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Application of endophytic bacterium *Bacillus velezensis* BTR11 to control bacterial leaf blight disease and promote rice growth

Trung Quang Do[1*], Tri Trong Nguyen[2] and Van Mai Dinh[3]

Abstract

Background Bacterial leaf blight (BLB) disease caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is one of the most destructive pathogens responsible for severe yield losses in rice. Application of bacterial biocontrol agent (BCA) with plant growth promotion (PGP) abilities is a promising method that alternates current conventional practices to manage BLB disease and increase crop yield. Hence, this study aimed to isolate and identify BCA with PGP abilities from internal plant tissues and rhizosphere soil of healthy and *Xoo*-infected rice and evaluate their antagonistic and PGP properties under greenhouse and field conditions.

Results A total of 14 bacterial isolates were isolated and presented in vitro antagonistic ability against the *Xoo*. *The* rice endophytic bacterium strain *Bacillius velezensis (*BTR11) had the highest antagonistic activity against the *Xoo*, produced indole acetic acid (IAA), and mineralized nutrients (K and P). The greenhouse experiment revealed that culture broth of strain BTR11 had a high protective effect (72.1%) against the BLB when applied at the protective-fighting mode, i.e., before and after the *Xoo* infection. Preliminary results of the field experiment showed that a protective effect against the bacterial blight disease was obtained as high as 75–85%, if the strain was used as an additive to the soil for the seedlings in combination with spraying when the disease began in the field. In addition, using the strain BTR11 also increased the rice yield to about 12% more than the un-treatment control.

Conclusions The study showed a significant potential for the application of endophytic bacteria in controlling BLB disease, while stimulating plant growth, thus reducing the use of agrochemicals in rice cultivation.

Keywords Bacterial leaf blight, *Xanthomonas oryzae* pv. *oryzae*, Rice endophytic bacteria, Biocontrol agent, Plant growth promotion

Background

Rice (*Oryza sativa* L.) is one of the important cereal crops that support food for half of the world's population (Fairhurst and Dobermann 2002). However, rice production is facing many problems such as bacterial leaf blight (BLB), which is caused by the bacterium *Xanthomonas oryzae* pv. *oryzae* (*Xoo*). This bacterium enters the leaves through wounds or stomata, grows causing blockage of the conduction vessels, making the rice leaves not receive nutrients and wither (Ke et al. 2017). The BLB resulted in yield loss and an increase in expenses for managing the diseases. Many strategies were applied to control the BLB such as generating disease-resistant rice varieties,

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applying chemical solutions containing Copper Hydroxide; Oxolinic acid; Thiodiaxole Zinc; Thiodiazol copper; and using antagonistic bacteria (Chung et al. 2015). Notably, the frequent and long-term use of plant protection chemicals is the cause of soil degradation and environmental pollution.

Controlling plant diseases using biological agents is an important direction to reduce the use of chemicals in agriculture (O'Brien 2017). Plant endophytes, e.g., bacteria (like *Bacillus*, *Klebsiella*, and *Streptomyces*) and fungi (such as *Chaetomium globosum*, *Penicillium chrysogenum*, and *Azospirillum* sp. B510) are gene sources with high potential for application in biological control, antagonizing many species of phytopathogens on different host plants (such as *Xoo*, *Burkholderia glumae*, *Fusarium oxysporum*, *Rhizoctonia solani*, and *Magnaporthe grisea*) (Do 2022). Endophytes inhibit pathogenic microorganisms by several mechanisms, such as (i) production of antibacterial substances, (ii) competition for nutrients and growth space, and (iii) stimulation of induced systemic resistance of plants (Afzal et al. 2019).

The endophytes of rice are highly diverse and include many strains with beneficial biological properties for the host plant (Bertani et al. 2016). Research on the endophytic flora of rice, as well as some other plant species, has shown that *Bacillus* species such as *B. amyloliquefaciens*, *B. subtilis*, *B. velezensis*, and *B. pumilus* have antibacterial activity against many plant pathogens, thus having potential applications in biological control (Bertani et al. 2016).

In Vietnam, rice yield is always severely affected due to the ravages of diseases such as rice blast disease, powdery mildew, BLB, tungro disease, etc., of which the BLB disease is especially dangerous. The BLB has been detected in many regions, especially in the North of Vietnam. Here, the BLB becomes serious and harmful in both spring and summer crops, on many different rice varieties, especially on hybrid rice varieties imported from China: hybrid Khang Dan, Q5. According to a statistical report of the Ministry of Agriculture and Rural Development, the area of rice infected with BLB disease in 2013 was 135.4 thousand hectares, an increase of 6.5 times compared to 2010 (Vu and Do 2022). Diseased plants increased slightly from 2010 to 2011 and suddenly increased in the period from 2011 to 2012 (Vu and Do 2022). The area of severely infected rice leading to the yield loss in 2013 was more than 9.5 thousand hectares (Vu and Do 2022). This situation is still happening and increasing in recent years, mainly in the Northern provinces and some provinces in the Mekong Delta.

