µL reaction mixture containing the tested bacterial cells (final $O.D._{600}$ = 0.1) and ROS assay buffer [0.2 mM L-012 (Wako, Japan), 10 µg/mL HRP (Sigma, USA), 50 mM Tris–HCl, and 0.03% Tween 20] was added to each well. Chitin at a final concentration of 500 µg/mL was served as the positive control, while $ddH_2O$ used for suspending the tested bacterial cells was used as the negative control. Chemiluminescence was detected in a Varioskan LUX microplate reader (Thermo Scientific, USA) within 1 h. Program settings: 5-s orbital shaking (medium intensity, 180 rpm), kinetic cycles (120 min, 120 s/cycle), and chemiluminescence detection (1 s/well).

#### Virulence factor determination

Production of Cel, Prt, and Peh was determined by using their assay medium respectively following the established methods [76] with minor modification. Briefly, the assay plate of Cel, Prt, and Peh was prepared by pouring about 30 ml of their assay medium to a 100 × 100 mm petri dish. After solidification, 5-mm diameter wells were introduced on the prepared assay plate. An aliquot of 20 µl of fresh cell culture of EC1 (positive control), A25-11, or B10-A1 grown in LB medium at $O.D._{600}$ of 1.0 was added to the wells in the assay plate. The LB medium was also added to a well in each plate as a negative control. For Cel assay, the plates incubated at 28 °C for 16 h were stained with 0.1% Congo red (*wt*/*vol*) for 10 min and then decolored with 1 M NaCl for multiple times. The plates were photographed when the clear zone created by EC1 was visible. For Prt assay, the plates were photographed after incubating at 28 °C for 16 h. For Peh assay, the plates incubated at 28 °C for 24 h were treated with 1 M HCl for multiple times. The plates were photographed when the clear zone created by EC1 was visible. Each assay was repeated at least three times.

#### qPCR (quantitative PCR) and RT-qPCR (Reverse transcription-quantitative PCR) assays

Surbiopure Plant Genomic DNA Purification Kit (catalog number: 031602-1C08) was used for total DNA extraction from two-week old rice samples. Verification of *Pantoea* biomass in rice leaves were performed by qPCR using primers targeting *PANsp_atpD* gene (Table 2).

For assessment of rice immunity gene expression under treatment by *Pantoea* strains, RT-qPCR analysis was performed. The culture of A25-11 or B10-A1 ($O.D._{600}$ = 0.5) was inoculated on detached rice leaves for 48 h, and total RNA was extracted from rice leaves by using Easypure® Universal Plant Total RNA Kit (TRANS. Catalog Number: ER302-02). RNA integrity was verified through 1% agarose gel electrophoresis. First-strand cDNA synthesis was performed with HiScript® III RT SuperMix kit (Vazyme, R323-01), followed by qRT-PCR using SYBR qPCR Master Mix (Vazyme, Q711-02), on a QuantStudio 6 Flex Real-Time PCR System (Thermo Fisher Scientific). The relative fold changes were calculated using the $2^{-\Delta\Delta C(t)}$ method [77]. At least three independent biological repeats were performed for each instance.

### Measurement of IAA

The *Pantoea* strains were cultured in TSB supplemented with 1 mg/mL L-tryptophan. The culture was incubated with shaking at 180 rpm in 28 °C for 3 days to reach an $O.D._{600}$ of about 2.0. The supernatant collected after centrifugation was adjusted to pH 3.0 and then extracted twice with an equal volume of ethyl acetate. Meanwhile, the harvested cells whose wet weight have been measured were suspended with PBS, subjected to vacuum disruption, and then centrifuged for collecting the supernatant, which was also adjusted to pH 3.0 and extracted twice with an equal volume of ethyl acetate. The ethyl acetate phase was vacuum-concentrated and re-dissolved in methanol for LC–MS analysis of intracellular and extracellular IAA contents following the established method [39].

