Control of *Magnaporthe oryzae* and Rice Growth Promotion by *Bacillus subtilis* JN005

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Abstract

It is quite important to develop the microorganism resources with biocontrol capacity for rice blast. This study evaluated *Bacillus subtilis* JN005 for growth promotion and biocontrol efficacy against *Magnaporthe oryzae*. Results showed that rice seeds treated with 1×10^7 cfu/mL suspension of *B. subtilis* JN005 had 16% germination energy, 14% germination rate, 15% germination index, and 270% vigor index compared to those treated with sterile water (control). In pot experiments, the JN005 strain-treated rice plants exhibited notable increase in plant height, root length, stem circumference, and fresh weight, as well as higher concentration of chlorophyll a, chlorophyll b, and total chlorophyll in rice leaves. Rice leaves inoculated with the JN005 strain resulted in increased activities of defense-related enzymes, including peroxidase (POD), phenylalanine ammonia-lyase (PAL), superoxide dismutase (SOD), and catalase (CAT) compared to the water and the *M. oryzae*-inoculated treatments. In vitro inoculated rice leaves with 1×10^7 cfu/mL bacterial suspension compared to sterile water or control treatment exhibited lower disease incidence in the curative and preventive groups by 79% and 76%, respectively. Field experiment showed that after spraying with 1×10^7 cfu/mL bacterial suspension, efficacy rates on controlling rice blast on plants were $(56.82 \pm 1.12)\%$ and $(58.39 \pm 3.05)\%$ at seedling and maturity stages, respectively, and that rice production yield was (524.40 ± 17.88) g/m². Therefore, *B. subtilis* JN005 could be a promising biological control agent for rice blast, thereby warranting further investigation of its efficacy.

Keywords *Bacillus subtilis* · Rice · *Magnaporthe oryzae* · Biological control

Introduction

Rice blast caused by the *Magnaporthe oryzae* is one of the most destructive plant diseases, effecting 10% to 30% annual global rice output loss (Asibi et al. 2019) that may result in severe economic and humanitarian issues (Miah et al. 2017). Although agrochemicals enable quick and effective control of plant diseases, their excessive use increases the

potential of resistance buildup in plant pathogens (Wang and Cernava 2020; Majeed 2018). In recent years, some promising and eco-friendly alternatives to chemical control have been developed to curtail plant diseases (Barratt et al. 2018; Emoghene and Futughe 2016). The use of biological control agents has become a trend owing to global development orientations on use of non-chemical alternatives with less negative impact to the environment.

Recent studies have identified bacterial groups as biocontrol agents against plant pathogens that mainly include *Pseudomonas* spp. and *Bacillus* spp. (Chen et al. 2020; Sun et al. 2017). *Bacillus* spp. are gram-positive, rod-shaped, endospore-forming bacteria that have potential to produce diverse bioactive compounds and ability to produce endospores, therefore make them preferable candidates for commercial exploitation as bacterial biocontrol agents (Sowanprecha et al. 2018; Jangir et al. 2018). To date, 26 microbiological preparations to control rice blast in China have been newly registered ([Handling Editor: Mikiyoshi Umehara.](http://</p></div><div data-bbox=)

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www.icama.org.cn/hysj/index.jhtml) that are composed of 22 *B. subtilis*, 3 *B. cereus*, and 1 *B. amyloliquefaciens* strains and are available in 21 wettable powder, 2 suspension concentrate, 2 oil dispersion, and 1 water dispersible granule forms.

Previous studies have shown that *B. subtilis* can promote plant growth and increase crop yield by solubilizing soil phosphorus, thus enhancing nitrogen fixation and producing indole acetic acid and siderophore (Hashem et al. 2019; Lastochkina et al. 2017; Ahmad et al. 2017). In addition, *B. subtilis* has strong antagonistic activities to many plant pathogens, such as *Rhizoctonia solani* (Tan et al. 2019), *Fusarium oxysporum* f. sp. *niveum* (Zhu et al. 2020), and *Colletotrichum fructicola* (Xu et al. 2020).