Therefore, this study aimed (1) to isolate, and identify bacteria with high antagonistic activity against *Xoo* bacterium and PGP abilities from local BLB disease-infected rice; (2) to determine the effectiveness of selected isolates against the BLB under the greenhouse and field conditions and their ability in promoting the growth of infected rice. The research on the application of BCA-PGP strains isolated from local conditions to produce a cheap, environmental friendly product is widening and providing a promising strategy to produce products such as biofertilizers, biopesticides, and biostimulants for sustainable agriculture in the future.

Methods

Isolation of bacteria from rice plants and rhizosphere soil

The rice plant samples together with plant rhizosphere soil including 3 healthy and 3 BLB disease-infected rice varieties (*Oryza sativa* L. BT7) were collected randomly from the same rice field plot at Giao Thuy, Nam Dinh. All samples were placed in an icebox and transported to the laboratory in a day. The plant samples (1 g of leaves and 1 g of roots) were sterilized on the surface by applying a procedure: soaked in 95% ethanol solution for 10 s, then immersed into 1% sodium hypochlorite (NaClO) solution for another 2 min; finally rinsed three times with sterilized distilled water. Surface-sterilized plant samples were cut into 5×5 mm fragments and placed on Petri plates containing nutrient agar (NA).

One g of rhizosphere soils was placed into a flash containing 9 ml of nutrient broth (NB) and placed on the shaker for 1 min. Then 1 ml of solution was taken and mixed with 9 ml of NB in another sterile test tube to prepare a 10-time serial dilution (up to 10^{-10}). The homogenized solution (0.1 ml) was filtered and spread onto NA plates by using sterilized plastic balls. All plates were incubated at 28 °C for 24 h to observe the bacterial colonies. The observed bacterial colonies were then sub-cultured several times onto new NA plates and were mixed with 50% sterile glycerol stocks to store at − 80 °C.

Inhibition of the *Xoo* by bacterial isolates

The stock of *Xanthomonas oryzeae* pv. *oryzeae* XR5 isolated from rice with BLB disease in Vietnam was obtained from the Central Institute for Natural Resources and Environmental Studies, Vietnam National University Hanoi, Vietnam. The isolated bacteria were investigated for their potential as BCAs on the *Xoo* strain XR5, a casual of BLB disease in rice. The *Xoo* was inoculated on NA plates and grown at 28 °C for 72 h. Then the bacterial cells were collected and suspended in 1 ml of sterilized distilled water until the $OD_{600}=1$. After that, 100 µl of *Xoo solution was used to spread on NA plates by using a sterilized glass spreader. A* sterile disc of filter paper (0.5 cm diameter) was placed in the middle of the *Xoo*-inoculated plates. Each bacterial culture ($OD_{600}=1$) was pipetted 10 µl and applied at the center of that filter

paper disc. The experiment was done in triplicates and the inoculated plates were incubated at 28 °C for 24 h. The clear halozone around the colonies indicates antagonistic activities of isolates against the *Xoo*. The diameter of the clear halozone was determined and used for the calculation of the antagonistic index of bacterial isolates.

Characterization of the plant-growth-promoting ability of anti-*Xoo* bacterial isolates

The isolated bacteria, which showed antagonistic activities against the *Xoo*, were further investigated for their plant-growth-promoting (PGP) characteristics including potassium and phosphate solubilization, and IAA and siderophore production. The potassium solubilization assay was done as a method described by Hu et al. (2006). Briefly, the selected bacteria were pipetted (10 µl/each) and placed in the center of the plate containing Aleksandrov media (glucose: 5 g/l; $MgSO_4{\cdot}7H_20$: 0.5 g/l; $FeCl_3$: 0.005 g/l; $CaCO_3$: 0.1 g/l; $Ca(H_2PO_4)_2$: 2 g/l; potassium aluminum silicate: 2 g/l; and bacteriological agar: 15 g/l). The inoculated plates were kept at 28 °C. After 2 days of incubation, the bacteria strains showed the ability in solubilizing potassium would produce a clear halozone around the colonies. A similar method was applied to screen for the phosphate solubilization ability of isolates on Pikovskaya agar medium (consisting of glucose, 10 g/l; $Ca_3(PO_4)_2$, 5 g/l; $(NH_4)_2SO_4$, 0.5 g/l; NaCl, 0.2 g/l; $MgSO_4{\cdot}7H_20$, 0.1 g/l; KCl, 0.2 g/l; yeast extract, 0.5 g/l; $MnSO_4{\cdot}H_2O$, 0.002 g/l; $FeSO_4{\cdot}7H_2O$, 0.002 g/l; and bacteriological agar, 15 g/l).