### Chemical treatments

Aminoindan-phosphonic acid hydrochloride (AIP, CAS: 1,416,354–35-2) was purchased from Shanghai Aladdin Biochemical Technology Co., Ltd (catalog number: A587182-250 mg). The fungicide 20% Tricyclazole (CAS: 41,814–78-2) was purchased from Jiangmen Plant Protection Co., Ltd. (pesticide registration number: PD20130273). AIP was dissolved in dimethyl sulfoxide (DMSO) to prepare a 400 mM stock solution, and diluted in distilled water to reach the working concentration (200 μM) For foliar application, 20 ml of AIP solution containing 0.02% (v/v) Tween 20 surfactant was sprayed over ten plants. For soil drench application, 5 ml of AIP solution was applied to saturate the soil per plant.

### *Statistical analysis*

Statistical analyses were performed with GraphPad_Prism 8. DPS 9.01 software, and one-way analysis of variance (ANOVA) tests or Student's *t*-test was carried out. $p$ values $< 0.05$ were considered as statistically significance.

#### Abbreviations

| | |
|---|---|
| ANI | Average nucleotide identity |
| ASVs | Amplicon sequence variants |
| Cel | Cellulose |
| IAA | Indole-3-acetic acid |
| JA | Jasmonic acid |
| LEfSe | Linear discriminant analysis Effect Size |
| NCBI | National Center for Biotechnology Information |
| PCoA | Principal coordinate analysis |
| Peh | Polygalacturonase |
| PERMANOVA | Permutational multivariate analysis of variance |
| Prt | Protease |
| qPCR | Quantitative PCR |
| RT-qPCR | Reverse transcription-quantitative PCR |
| SA | Salicylic acid |
| SRA | Sequence read archive |
| SynCom | Simplified synthetic community |
| PA | Prune agar |
| TSB | Tryptic Soy Broth |
| LB | Luria–Bertani medium |
| AIP | Aminoindan-phosphonic acid |
| PAL | Phenylalanine ammonia-lyase |

## Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40793-025-00799-y.

Supplementary Material 1

#### Acknowledgements

Not applicable.

#### Author contributions

YD, JFL, ZL conceptualized the study. YZD acquired funding. WS, QL, XX, ZZ, YZ, and JZ help in conducting experiments and analyzing data. HLC and XFZ analyzed and interpreted the 16S rRNA amplicon sequences data. WS and XJ conducted the field experiments. WDS, QL, and YZD drafted the original manuscript. WDS, QL, and HLC revised the manuscript. All authors approved the submitted manuscript.

#### Funding

This research was financially supported by National Key Research and Development Program of China (2022YFA1304402; 2023YFD1400200).

#### Data availability

The 16S rRNA amplicon sequences data have been deposited in the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) under accession number PRJNA1213804. Supporting data for this study are available in the supplementary information.

## Declarations

#### Ethics approval and consent to participate

Not applicable.

#### Consent for publication

Not applicable.

#### Competing interests

The authors declare no competing interests.

#### Author details

[1]Guangdong Provincial Key Laboratory of Microbial Signals and Disease Control, Integrative Microbiology Research Centre, South China Agricultural University, Guangzhou 510642, China
[2]Guangdong Provincial Key Laboratory of Plant Stress Biology, State Key Laboratory of Biocontrol, School of Life Sciences, Sun Yat-Sen University, Guangzhou 510275, China
[3]Yangjiang Institute of Agricultural Sciences, Yangjiang 529500, China

Received: 17 February 2025 / Accepted: 11 October 2025
Published online: 07 November 2025

#### References

1. Fukagawa NK, Ziska LH. Rice: importance for global nutrition. J Nutr Sci Vitaminol (Tokyo). 2019;65(Supplement):S2–3.
2. Liu Z, Zhu Y, Shi H, Qiu J, Ding X, Kou Y. Recent progress in rice broad-spectrum disease resistance. Int J Mol Sci. 2021. https://doi.org/10.3390/ijms222111658.
3. Talbot NJ. Appressoria. Curr Biol. 2019;29(5):R144–6.
4. Foster AJ, Talbot NJ. Getting a grip on blast. Nat Microbiol. 2020;5(12):1457–8.
5. Wilson RA. Magnaporthe oryzae. Trends Microbiol. 2021;29(7):663–4.
6. Xue Y, Hu M, Chen S, Hu A, Li S, Han H, et al. *Enterobacter asburiae* and *Pantoea ananatis* causing rice bacterial blight in China. Plant Dis. 2021;105(8):2078–88.