Hence, this study aimed to assess the potential of *B. subtilis* JN005 (GenBank: KU302780) for rice blast control and rice growth improvement. This study used *B. subtilis* JN005 to find out if it can effectively inhibit *M. oryzae* growth. The strain was isolated from a healthy rice plant among diseased rice hills of the susceptible cultivar Xiangzaoxian 24, a single-season cropping rice, in the mountainous areas of Taojiang, Hunan, China.

Materials and Methods

Microorganisms, Media, Plant Materials, and Fungicides

B. subtilis JN005 and *M. oryzae* are stored in the Laboratory of Plant Pathogenic Microorganisms and Rice Diseases, Hunan Agricultural University, Changsha, China. Unless otherwise specified, *B. subtilis* JN005 was grown on Nutrient Agar (NA) (Zhang et al. 2019). The phytopathogenic fungus *M. oryzae* was cultured and maintained on Potato Dextrose Agar (PDA) (He et al. 2019). The test rice variety was “Xiangwanxian No 12” from Hunan Hope Seed Industry Technology Co., Ltd., China. The fungicides used were *B. subtilis* WP from Deqiang Biological Co., Ltd., China, 40% isoprothiolane EC from Jiangsu Longdeng Chemical

Fig. 1 Effect of *Bacillus subtilis* JN005 suspension of 1×10^7 cfu/mL treatment (right) on rice seed germination in comparison to the control or sterile water treatment (left)

Co., Ltd., China, and 75% tricyclazole WP from Shanghai Hualian Biopharmaceutical Co., Ltd., China.

Effect of *B. subtilis* JN005 on Rice Seed Germination

Rice seeds were soaked in a beaker containing water. Floating seeds were taken out and discarded, while the seeds that sunk were used in research. After removing the water, the seeds were sterilized by immersing in 70% alcohol for 30 s, then into 1% sodium hypochlorite for 1 min, and then rinsed off twice with sterile water (Anhar et al. 2019). Afterward, seeds were soaked in sterile water for 24 h, and then 100 seeds were evenly dispersed on petri dishes ($d=9$ cm) with two sheets of filter papers moistened with 10 mL of *B. subtilis* JN005 suspension at various concentrations: 1×10^8 cfu/mL, 1×10^7 cfu/mL, and 1×10^6 cfu/mL. Each petri dish was covered with a double wet gauze and then incubated for seven days at 28 °C in a dark culturing box. Controls were prepared by moistening the filter papers with 10 mL sterile water. Each treatment had three replicates and each petri dish was weighed every 12-h period, and the evaporated solution of each treatment was replenished to keep the solution concentration consistent during the treatment. Buds were cut, dried, and weighed on the seventh day. The germination energy (GE) was calculated on the fifth day based on the GB/T3543.4–1995 Rules for Agricultural Seed Testing—Germination Test in China. The germination rate (GR), germination index (GI), and vigor index (VI) were calculated on the seventh day:

$$\text{GE (\%)} = (\text{number of germinated seeds on the 5th day}) / (\text{total number of seeds}) \times 100$$

$$\text{GR (\%)} = (\text{number of germinated seeds on the 7th day}) / (\text{total number of seeds}) \times 100$$

$$\text{GI} = \sum [(\text{days of germination}) / (\text{corresponding number of germinated seeds})]$$

$$VI = GI \times (\text{dry weight of buds})$$

Effect of *B. subtilis* JN005 on Rice Seedlings

Surface sterilized rice seeds were sown into plastic pots containing field soil at 28 °C and 12-h light per day. Each pot (20 cm × 15 cm × 10 cm) was irrigated every seven days with 50 mL of *B. subtilis* JN005 suspension at various concentrations (1×10^8 cfu/mL, 1×10^7 cfu/mL, and 1×10^6 cfu/mL) and 50 mL water for the control setup. Three pots were used for each treatment. Growth parameters of 35-day-old seedlings were measured, including plant height, root length, stem circumference, and fresh weight. Chlorophyll estimation was carried out following the method of Bisht et al. (2020). In brief, 0.10 g of fresh rice leaf tissue was harvested from each treatment, and was homogenized in 80% acetone, and centrifuged for 5 min. Then, the supernatant was obtained, and absorbance was measured through the UV–Vis spectrophotometer at 645 nm and 663 nm.