For quantitative measurement of phosphate solubilization, the method is described by Nautiyal (1999). Briefly, 1 ml of bacterial culture (10^8 cfu/ml) was pipetted and inoculated with 100 ml of the National Botanical Research Institute's Phosphate medium (NBRIP; glucose, 10 g/l; $Ca_3(PO_4)_2$, 5 g/l; $(NH4)_2SO_4$, 0.1 g/l; $MgSO_4{\cdot}7H_2O$, 0.25 g/l; KCl, 0.2 g/l; and $MgCl{\cdot}H_2O$, 5 g/l) in an Erlenmeyer flask (250 ml). The flasks containing inoculated media were kept on the shaker at 180 rpm, at 30 °C for 2 days. The culture was filtered through a Whatman filter paper (No.1) and centrifuged at 10,000 rpm for 20 min at room temperature. Then a mixture was prepared by mixing 1 ml of supernatant, 2.5 ml of Barton's reagent, and 46.5 ml of sterile distilled water. The mixture was gently shaken and kept at room temperature for 1 h. The yellow color development indicated the positive reaction and correlated with the amount of P solubilized in the supernatant. The spectrophotometer at 430 nm was applied to detect the intensity of the yellow color, which was used to extrapolate the concentration of solubilized P from the standard curve.

The IAA production of isolates was measured by using the colorimetrical method (Patten and Glick 2002). Briefly, 1 ml of overnight-grown bacteria culture was pipetted into a new sterilized flask containing 100 ml of NB medium supplemented with 1 g/l of L-tryptophan. The inoculated culture was incubated at 30 °C for 2 days. After incubation, 1.5 ml of bacterial culture was pipetted and transferred to a new 1.5 ml Eppendorf tube. Then, the Eppendorf tubes were centrifuged at 1000 rpm for 1 min at 4 °C to pellet the bacterial cells. The collected supernatant (1 ml) was transferred into a new cuvette and then added equal amount (1 ml) of Salkowski's reagent (was made by mixing 2 ml of 0.5 M $FeCl_3$ with 98 ml of 35% perchloric acid). The cuvettes were kept at room temperature for 30 min to develop the pink color, which was an indicator of IAA production. The wavelength (535 mm) was applied for a spectrophotometer to measure the absorbance of the supernatant, which could extrapolate the IAA concentration from the IAA standard curve.

The siderophore production test was investigated on chrome azurol S (CAS) medium as the method described by Do (2022). The components of CAS medium were prepared in order: 100 ml of Minimal Media 9 salt solution, 750 ml of water, 32.2 g PIPES (piperazine-1,4-bis (2-ethanesulfonic acid), pH 6.8, 15 g/l agar. The solution was autoclaved and cooled under tap water. When the temperature of CAS media was around 50 °C, 30 ml of blue dye solution was added, mixed well, and poured onto sterilized plates. A loopful of bacteria isolates cultured overnight were streaked on prepared CAS medium plates and incubated at 28 °C for 24 h. The positive reaction was recognized by the clear halo-zone forming around the colonies due to the iron removal from the dye (Arora and Verma 2017).

Molecular identification of selected endophytic bacteria

The selected BCAs-PGP endophytic bacteria were identified by molecular method. The extraction of genomic DNA was done by using PureLink™ Genomic DNA Mini Kit (Thermo Fisher). The extracted DNA was used to amplify fragments of the 16S rRNA gene using the universal primers, 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′). The PCR products (about 1.350 kb) were separated on the agarose gel and purified by using Wizard™ SV Gel and PCR Clean-Up System (Thermo Fisher). The purified PRC products were sequenced at 1st Base company (Singapore). The bacterial strains' identification was done by comparing obtained sequences with 16S rRNA sequences available in the National Centre for Biotechnology Information (NCBI) server using the Basic Local Alignment Search Tool (Blastn) function.

Study on the prevention of BLB disease of selected strain under greenhouse conditions

The experiment was carried out on Bac Thom rice variety No. 7 (BT7), grown under net house conditions at the Vietnam National University of Forestry in the Spring–Summer 2021 crop and cared for according to routine procedures. Experimental plots of 1 m^2 size (1 m × 1 m) were cleaned of weeds, harrowed, and fertilized according to routine procedures. BT7 rice seeds were incubated for 7–10 days, and transplanted into soil plots at the rate of 30 clusters/m^2; the distance between plants and rows was 16 × 16 cm and 20 × 20 cm, respectively. Maintained a surface water level of 5 cm and performed routine care.