7. Jiang N, Yan J, Liang Y, Shi Y, He Z, Wu Y, et al. Resistance genes and their interactions with bacterial blight/leaf streak pathogens (*Xanthomonas oryzae*) in rice (*Oryza sativa* L.)-an updated review. Rice. 2020;13(1):3.
8. Yin Y, Miao J, Shao W, Liu X, Zhao Y, Ma Z. Fungicide resistance: progress in understanding mechanism, monitoring, and management. Phytopathology. 2023;113(4):707–18.
9. Jayasiri M, Yadav S, Propper CR, Kumar V, Dayawansa NDK, Singleton GR. Assessing potential environmental impacts of pesticide usage in paddy ecosystems: a case study in the deduru oya river basin, Sri Lanka. Environ Toxicol Chem. 2022;41(2):343–55.
10. Zhong Z, Chen M, Lin L, Chen R, Liu D, Norvienyeku J, et al. Genetic variation bias toward noncoding regions and secreted proteins in the rice blast fungus *Magnaporthe oryzae*. mSystems. 2020. https://doi.org/10.1128/msystems.00346-20.
11. Bezerra GA, Chaibub AA, Oliveira MIS, Mizubuti ESG, Filippi MCC. Evidence of *Pyricularia oryzae* adaptability to tricyclazole. J Environ Sci Health B. 2021;56(10):869–76.
12. Xu Z, Xu X, Wang Y, Liu L, Li Y, Yang Y, et al. A varied AvrXa23-like tale enables the bacterial blight pathogen to avoid being trapped by *Xa23* resistance gene in rice. J Adv Res. 2022;42:263–72.
13. Bulgarelli D, Schlaeppi K, Spaepen S, Loren V, van Themaat E, Schulze-Lefert P. Structure and functions of the bacterial microbiota of plants. Annu Rev Plant Biol. 2013;64:807–38.
14. Frontiers Production O. Erratum: From microbiome to traits: designing synthetic microbial communities for improved crop resiliency. Front Plant Sci. 2020;11:614083.
15. Paasch BC, He SY. Toward understanding microbiota homeostasis in the plant kingdom. PLoS Pathog. 2021;17(4):e1009472.
16. Wang W, Zhai Y, Cao L, Tan H, Zhang R. Endophytic bacterial and fungal microbiota in sprouts, roots and stems of rice (*Oryza sativa* L.). Microbiol Res. 2016;188:1–8.
17. Sahu KP, Patel A, Kumar M, Sheoran N, Mehta S, Reddy B, et al. Integrated metabarcoding and culturomic-based microbiome profiling of rice phyllosphere reveal diverse and functional bacterial communities for blast disease suppression. Front Microbiol. 2021;12:780458.
18. Dastogeer KMG, Yasuda M, Okazaki S. Microbiome and pathobiome analyses reveal changes in community structure by foliar pathogen infection in rice. Front Microbiol. 2022;13:949152.
19. Wang D, Wan Y, Liu D, Wang N, Wu J, Gu Q, et al. Immune-enriched phyllosphere microbiome in rice panicle exhibits protective effects against rice blast and rice false smut diseases. Imeta. 2024;3(4):e223.
20. Wang D, Wei L, Ma J, Wan Y, Huang K, Sun Y, et al. *Bacillus cereus* NJ01 induces SA- and ABA-mediated immunity against bacterial pathogens through the EDS1-WRKY18 module. Cell Rep. 2024;43(4):113985.
21. Khan RAA, Alam SS, Jaman MS, Li Y, Ahmad M. Editorial: Biocontrol of phytopathogens-recent progress for improvement in efficacy and understanding action mechanism. Front Microbiol. 2024;15:1407711.