Effect of *B. subtilis* JN005 on Defense Enzymes Activity

Rice plants grown in plastic pots (20 cm × 15 cm × 10 cm) containing field soil at 28 °C and 12-h light per day were sprayed with various liquid treatments at 25 days. The experiment was designed with three replications and four treatments, viz, water (T1 = negative control), 2×10^5 conidia/mL *M. oryzae* suspension (T2 = positive control), 1×10^7 cfu/mL *B. subtilis* JN005 suspension (T3), and 1×10^7 cfu/mL *B. subtilis* JN005

Disease index

$$= \sum \left[(\text{number of infected plants in each category} \times \text{numerical values of each category}) / (\text{total number of plants} \times \text{highest numerical values}) \right] \times 100$$

suspension + 2×10^5 conidia/mL *M. oryzae* spore suspension (T4). Rice leaf samples were collected at 1, 3, 5, and 7 days after treatment. The activities of catalase (CAT), phenylalanine ammonia-lyase (PAL), peroxidase (POD), and superoxide dismutase (SOD) were examined using a relative kit (Nanjing Jiancheng Bioengineering Institute, China).

Effect of *B. subtilis* JN005 on Rice Blast Development on Detached Rice Leaves

Detached healthy rice leaves at the five-leaf stage were poked slightly with an inoculating needle and then placed into petri dishes with 0.1% 6-benzylaminopurine solution. For the preventive group, leaf surfaces were sprayed with *B. subtilis* JN005 suspension of 1×10^7 cfu/mL first and were inoculated with *M. oryzae* spore suspension of 2×10^5 conidia/mL after 24 h. For the curative group, leaf surfaces were sprayed with

the bacterial suspension 24 h after being inoculated with the spore suspension. Other treatments used were sterile water (control), 40% isoprothiolane EC, and 75% tricyclazole WP in place of the JN005 strain suspension. All petri dishes were placed at conditions of 85%–100% relative humidity and temperature of 28 °C, with a 12-h photoperiod for seven days. Disease incidence was measured from 15 leaves for each treatment.

Disease incidence (%)

$$= (\text{number of disease spots/number of spots investigated}) \times 100$$

Effect of *B. subtilis* JN005 on Rice Blast in the Field

A two-year field experiment was carried out at experimental site of Taojiang, Hunan province, China (N28°42'55.56", E112°15'74.24"), where rice blast occurred frequently to evaluate the efficacy of *B. subtilis* JN005 on rice blast. The experimental site had sandy loam soil with 5.6 pH and 1.09% organic matter.

Before being sown in 30 m² field plots, the rice seeds were dressed separately in *B. subtilis* JN005 suspension (1×10^7 cfu/mL), *B. subtilis* WP fungicide, and water. All treatments were replicated three times in a randomized complete block design (RCBD). In the same treatment of field plots, rice plants at tillering and heading stages received treatments of *B. subtilis* JN005 suspension (1×10^7 cfu/mL), *B. subtilis* WP, and water. The disease severity survey was conducted at seedling and maturity stages, and rice yield was investigated at maturity stage. Leaf blast and panicle blast were scored following the 0–9 scale of the International Standard Evaluation System of Rice (SES) by the International Rice Research Institute (IRRI).

Efficacy (%)

$$= (\text{control disease index} - \text{treatment disease index}) / \text{control disease index} \times 100$$

Statistical Analyses

All analyses were carried out using the Dunnett's T3 test ($P \leq 0.05$) following one-way ANOVA of the IBM SPSS 22.0 software for Windows.