The bacterial strain of *Xoo* was grown in Tryptic Soy Broth (TSB) medium (tryptone 17 g/l; soya peptone 3 g/l; NaCl 5 g/l; K_2HPO_4 2.5 g/l; glucose 2.5 g/l; pH 7.3; the number of ingredients used fraction ½) at 30 °C, shaking at 160 rpm for 24 h. The culture solution was then diluted with sterile ½TSB medium to $OD_{600} = 0.2$. Then, 3-week-old rice seedlings were infected with the *Xoo* by using scissors that dipped into the *Xoo* culture to cut off the tips of rice leaves about 3–5 cm (Ke et al. 2017).

The strain of selected bacteria was grown in NB medium under optimal conditions. The culture solution was diluted with water at the rate of 5% and sprayed. The experimental formulas were prepared as in Table 1.

The isolated strains were examined for plant growth promotion (PGP) traits under in vitro conditions such as the ability to produce Indole-3-acetic acid (IAA), ammonia, siderophore, ACC deaminase, and phosphate solubilization. Experiments were repeated 3 times in a completely randomized block design. The prevalence of leaf blight in the treated experiments was evaluated by randomly selecting 10 rice stalks in the middle of the experimental plots and measuring the degree of leaf damage in the experimental plots. The stalks were harvested before spraying (2 days) and after spraying (3, 5, 7, 14, 21, and 28 days). Control effectiveness (H) was assessed according to the Abbott formula (Abbott 1987) as follows:

$$H\,(\%) = [(C - T)/T] \times 100$$

where C and T are the disease index on seedlings in the control and treated experiment, respectively, calculated by lesion length (mm).

Study on the prevention of BLB disease of selected bacteria under field conditions

The field experiment was conducted in Giao Yen commune, Giao Thuy district, Nam Dinh province in the Spring–summer crop of 2021. The field experiment was carried out on Bac Thom rice variety BT7. Selected bacteria were grown in NB medium under optimal conditions. Bacterial cells were collected by centrifugation and dissolved in 0.9% NaCl solution until $OD_{600} = 0.5$. The bacterial solution was irrigated into the soil 2 days before sowing rice seeds (ratio 100 ml/m^2, mixed well in 10 cm topsoil).

Seedlings were grown on soil inoculated with selected bacteria for 15 days before being transplanted in the field. The experimental area was 3 replicated plots, 30 m^2 per plot (length × width was 3 × 10 m). In each plot, rice was transplanted in rows with a density of 42 stalks/m^2, the distance between plants was 15 × 15 cm, and the between rows was 16 × 16 cm. Rice seedlings were cared for according to routine procedures. At the flowering stage (70–75 days after planting), which was reported to be susceptible to BLB disease of rice (Sigh et al. 2010), the experiments with different treatments were prepared as in Table 2.

Monitored disease progression in experimental and control fields, and evaluated the effectiveness of controlling the BLB disease every 7 days from spraying until harvest. Specifically, each formula marked 10 points for investigation, each survey point had 10 random branches, counted the number of diseased leaves, and classified the BLB disease level. Calculation of disease index and disease rate according to QCVN 01-166:2014/BNNPTNT (Method of investigation and detection of rice pests) using the following formulas:

$$\text{Disease index (\%)} = [(n_1 + 3n_3 + 5n_5 + 7n_7 + 9n_9)/(N \times K)] \times 100$$

Table 1 Experiment to investigate the application of strain, *Bacillius velezensis* (BTR11) against the *Xanthomonas oryzeae* pv oryzeae XR5 (*Xoo*) under greenhouse conditions

No	Treatment	Description
1	Formula 1	Sprayed bacteria solution of 2 days before *Xoo* infection and 5 consecutive days after *Xoo* infection
2	Formula 2	Only sprayed with bacteria solution for 5 consecutive days after *Xoo* infection
3	Control A	Sprayed with Bismerthiazol solution (0.025 g mixed in 50 ml of spray water for 1 m^2) 2 days before *Xoo* infection and 5 consecutive days after *Xoo* infection
4	Control B	Sprayed with Bismerthiazol solution (0.025 g mixed in 50 ml of spray water for 1 m^2) for 5 consecutive days after *Xoo* infection
5	Control C	Infected with *Xoo*, not treated

Table 2 Efficiency of controlling bacterial leaf blight (BLB) disease of strain, *Bacillius velezensis* BTR11 under field conditions

No	Treatments	Description
1	T1	Sprayed with a solution of diluted bacteria (OD_{600} = 0.2), frequency once every 2 days, a total of 5 times
2	Negative control (T2)	Non-sprayed
3	Positive control (T3)	Sprayed with the chemical insecticide Kasumil 2SL (Japan) with the factory's instruction (40 ml of the drug mixed in a 16 l spray bottle for 300–400 m^2)

In which: n_1, n_3, ... n_9 is the number of infected leaves at different disease lesions (level), respectively; N is the total number of investigated leaves; K is the highest disease lesion observed.