22. Yu L, Yang C, Ji Z, Zeng Y, Liang Y, Hou Y. First report of new bacterial leaf blight of rice caused by *Pantoea ananatis* in southeast China. Plant Dis. 2021;106(1):310.
23. Gao L, Li W, Ren B, Hu Y, Ma X, Jia Y, et al. First report of leaf blight caused by *Pantoea agglomerans* on wheat in China. Plant Dis. 2022;107(4):1215.
24. Luna E, Lang JM, McClung A, Wamishe Y, Jia Y, Leach JE. First report of rice bacterial leaf blight disease caused by *Pantoea ananatis* in the United States. Plant Dis. 2023. https://doi.org/10.1094/PDIS-08-22-2014-PDN.
25. Smits THM, Duffy B, Blom J, Ishimaru CA, Stockwell VO. Pantocin A, a peptide-derived antibiotic involved in biological control by plant-associated *Pantoea* species. Arch Microbiol. 2019;201(6):713–22.
26. Lu L, Chang M, Han X, Wang Q, Wang J, Yang H, et al. Beneficial effects of endophytic *Pantoea ananatis* with ability to promote rice growth under saline stress. J Appl Microbiol. 2021;131(4):1919–31.
27. Lv L, Luo J, Ahmed T, Zaki HEM, Tian Y, Shahid MS, et al. Beneficial effect and potential risk of *Pantoea* on rice production. Plants. 2022. https://doi.org/10.3390/plants11192608.
28. Finkel OM, Castrillo G, Herrera Paredes S, Salas Gonzalez I, Dangl JL. Understanding and exploiting plant beneficial microbes. Curr Opin Plant Biol. 2017;38:155–63.
29. Copeland C, Schulze-Lefert P, Ma KW. Potential and challenges for application of microbiomes in agriculture. Plant Cell. 2025. https://doi.org/10.1093/plcell/koaf185.
30. Liu L, Sonbol FM, Huot B, Gu Y, Withers J, Mwimba M, et al. Salicylic acid receptors activate jasmonic acid signalling through a non-canonical pathway to promote effector-triggered immunity. Nat Commun. 2016;7:13099.
31. De Vleesschauwer D, Gheysen G, Hofte M. Hormone defense networking in rice: tales from a different world. Trends Plant Sci. 2013;18(10):555–65.
32. Salman EK, Ghoniem KE, Badr ES, Emeran AA. The potential of dimetindene maleate inducing resistance to blast fungus *Magnaporthe oryzae* through activating the salicylic acid signaling pathway in rice plants. Pest Manag Sci. 2022;78(2):633–42.
33. Islam MR, Chowdhury R, Roy AS, Islam MN, Mita MM, Bashar S, et al. Native *Trichoderma* induced the defense-related enzymes and genes in rice against *Xanthomonas oryzae* pv. *oryzae* (*Xoo*). Plants (Basel). 2023. https://doi.org/10.3390/plants12091864.
34. Zhu Q, Tang MJ, Yang Y, Sun K, Tian LS, Lu F, et al. Endophytic fungus *Phomopsis liquidambaris* B3 induces rice resistance to RSRD caused by *Fusarium proliferatum* and promotes plant growth. J Sci Food Agric. 2021;101(10):4059–75.
35. IRRI. Standard evaluation system for rice (SES). International Rice Research Institute. 2002.
36. Kini K, Agnimonhan R, Dossa R, Silue D, Koebnik R. Genomics-informed multiplex PCR scheme for rapid identification of rice-associated bacteria of the genus *Pantoea*. Plant Dis. 2021;105(9):2389–94.
37. Ke Y, Hui S, Yuan M. *Xanthomonas oryzae* pv. *oryzae* inoculation and growth rate on rice by leaf clipping method. Bio-Protoc. 2017;7(19):e2568.