Results

Effect of *B. subtilis* JN005 on Rice Seed Germination

Significant growth promotion was observed in rice seeds treated with *B. subtilis* JN005 suspension (Fig. 1 and

Table 1 Effect of *Bacillus subtilis* JN005 in suspension forms on rice seed germination

Treatments	Germination energy (%)	Germination rate (%)	Germination index	Vigor index
Control	81.00 ± 0.58c	82.33 ± 1.45c	145.93 ± 0.45c	10.22 ± 0.03c
1 × 10 ⁶ cfu/mL	87.67 ± 0.67b	88.33 ± 0.67b	158.68 ± 1.50ab	24.40 ± 4.40b
1 × 10 ⁷ cfu/mL	93.67 ± 0.67a	94.00 ± 1.00a	167.11 ± 2.82a	37.81 ± 2.57a
1 × 10 ⁸ cfu/mL	89.33 ± 0.67b	90.33 ± 0.33b	163.04 ± 1.33ab	34.80 ± 1.70a

Data were mean ± SE (*n* = 3), different lowercase letters within a column indicated significant difference at 0.05 level

Table 2 Effect of *Bacillus subtilis* JN005 suspensions on the growth parameters of rice seedlings

Treatments	Plant height (cm)	Root length (cm)	Stem circumference (cm)	Aboveground fresh weight (g)	Underground fresh weight (g)
Control	28.45 ± 0.12d	2.88 ± 0.02c	2.59 ± 0.06c	8.51 ± 0.10d	1.79 ± 0.05c
1 × 10 ⁶ cfu/mL	37.84 ± 0.27c	5.30 ± 0.36b	3.50 ± 0.02b	17.01 ± 0.89c	2.74 ± 0.13b
1 × 10 ⁷ cfu/mL	41.67 ± 0.02a	6.54 ± 0.01a	4.00 ± 0.10a	19.59 ± 0.47a	3.40 ± 0.13a
1 × 10 ⁸ cfu/mL	40.27 ± 0.16b	5.95 ± 0.02a	3.82 ± 0.05a	18.36 ± 0.20b	2.99 ± 0.00b

Data were mean ± SE (*n* = 3), different lowercase letters within a column indicated significant difference at 0.05 level

Fig. 2 Effect of *Bacillus subtilis* JN005 suspension at 1 × 10⁷ cfu/mL treatment on rice seedlings (right) in comparison to the control or water treatment (left)

Table 1). Compared to the control or sterile water treatment (Table 1), rice seeds treated with *B. subtilis* JN005 suspension at a population density of 1 × 10⁷ cfu/mL

showed the highest germination energy at (93.67 ± 0.67)%, germination rate at (94.00 ± 1.00)%, germination index at (167.11 ± 2.82), and vigor index at (37.81 ± 2.57). Values obtained were 16%, 14%, 15%, and 270% higher than values measured in germination energy, germination rate, germination index, and vigor index, respectively, from the control or sterile water treatment. Except on vigor index, the effects on rice seed germination from 1 × 10⁶ cfu/mL bacterial cells treatments were not significantly different when compared with 1 × 10⁸ cfu/mL, but both treatments showed significantly higher values than the control treatment.

Effect of *B. subtilis* JN005 on Rice Seedlings

Growth parameters were measured with the application of *B. subtilis* JN005 suspension in potted rice seedlings, namely plant height, root length, stem circumference, and fresh weight (Table 2). Compared with water treatment, results show that *B. subtilis* JN005 suspension treatments exhibited growth-promoting effects on rice seedlings (Fig. 2). With the decrease of the concentration of *B. subtilis* JN005 suspension, the growth-promoting effect first increased and all

Table 3 Effect of *Bacillus subtilis* JN005 suspension on chlorophyll content of rice seedlings

Treatments	Chlorophyll a (mg/L)	Chlorophyll b (mg/L)	Total chlorophyll (mg/L)
Control	1.32 ± 0.06d	0.53 ± 0.03d	1.85 ± 0.03d
1 × 10 ⁶ cfu/mL	1.98 ± 0.02c	0.67 ± 0.02c	2.65 ± 0.04c
1 × 10 ⁷ cfu/mL	3.45 ± 0.09a	1.35 ± 0.02a	4.80 ± 0.07a
1 × 10 ⁸ cfu/mL	2.17 ± 0.01b	0.88 ± 0.01b	3.05 ± 0.03b