$$\text{Disease rate (\%)} = (\text{Number of infected leaves} / \text{Number of investigated leaves}) \times 100$$

The rice yield and factors constituting it were assessed according to QCVN 01-55:2011/BNNPTNT including the number of panicles/m^2, number of firm seeds, the weight of 1000 seeds, theoretical yield, and actual yield (n = 30 samples). Samples were collected according to TCVN 5451: 2008.

$$\text{Theoretical yield (quintal/ha)} = (\text{Number of panicles/m}^2 \times \text{Number of firm seeds/panicle} \times \text{Weight of 1000 seeds})/10000$$

$$\text{Actual yield (quintal/ha)} = (\text{yield of plots/30 m}^2) \times 10000\ \text{m}^2$$

Data analysis

Experimental data were processed using Excel and IRRISTAT 5.0 software. Tukey's honestly significant difference (HSD) method in SPSS (version 17) was applied to compare the means in all experiments.

Results

Isolation of bacterial biocontrol agents against the *Xoo*

From collected soil and rice plant samples, a total of 27 colonies were isolated (12 colonies from rice-plant tissue and 15 colonies from rhizosphere). Among those, 14 isolates presented positive responses to in vitro antagonism tests against the *Xoo* (Table 3). As can be seen from Table 3, the antagonized activity varied among isolates and the diameter of the antagonistic halozone ranged from 6.15 to 21.15 mm. The *Xoo*-antagonized bacteria from BLB-diseased plants were 10 isolates, while the remaining ones (4 isolates) were from healthy plants. The largest halozone diameter of in vitro antagonism test against the *Xoo* ($\Delta\gamma \geq 20$ mm) was observed for endophytic isolates BTL8 and BTR11 (Table 3). Additionally, for siderophore production, BTS2, BTR9, and BTR11 were observed to produce the largest halozone diameter (in a range of 16–25 mm) (Table 3). Therefore, the BTL8 and BTR11 strains were selected for the screening of the PGP potential of endophytes.

The results showed that all selected isolates produced IAA with amounts from 1.76 to 4.23 µg/ml. Only strain BTR11 presented Potassium and Phosphate solubilization with the highest value of solubilized P in the liquid culture at 23.43 ± 0.31 µg/ml (Table 4). Hence, the strain of BTR11 was chosen for further studies.

The result of species identification indicated that strain BTR11 was the closest to *Bacillus velezensis* strain EGI198. The sequence was deposited on the Genbank with accession number OR098461.

Evaluation of the effectiveness of strain BTR11 in controlling BLB disease under greenhouse conditions

The greenhouse experiment was monitored for 28 days and the progression of blight was evaluated based on the disease symptoms such as yellowing, and wilting at the leaves' tips, and measured the lesions' length. The effectiveness of disease control based on the lesion length measurement was illustrated in Fig. 1A and presented in Fig. 1B. The results showed that the most effective disease control was observed in Formula 1 (efficiency was 72.1%) and Control A (efficiency was 75.3%), in which the control agents (BTR11 cell suspension and Bismethiazole drug, respectively) were sprayed before and after *Xoo* infection.

As shown in Fig. 1B, the efficiency of disease control in Formula 2 was significantly reduced to about 20% and the one of control B using the chemical drug Bismerthiazol under the same conditions still achieved a high efficiency of 69.4%. As we expected, the efficiency in Control C was nearly zero. These results suggest the direction of using BCAs in a preventive mode, that is, creating conditions for rice plants to be exposed to antagonistic microorganisms early before infection and combining spraying to treat diseases when the plants showed symptoms of the BLB disease.

Table 3 In vitro screening for antagonistic activity and siderophore production of bacteria isolated from plant tissues (root, leaf) and rhizosphere soils of healthy and bacterial leaf blight (BLB) disease-infected rice

No	Bacteria isolates	Plant condition	Diameter of antagonistic halozone (mm)	Siderophore production
1	BTR1	Healthy rice	6.15 ± 0.53	–
2	BTS3		13.32 ± 1.21	+
3	BTL4		7.61 ± 0.74	+
4	BTL5		11.23 ± 0.65	–
5	BTL6	BLB-diseased rice	7.35 ± 0.58	–
6	BTR3		13.41 ± 0.81	–
7	BTR6		14.13 ± 0.73	+
8	BTS10		8.24 ± 1.41	+
9	BTS2		6.38 ± 0.93	+ +
10	BTR10		15.27 ± 0.58	–
11	BTL7		7.61 ± 0.67	–
12	BTL8		18.13 ± 0.82	+
13	BTL9		14.16 ± 1.03	+ +
14	BTR11		21.15 ± 0.97	+ +