38. Liu J, Lefevere H, Coussement L, Delaere I, De Meyer T, Demeestere K, et al. The phenylalanine ammonia-lyase inhibitor AIP induces rice defence against the root-knot nematode *Meloidogyne graminicola*. Mol Plant Pathol. 2024;25(1):e13424.
39. Cui G, Huang C, Bi X, Wang Y, Yin K, Zhu L, et al. Aminotransferase SsAro8 regulates tryptophan metabolism essential for filamentous growth of sugarcane smut fungus *Sporisorium scitamineum*. Microbiol Spectr. 2022;10(4):e0057022.
40. McQueen-Mason S, Durachko DM, Cosgrove DJ. Two endogenous proteins that induce cell wall extension in plants. Plant Cell. 1992;4(11):1425–33.
41. Fu J, Liu H, Li Y, Yu H, Li X, Xiao J, et al. Manipulating broad-spectrum disease resistance by suppressing pathogen-induced auxin accumulation in rice. Plant Physiol. 2011;155(1):589–602.
42. Berg G, Koberl M, Rybakova D, Muller H, Grosch R, Smalla K. Plant microbial diversity is suggested as the key to future biocontrol and health trends. FEMS Microbiol Ecol. 2017;93(5):fix050.
43. Rolli E, Vergani L, Ghitti E, Patania G, Mapelli F, Borin S. "Cry-for-help" in contaminated soil: a dialogue among plants and soil microbiome to survive in hostile conditions. Environ Microbiol. 2021;23(10):5690–703.
44. Rizaludin MS, Stopnisek N, Raaijmakers JM, Garbeva P. The chemistry of stress: understanding the "cry for help" of plant roots. Metabolites. 2021. https://doi.org/10.3390/metabo11060357.
45. Berendsen RL, Vismans G, Yu K, Song Y, de Jonge R, Burgman WP, et al. Disease-induced assemblage of a plant-beneficial bacterial consortium. ISME J. 2018;12(6):1496–507.
46. Yuan J, Zhao J, Wen T, Zhao M, Li R, Goossens P, et al. Root exudates drive the soil-borne legacy of aboveground pathogen infection. Microbiome. 2018;6(1):156.
47. Liu Y, Zhang H, Wang J, Gao W, Sun X, Xiong Q, et al. Nonpathogenic *Pseudomonas syringae* derivatives and its metabolites trigger the plant "cry for help" response to assemble disease suppressing and growth promoting rhizomicrobiome. Nat Commun. 2024;15(1):1907.
48. Carrion VJ, Perez-Jaramillo J, Cordovez V, Tracanna V, de Hollander M, Ruiz-Buck D, et al. Pathogen-induced activation of disease-suppressive functions in the endophytic root microbiome. Science. 2019;366(6465):606–12.
49. Liu H, Li J, Carvalhais LC, Percy CD, Prakash Verma J, Schenk PM, et al. Evidence for the plant recruitment of beneficial microbes to suppress soil-borne pathogens. New Phytol. 2021;229(5):2873–85.
50. Murty MG. Phyllosphere of cotton as a habitat for diazotrophic microorganisms. Appl Environ Microbiol. 1984;48(4):713–8.
51. Schauer S, Kutschera U. A novel growth-promoting microbe, *Methylobacterium funariae* sp. nov., isolated from the leaf surface of a common moss. Plant Signal Behav. 2011;6(4):510–5.
52. Vorholt JA. Microbial life in the phyllosphere. Nat Rev Microbiol. 2012;10(12):828–40.
53. Schmidt JE, Puig AS, DuVal AE, Pfeufer EE. Phyllosphere microbial diversity and specific taxa mediate within-cultivar resistance to *Phytophthora palmivora* in cacao. mSphere. 2023;8(5):e0001323.