Data were mean ± SE (*n* = 3), different lowercase letters within a column indicated significant difference at 0.05 level

the indexes reached the maximum and then decreased when the concentration reached 1×10^7 cfu/mL. The *B. subtilis* JN005 suspension of 1×10^7 cfu/mL increased root plant height (46%), root length (127%), stem circumference (54%), aboveground fresh weight (130%), and underground fresh weight (90%) as compared to the control after 35 days. The *B. subtilis* JN005 inoculation induced changes in chlorophyll concentration. When rice seedlings leaves were inoculated with three concentrations of *B. subtilis* JN005, the chlorophyll concentration became elevated compared to those uninoculated ones. Maximum significant improvement was observed in 1×10^7 cfu/mL bacterial cells inoculated leaves where Chl a, Chl b, and Total Chl increased by 161%, 155%, and 159%, respectively, compared to the sterile water or control treatment (Table 3).

Effect of *B. subtilis* JN005 on Defense Enzymes Activity

In theory, *B. subtilis* JN005 induces the CAT, PAL, POD, and SOD activities in rice leaves. Results show that activities of CAT, PAL, and SOD increased gradually, peaking at three days after application of *B. subtilis* JN005

suspension of 1×10^7 cfu/mL (Fig. 3A, B, and C). Figure 3D shows that the activities of POD in the rice leaves increased dramatically after treatment with *B. subtilis* JN005 suspension, and activity was highest at 1×10^7 cfu/mL bacterial cells on the fifth day. The POD activity showed longer duration, while other enzymes activities were opposite. The activities of CAT, PAL, SOD, and POD significantly increased in the plants treated with the combination suspensions of *B. subtilis* JN005 and *M. oryzae* spores (T4) compared to pathogen-inoculated positive control (T2). Rice leaves treated with the *B. subtilis* JN005 suspension alone (T3) also showed significantly higher enzymatic activity compared to the water treatment (T1) and the pathogen-inoculated treatment (T2).

Effect of *B. subtilis* JN005 on Rice Blast Development on Detached Rice Leaves

B. subtilis JN005 suspensions were tested for its ability to control *M. oryzae* through in vitro inoculation on rice leaves. Typical blast disease spots on rice leaves were observed (Fig. 4), and disease incidence was 100.00% in both the preventive and the curative groups inoculated with

Fig. 3 Effect of *Bacillus subtilis* JN005 on the activities of defense enzymes: **A** catalase (CAT), **B** phenylalanine ammonialyase (PAL), **C** superoxide dismutase (SOD), and **D** peroxidase (POD). T1 Water,

T2 2×10^5 conidia/mL *M. oryzae* suspension, T3 1×10^7 cfu/mL *B. subtilis* JN005 suspension, T4 1×10^7 cfu/mL *B. subtilis* JN005 suspension + 2×10^5 conidia/mL *M. oryzae* spore suspension

Fig. 4 Effect of *Bacillus subtilis* JN005 on rice blast development on detached rice leaves. T1 Sterile water, T2 40% isoprothiolane EC, T3 75% tricyclazole WP, T4 1×10^7 cfu/mL *B. subtilis* JN005 suspension

sterile water as control. In treatments with 1×10^7 cfu/mL bacterial suspension, symptoms observed were significantly reduced, with disease incidence of only ($20.74 \pm 0.74\%$) and ($24.44 \pm 1.28\%$) in the preventive and in the curative groups, respectively. These results suggest that the JN005 strain displayed preventive effect and weak curative effect against the leaf blast. Additionally, the JN005 strain showed a comparable control effect of leaf blast with 40% isoprothiolane EC, but it was not as excellent as the 75% tricyclazole WP treatment (Table 4).