Data were value ± SD ($n = 3$). Samples: R, root tissues; L, leaf tissues; S, rhizosphere soil. BLB, bacterial leaf blight; *Xoo*, *Xanthomonas oryzae* pv. *oryzae*. BT: Bac thom rice variety. Halozone diameter (mm) for siderophores production: ++, 16–25 mm; +, 5–15 mm; –, no siderophores

Table 4 Characterization of *Xanthomonas oryzeae* pv. *oryzeae* XR5 (*Xoo*)-anti isolates for plant growth-promoting properties

No	Isolate	Potassium solubilization	Phosphate solubilization ability (μg/ml)	Indole-3-acetic acid production (μg/ml)
1	BTL8	–	6.03 ± 0.12 b	1.76 ± 0.08 b
2	BTR11	+	23.43 ± 0.31 a	4.23 ± 0.26 a

Values are mean ± SD ($n = 3$). Values in the same column with the same letters are not significantly different among treatments as determined by the Tukey honest significant difference test ($P < 0.05$). (+) A positive reaction forms a clear zone around the colony for potassium solubilization ability. (–) A negative reaction for potassium solubilization ability

Evaluation of the effectiveness of strain BTR11 in controlling BLB at field conditions

Unlike the greenhouse experiment, in the field experiment, the BTR11 strain was introduced into the soil to enhance the penetration of bacteria into the roots from the very beginning of the growth of rice seedlings. The results showed that BLB disease appeared in all experimental fields with the disease rate increasing gradually during the sample collection (Fig. 2). The results showed a significant difference between *Xoo* infection of the rice exposed early to strain BTR11 (T1) and the non-treated rice (T2). At 7 days after spraying, in the treated field, the BLB disease rate (19.83%) and the BLB disease index (9.38%) were statistically significant than the ones of the non-treated field (31.72 and 18.25%, respectively) (Fig. 2A, B). After that, the results presented a rapid increase in disease rate and disease index and reached the highest at this stage at 67.35 and 44.75%, respectively, in the untreated field (T2). Meanwhile, a steady decrease in them was observed for fields treated with either strain BTR11 (T1) or chemical insecticide Kasumil 2SL (T3). The evaluation results at the time of maturity, the field sprayed with strain BTR11 (T1) had a disease rate and disease index of 12.96 and 6.63%, respectively, equivalent to the field sprayed with Kasumin 2SL (T3) was 10.45 and 4.12%.

Moreover, the effectiveness of leaf blight control in fields sprayed with BTR11 strain (T1) and fields sprayed with Kasumin 2SL (T3) was similar throughout the investigation, reaching 75–85% (Fig. 2C). The disease control efficiency was higher than that in the greenhouse experiment, reflecting the effect of early exposure to the endophytic bacterial strain BTR11 by treating the soil used to grow rice seedlings with this strain.

As a result of controlling the BLB disease, the yield of the field treated with strain BTR11 was significantly higher than the non-treated field (control). The effect of strain BTR11 on the development of rice seed, panicle, and yield was presented in Fig. 3 and in Table 5.

As shown in Table 5, in the experimental treatment (T1), all investigated criteria gave higher values than the ones in the control (T2). The strain BTR11 in this study showed the effect as a probiotic for rice, with high potential for application in organic rice farming, helping to reduce the use of chemicals in agriculture.

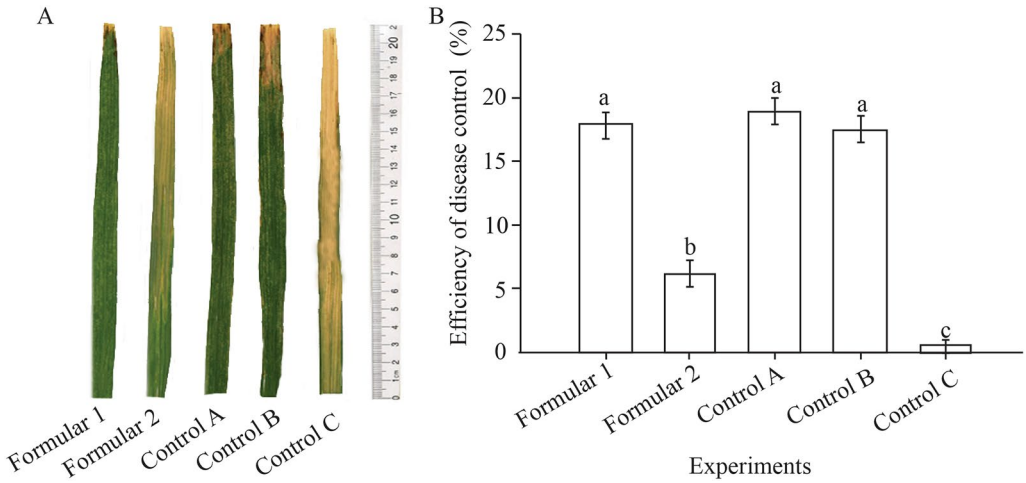

Fig. 1 Disease severity control of rice inoculated with strain, *Bacillius velezensis* BTR11 under greenhouse conditions. **A** Representative for *Xanthomonas oryzeae* pv. *oryzeae* XR5 (*Xoo*) suppression of different treatments. **B** Effectiveness of *Xoo* suppression of different treatments. Plotted data are means ± SD ($n = 3$). The same letter(s) are not significantly different as determined by Tukey's honestly significant difference test ($p < 0.05$)