54. Liu X, Matsumoto H, Lv T, Zhan C, Fang H, Pan Q, et al. Phyllosphere microbiome induces host metabolic defence against rice false-smut disease. Nat Microbiol. 2023;8(8):1419–33.
55. Talbot NJ. On the trail of a cereal killer: exploring the biology of *Magnaporthe grisea*. Annu Rev Microbiol. 2003;57:177–202.
56. Urashima AS, Grosso CRF, Stabili A, Freitas EG, Silva CP, Netto DCS, et al. Effect of *Magnaporthe grisea* on seed germination, yield and quality of wheat. Dordrecht: Springer, Netherlands; 2009.
57. Danso Ofori A, Su W, Zheng T, Datsomor O, Titriku JK, Xiang X, et al. Roles of phyllosphere microbes in rice health and productivity. Plants. 2024. https://doi.org/10.3390/plants13233268.
58. Sivakumar N, Sathishkumar R, Selvakumar G, Shyamkumar R, Arjunekumar K. Phyllospheric microbiomes: diversity, ecological significance, and biotechnological applications. Cham: Springer International Publishing; 2020.
59. Krishnappa C, Balamurugan A, Velmurugan S, Kumar S, Sampathrajan V, Kundu A, et al. Rice foliar-adapted *Pantoea* species: promising microbial biostimulants enhancing rice resilience against foliar pathogens, *Magnaporthe oryzae* and *Xanthomonas oryzae* pv. *oryzae*. Microb Pathog. 2024;186:106445.
60. Meepagala KM, Anderson CM, Techen N, Duke SO. *Pantoea ananatis*, a plant growth stimulating bacterium, and its metabolites isolated from *Hydrocotyle umbellata* (dollarweed). Plant Signal Behav. 2024;19(1):2331894.
61. Usuda Y, Nishio Y, Nonaka G, Hara Y. Microbial production potential of *Pantoea ananatis*: from amino acids to secondary metabolites. Microorganisms. 2022. https://doi.org/10.3390/microorganisms10061133.
62. Coutinho TA, Venter SN. *Pantoea ananatis*: an unconventional plant pathogen. Mol Plant Pathol. 2009;10(3):325–35.
63. Marowa P, Ding A, Kong Y. Expansins: roles in plant growth and potential applications in crop improvement. Plant Cell Rep. 2016;35(5):949–65.
64. Batool R, Umer MJ, Zhang Y, Guo J, Wang Z. Phytol-induced interplant signaling in maize facilitates *EXP-A20*-driven resistance through *ACO31*-dependent ethylene accumulation against *Ostrinia furnacalis*. Plant J. 2025;121(1):e17186.
65. Chen X, Chen J, Liao D, Ye H, Li C, Luo Z, et al. Auxin-mediated regulation of arbuscular mycorrhizal symbiosis: a role of SlGH3.4 in tomato. Plant Cell Environ. 2022;45(3):955–68.
66. Chai Q, Shang X, Wu S, Zhu G, Cheng C, Cai C, et al. 5-aminolevulinic acid dehydratase gene dosage affects programmed cell death and immunity. Plant Physiol. 2017;175(1):511–28.
67. Nadarajah K, Abdul Rahman NSN. The microbial connection to sustainable agriculture. Plants. 2023. https://doi.org/10.3390/plants12122307.
68. Dos Santos HRM, Argolo CS, Argolo-Filho RC, Loguercio LL. A 16S rDNA PCR-based theoretical to actual delta approach on culturable mock communities revealed severe losses of diversity information. BMC Microbiol. 2019;19(1):74.
69. Segata N, Izard J, Waldron L, Gevers D, Miropolsky L, Garrett WS, et al. Metagenomic biomarker discovery and explanation. Genome Biol. 2011;12(6):R60.
70. Hu M, Li J, Chen R, Li W, Feng L, Shi L, et al. *Dickeya zeae* strains isolated from rice, banana and clivia rot plants show great virulence differentials. BMC Microbiol. 2018;18(1):136.
71. Venkatachalam S, Ranjan K, Prasanna R, Ramakrishnan B, Thapa S, Kanchan A. Diversity and functional traits of culturable microbiome members, including cyanobacteria in the rice phyllosphere. Plant Biol (Stuttg). 2016;18(4):627–37.
72. Hartman K, van der Heijden MG, Roussely-Provent V, Walser JC, Schlaeppi K. Deciphering composition and function of the root microbiome of a legume plant. Microbiome. 2017;5(1):2.
73. Guo M, Chen Y, Du Y, Dong Y, Guo W, Zhai S, et al. The bZIP transcription factor MoAP1 mediates the oxidative stress response and is critical for pathogenicity of the rice blast fungus *Magnaporthe oryzae*. PLoS Pathog. 2011;7(2):e1001302.
74. Chen X, Jia Y, Wu BM. Evaluation of rice responses to the blast fungus *Magnaporthe oryzae* at different growth stages. Plant Dis. 2019;103(1):132–6.
75. Lopez-Moya F, Martin-Urdiroz M, Oses-Ruiz M, Were VM, Fricker MD, Littlejohn G, et al. Chitosan inhibits septin-mediated plant infection by the rice blast fungus *Magnaporthe oryzae* in a protein kinase C and Nox1 NADPH oxidase-dependent manner. New Phytol. 2021;230(4):1578–93.
76. Lv M, Ye S, Hu M, Xue Y, Liang Z, Zhou X, et al. Two-component system ArcBA modulates cell motility and biofilm formation in *Dickeya oryzae*. Front Plant Sci. 2022;13:1033192.
77. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods. 2001;25(4):402–8.


## Publisher's Note