Table 4 In vitro inoculation of *Bacillus subtilis* JN005 on detached rice leaves to showcase leaf blast incidence

Treatment	Dilution ratio	The preventive group Disease incidence (%)	The curative group Disease incidence (%)
Control (sterile water)	0	$100.00 \pm 0.00a$	$100.00 \pm 0.00a$
40% isoprothiolane EC	500	$25.18 \pm 0.74b$	$22.96 \pm 0.74b$
75% tricyclazole WP	1500	$9.63 \pm 1.48d$	$13.33 \pm 1.28c$
1×10^7 cfu/mL <i>B. subtilis</i> JN005	0	$20.74 \pm 0.74c$	$24.44 \pm 1.28b$

Data were mean $\pm$ SE ($n=3$), different lowercase letters within a column indicated significant difference at 0.05 level

Table 5 Biocontrol effects of *Bacillus subtilis* JN005 and WP on field-grown rice plants in two-year trial

Treatments	Seedling		Maturity		Rice yield (g/m ²)
	Disease index	Efficacy (%)	Disease index	Efficacy (%)	
Control	$3.66 \pm 0.21a$	—	$5.28 \pm 0.69a$	—	$340.55 \pm 12.55b$
<i>B. subtilis</i> JN005, 1×10^7 cfu/mL	$1.58 \pm 0.13b$	56.82 ± 1.12	$2.16 \pm 0.13b$	58.39 ± 3.05	$524.40 \pm 17.88a$
<i>B. subtilis</i> WP	$1.32 \pm 0.32b$	65.45 ± 7.18	$1.64 \pm 0.25b$	62.74 ± 7.13	$488.15 \pm 13.69a$

Data were mean $\pm$ SE ($n=6$), different lowercase letters within a column indicated significant difference at 0.05 level

Effect of *B. subtilis* JN005 on Rice Blast in the Field

The biocontrol efficacy of *B. subtilis* JN005 was conducted in field trials as well and the results are shown in Table 5. Compared with fungicide, *B. subtilis* WP and *B. subtilis* JN005 suspension (1×10^7 cfu/mL) showed similar levels of disease control. At seedling and maturity stages, rice treated with water (control) showed the most serious disease symptoms with disease indexes of (3.66 ± 0.21) and (5.28 ± 0.69), respectively. Meanwhile, rice treated with *B. subtilis* JN005 suspension (1×10^7 cfu/mL) had disease indexes of (1.58 ± 0.13) and (2.16 ± 0.13) at seedling and maturity stages, respectively, which were comparable to indexes of (1.32 ± 0.32) and (1.64 ± 0.25) measured in rice treated with *B. subtilis* WP. After spraying *B. subtilis* JN005 (1×10^7 cfu/mL), rice blast was controlled up to ($56.82 \pm 1.12\%$) and ($58.39 \pm 3.05\%$) at seedling and maturity stages, respectively. The spray application of *B. subtilis* JN005 and fungicide *B. subtilis* WP in rice resulted in the plants' overall vegetative growth improvement. Compared to the control, significant increases in rice yields were obtained from treatments of *B. subtilis* JN005 suspension (1×10^7 cfu/mL) at 54% and fungicide *B. subtilis* WP treatment at 43%. The values are mean of data trials conducted in 2 years.

Discussion

The fungus *M. oryzae* is one of the most destructive plant pathogens. It can cause outbreak of rice blast disease that could pose threats to global food security. *Bacillus* spp. has

a well-recognized potential against crop diseases and most of biocontrol agents have been developed from *Bacillus* spp. For example, *B. subtilis* GB03, known by its product name Companion, can antagonize *Fusarium*, *Pythium*, and *Rhizoctonia* (Dhakal and Singh 2019).

This research illustrated that the germination energy, germination rate, germination index, and vigor index of rice seeds treated with *B. subtilis* JN005 suspension (1×10^7 cfu/mL) have high values compared to measurements from sterile water or control treatment. Similarly, various growth parameters of rice seedlings were enhanced, such as plant height, root length, stem circumference, and fresh weight in rice seeds with all *B. subtilis* JN005 treatments compared to the control treatment. Leaves of rice seedlings inoculated with *B. subtilis* JN005 resulted in increased chlorophyll concentration than the water treatment. Therefore, results clearly indicate that germination and growth of rice seeds reinforce the role of *Bacillus* spp. as a plant growth promoter, as well as the findings of some previous studies (Naseer et al. 2020; Liu et al. 2020; Gholamalazadeh et al. 2017).