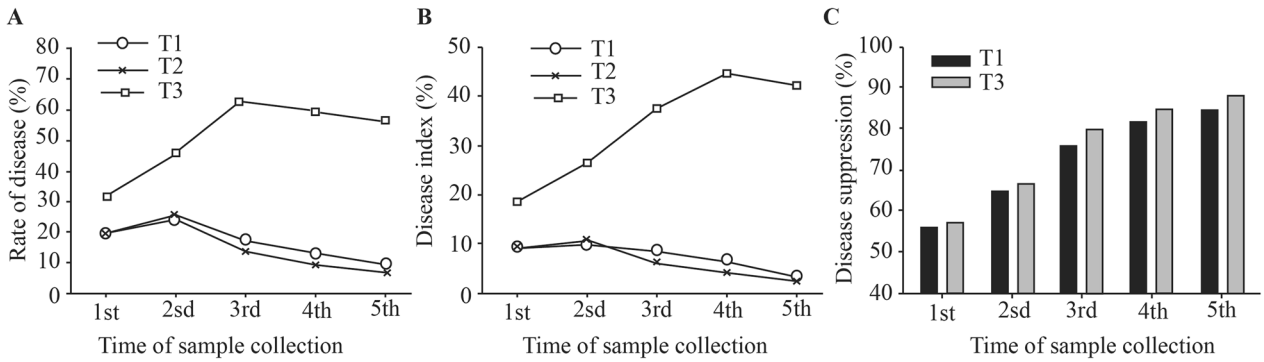

Fig. 2 Effectiveness of rice leaf blight control under field conditions. T1: soil inoculated with strain, *Bacillius velezensis* BTR11 + spay solution of strain BTR11 at flowering stage every 2 days, a total of 5 times, T2: soil inoculated with strain BTR11 + not spay solution of strain BTR11 at flowering stage; T3: soil inoculated with strain BTR11 + Sprayed with the chemical insecticide Kasumil 2SL at flowering stage

Discussion

Bacterial endophytes that colonize inside the plant tissue could play roles as biocontrol agents and plant growth-promoting factors as well (Berg et al. 2005). The crosstalk between host plants and endophytes is carried out and normally mediated through the production of secondary metabolites by microorganisms or host cells acting as informatics transfers (Reinhold-Hurek and Hurek 2011).

It was reported that the endophytes could be potential BCAs against *Xoo* and simultaneously promote the growth of rice plants, which were infected with the *Xoo* (Ngalimat et al. 2021). The results of this study suggest endophytic bacteria *Bacillus velezensis* BTR11, which was isolated from BLB-diseased infected rice and produced the highest diameter of *Xoo*-antagonistic halozone, could be applied as a biocontrol agent against BLB disease-infected rice. These results were consistent with previous reports, in which the isolated endophytes showed potential application as an effective biocontrol agent in inhibiting the development of *Xoo* in rice (Azman et al. 2017). For example, Nagendran et al. (2013) demonstrated that the application of the endophytic bacterium, *Bacillus subtilis* var. amyloliquefaciens (FZB24) at the stage of rice seeds treatment produced the lowest severity of BLB of rice (31.36%) under glasshouse condition. In another report by Do (2022), the endophytic bacteria, *Bacillus velezensis* ND06 and *Pseudomonas putida* ND10 strains isolated from healthy rice plants also presented antagonistic activity against rice blast disease (*Magnaphorthe oryzae*) and promoted the rice plant development under

Fig. 3 A representative for the effect of strain, *Bacillius velezensis* BTR11 on the development of rice seed in field trials

Table 5 Effect of strain *Bacillius velezensis* (BTR11) to yield of rice under field conditions

Formula	Number of panicles/m^2	Number of firm seeds/panicle	Weight of 1000 seeds (g)	Theoretical yield (quintal/ha)	Practical yield (quintal/ha)
T1	286.31	118.34	20.73	65.49	61.31
T2	267.92	104.18	19.32	53.93	49.27

greenhouse conditions. These results suggest a promising of endophytic bacteria BTR11 in alternating the chemical drugs used to control the phytopathogen in agriculture.