In fact, *Bacillus* spp. promote plant health and plant growth through nitrogen fixation, phosphate solubilization, and phytohormone production (Tiawari et al. 2019). Nitrogen-fixing microorganisms can absorb elemental nitrogen from the atmosphere and form compounds that serve as plant nutrients (Kuan et al. 2016). Phosphate solubilization microorganisms can help plant roots to accelerate the absorption of organic and inorganic phosphates from soil (Garcia and Delgado 2016). *Bacillus* spp. may directly raise plant yield via mechanisms that impart the production of phytohormones or plant growth regulators, such as gibberellins, auxins, and ethylene (Park et al. 2017; Shahzad et al. 2017; Vicente et al. 2019). Additionally, this study revealed that rice yields of plants with *B. subtilis* JN005 treatments (524.40 ± 17.88 g/m²) were higher than the water control (340.55 ± 12.55 g/m²) and fungicide *B. subtilis* WP (488.15 ± 13.69 g/m²) treatments. Similarly, rice yields in treatments *B. siamensis* 624.10 g/m² were found significantly higher than the untreated control 484.10 g/m² (Thampiban et al. 2018).

One effective biological control for multiple pathogens is the activation of the defense systems in the host plant (Jamali et al. 2020). This study showed that the accumulations of CAT, PAL, SOD, and POD in rice leaves were higher in the *B. subtilis* JN005 treatments compared to both the pathogen challenged and sterile water or control treatments. The results suggest that *B. subtilis* JN005 helped reduce the oxidative damage caused by the pathogen, *M. oryzae* through the upregulation of CAT, PAL, SOD, and POD activities. The JN005 strain triggered the plant's defense mechanism that helped the plant to improve its resistance against pathogen infection. These findings are parallel with earlier reports implying the potential of biocontrol *Bacillus* spp. toward strengthening the defense enzymes activities that build up

the defensive mechanism of rice plants against diseases (Rais et al. 2017; Elshakh et al. 2016).

Isoprothiolane and tricyclazole are extensively used fungicides on rice blast (Rijal and Devkota 2020; Elamawi et al. 2018). Inoculation experiment of detached rice leaves showed no significant difference between the JN005 strain 1×10^7 cfu/mL suspension and fungicide 40% isoprothiolane EC in controlling *M. oryzae*, but tricyclazole 75% WP was found to be most effective among three treatments. Additionally, *B. subtilis* JN005 had better preventive effect than its curative effect for leaf blast control. The results of a 2-year field experiment studies revealed lower blast disease indexes in the *B. subtilis* JN005-treated group at (1.58 ± 0.13) and (2.16 ± 0.13) during seedling and maturity stages of rice plants, respectively, compared with (1.32 ± 0.32) and (1.64 ± 0.25) derived from the *B. subtilis* WP-treated group. *B. subtilis* JN005 and *B. subtilis* WP have almost similar effects to blast disease; thus, the JN005 strain exhibits great promise in suppressing disease severity following *M. oryzae* infection. Consequently, the JN005 strain may be potentially used for the biocontrol of rice blast and its antagonistic activity indicates a prospect for practical applications in agriculture sustainable development.

Reportedly, *Bacillus* spp. excrete extracellular metabolites, such as antibiotics, cell wall hydrolases, and siderophores that have antagonistic activity on plant pathogens (Miljaković et al. 2020; Kaspar et al. 2019). For example, *B. amyloliquefaciens* strains VB7 reduced stem rot incidence in greenhouse-grown carnations and that antimicrobial compounds detected include fatty acids and phenols (Vinodkumar et al. 2017). Hence, a future study on *B. subtilis* JN005 will be conducted to determine which antibiotics it produces that curtail the incidence of plant diseases.

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Author Contributions All authors contributed substantially.

Declarations

Conflict of interest All of the authors declare that they do not have any conflict of interest.

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