In addition, *Bacillus* sp. with the ability to produce specific metabolites, such as surfactins, difficidin, and bacilysin was reported as an effective bio-agent against the *Xoo* antagonistic bacteria (Wu et al. 2018). In this study, endophytic *B. velezensis* BTR11 presented the ability to produce siderophore and phytohormone indicating the potential as a biocontrol agent against *Xoo.* Production of siderophore to reduce available iron in the rhizosphere zones of the host plants was reported as an antagonistic strategy of endophytic bacteria in competing with phytopathogens (Shanmugaiah et al. 2016). The other possible explanation is the siderophore could interact synergistically with other metabolites or bioactive compounds like antibiotics and cell-wall degrading enzymes in the nutritional competition (Yasmin et al. 2016). Furthermore, the IAA produced by strain BTR11 could play roles in suppressing the development of BLB disease. This was strengthened by the study of Khare and Arora (2010), which showed that IAA produced by *P. aeruginosa* suppressed the development of *Macrophomina phaseolina* (Tassi) Goid caused charcoal rot disease of chickpea. Moreover, the antagonistic mechanism of strain BTR11 could be the ability to stimulate plant growth such as the production of IAA, potassium solubilization, and phosphate solubilization. Notably, the difference in the productivity of siderophores from microbes isolated from different sources and studies might be due to the difference in disease suppression on BLB-diseased rice.

Together with the investigation of the antagonistic ability of BCAs, the evaluation of the PGP efficiency of potential BCAs under greenhouse conditions was necessary and should be done before transferring into the field. In this study, the results of the greenhouse experiment presented higher efficiency of *B. velezensis* BTR11 in suppressing BLB pathogen compared to the one of non-inoculated control and were equivalent to the one of positive control of Bismerthiazol treated plants. This result is in agreement with the reports of Velusamy et al. (2006), in which a reduction of BLB suppression in nethouse (58.8%) and in the field (64.5%) experiments were reported by applying a solution of bacteria *P. fluorescens* PTB9 that produced 2,4-diacetylphloroglucinol. Similarly, Vidhyasekaran et al. (2001) demonstrated the use of *P. fluorescens* Pf1 treatment under either greenhouse or field conditions increased the yield and effectively controlled the BLB disease, and even more effective than positive control used streptocyclin instead.

Consistently, the results of field experiments showed that rice plants inoculated with *B. velezensis* BTR11 produced a significant decrease in diseased leaf area than the non-inoculated plant (control) but non-significant than the positive control, which applied chemical insecticide Kasumil 2SL. Strain BTR11 presenting efficiency in suppressing the BLB disease as high as one of positive control under field conditions could be used as a promising BCAs to inhibit *Xoo* in rice. These results could be the inoculation of soil used to cultivate the rice plants with strain BTR11 created protection for the rice seedlings against BLB at the early growth stage while spraying with this bacterial solution at the later growth stage protected the rice plant from subsequent disease incidence. In addition, the application of strain BTR11 led to the increase of biomass, and subsequently, the yield increase might be due to its ability to produce IAA and siderophores, solubilizing mineral nutrients (K and P). This explanation was demonstrated by the reports, in which inoculation of *Bacillus* and *Pseudomonas* strains promoted the growth of rice, cucumber, lettuce, potato, and tomato (Do 2022). Notably, there are more and more

isolates from rhizosphere and plant tissues that played roles in yield increase and/or reduction in plant diseases being explored (Beneduzi et al. 2012).

Conclusions

The rice endophytic strain *Bacillus velezensis* BTR11 had a high activity against *Xoo* bacterium shown in in vitro experiments. The BTR11 strain could produce siderophore, IAA, and solubilize K and P. In addition, the application of the BTR11 strain to control rice blight under net house conditions showed that using the method of disease prevention and control (spraying before and after *Xoo* infection) gave a control efficiency of 72.1%, equivalent to chemical drugs applied under the same conditions. Meanwhile, if used according to the anti-disease method (sprayed after *Xoo* infection), the control efficiency was only 20.4%. Experimenting in field conditions combining treatment of soil with strain BTR11 with preventive spraying when rice is in the growing stage and spraying when detecting blight has shown effective disease control, equivalent to the chemical drug Kasumin 2SL, in conditions of natural infection. The treatment with the BTR11 strain also increased rice yield.

Abbreviations

IAA	Indole-3-acetic acid
NBRIP	National Botanical Research Institute's Phosphate
PGP	Plant growth promoting
BLB	Bacterial leaf blight
BCAs	Bacterial biocontrol agents
Xoo	*Xanthomonas oryzae* pv. *oryzae*
NA	Nutrient agar

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Author contributions

TQD was involved in the conceptualization, supervision, final interpretation of data, and editing of the manuscript. VMD isolated fungal pathogens and bacteria and carried out greenhouse experiments. TTN re-produced Fig. 1 and revised Table 1, double-checked the data analyses, and revised the manuscript. All authors read and approved the final manuscript.

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