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Antagonistic activities of endophytic bacteria isolated from rice roots against the fungus *Magnaporthe oryzae*, a causal of rice blast disease

Quang Trung Do*

Abstract

Background: The blast disease of rice caused by the fungus *Magnaporthe oryzae* is one of the most destructive diseases in Nam Dinh province, Vietnam. This study aimed to isolate and screen antagonistic bacteria isolated from the rice (*Oryza sativa*) against *M. oryzae*.

Results: In the present study, 14 endophytic bacteria were isolated from rice roots of a Ngoc Xuan variety in Nam Dinh province. The result showed that 6 isolates showed in vitro antagonistic activity against *M. oryzae*. Of 6, 2 strains, ND06 and ND10, molecularly identified as *Bacillus velezensis* and *Pseudomonas putida*, produced a significant inhibition on the pathogenic growth with growth inhibition of 62.87% and 64.25%, respectively, while the other 4 (ND03, ND07, ND09, and ND11) showed a weak inhibition. In addition, the ND06 and ND10 strains also presented antagonistic activity against *M. oryzae* under greenhouse conditions. Moreover, screening plant growth-promoting (PGP) traits of 2 isolates exhibited all 5 PGP traits including IAA production, phosphate solubilization, and production of ammonia, 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase, and siderophore. In addition, the greenhouse experimental results indicated that the cultivar rice seedlings inoculated with ND06 or ND10 strain produced a significant enhancement of the agronomic parameters (root length, shoot length, dry matter, and chlorophyll content).

Conclusions: The results indicated that the rice root endophytic bacteria (ND06 and ND10) possessed contemporarily multiple PGP traits and antifungal activity. These 2 strains should be further characterized in order to confirm the beneficial traits to develop as a potential biofertilizer and/or biocontrol agent for rice sustainable production.

Keywords: *Magnaporthe oryzae*, Endophytic bacteria, IAA production, Phosphate solubilization, Siderophore production

Background

Rice (*Oryza sativa*) is an important cereal crop that provides food for more than three billion people around the world, especially in Asian countries (Kyndt et al. 2014). However, it was reported that rice yield production was strongly affected by both abiotic factors (such as drought,

cold, acidity, salinity) and biotic factors (like pests, weeds, and diseases) (Onyango 2014). Among those factors, fungal diseases are the most worrying factors because of a significant loss of crop yield and production of potentially toxic compounds that were the results of fungal infection (Suprpta 2012). Rice blast is one of the most destructive diseases of rice that cause great damage to rice yield and threatens the stability of rice production worldwide because this pathogen can infect and damage leaves, stems, nodes, and panicles at all stages of rice development (Dai et al. 2010). The mycelial fungus, *Magnaporthe*

*Correspondence: trungcnsinh@gmail.com

Central Institute for Natural Resources and Environmental Studies, Vietnam National University Hanoi, 19 Le Thanh Tong, Hoan Kiem, Ha Noi, Vietnam

oryzae, is considered to be the main causal agent of rice blast disease (Dean et al. 2012). The fungal spores can be easily dispersed leading to widespread by the wind and rain. Therefore, this pathogen infection usually occurs during periods of prolonged rain or high humidity (Talbot and Wilson 2009).

The common method used to manage this phytopathogen is chemical control involving the use of chemical fungicides (Pooja and Katoch 2014). However, this method is raising several public concerns such as the emergence of fungicide resistant strains (Skamnioti and Gurr 2009), environmental pollution, residual toxicity, soil quality reduction, natural ecosystem damage, and human health issues as well (Fattahi et al. 2015). Therefore, developing an alternative approach that is cost-effective, environment-friendly, and also has minimal side effects is attracting the scientist's interest.

Nowadays, using natural products as sources to develop or synthesize fungicides is an emerging method that offers several advantages such as low toxicity to living microorganisms and safe for the environment (Yoon et al. 2013). Utilization of potential microbes antagonizing phytopathogens as biocontrol agents is another alternative approach to manage the diseases (Suprpta 2012). The antagonistic microbes have many mechanisms to combat the plant pathogens through competition, antibiosis, and hyperparasitism (Montesinos 2003). Hence, compared to methods using synthetic fungicides, the biocontrol method is more cost-effective, safe, and environmentally friendly.

Recently, the method using beneficial microbes known as plant growth-promoting bacteria (PGPB) to biocontrol phytopathogens is being exploited. An important group of PGPB is endophytic bacteria that are getting more interest in investigating their roles in the plant-endophyte interactions. Endophytic bacteria not only do not harm plants but also stimulate plant growth because of their good properties such as nitrogen fixation (Ladha and Reddy 2003), phosphate solubilization (Verma et al. 2011), synthesis of plant hormones such as IAA (Bal et al. 2013) or some secondary substances such as siderophore; inhibition of plant diseases (Sayyed et al. 2012). Although several endophytic bacteria were explored for their potential application as biocontrol agents in agriculture, the diversity and PGP effects of endophytic bacteria isolated from different plant species and locations could vary widely and remain poorly understood. Therefore, screening and characterizing the community and beneficial functions of root-colonizing endophytic bacteria will provide more scientific knowledge of plant and microbial interactions.

In this study, strains of rice root endophytic bacteria that have the ability to control the *Magnaporthe oryzae*, a

causal agent of blast disease in rice, and also have properties to stimulate plant growth in order to develop microbial fertilizers in the future were isolated and screened.

Methods

Isolation of endophytic bacteria from rice root

Samples of rice roots were collected from healthy rice (variety Ngoc Chau) cultivated in fields at Quat Lam, Giao Thuy, Nam Dinh, Vietnam, and it had an active irrigation system.

Sample collection, sample processing, and surface disinfection for endophytic bacteria isolation were carried out as the method described by Bertani et al. (2016), with some minor modifications. Specifically, the sample of rice roots was collected at the time the rice begins to branch. The sample collection points were located on the diagonal line of the field plot, 30 m apart and 10 m from the shore. At each point, 3 different rice groups with the entire root system were collected and put in a sealed plastic bag and kept in a cool container until isolating the endophytic bacteria.

The root samples were completely removed from the soil under running tap water. The root samples were then, soaked in 70% ethanol for 2 min before being soaked in a 3% NaOCl solution for 5 min with the addition of 2–3 drops of Tween 20. Finally, the root samples were washed 5 times with sterile distilled water. To make sure the disinfection process was highly effective, completely removing microorganisms from the root surface, 100 µl of the last wash water was spread over a plate containing TSA medium (Tryptic Soy Agar, Difco, USA). The appearance of microorganisms on plates after 3 days of incubation at 28 °C was checked. In addition, the roots after disinfection were dried on sterile absorbent paper and placed on TSA media plates. These plates were cultured at 28 °C for 3 days.

In parallel, disinfected rice roots were gently grounded with autoclaved pestle and mortar using 10 mL of 1X PBS (Phosphate-buffered saline) solution. The ground suspension (100 µl) was diluted in PBS solution (10 times, 100 times), then spread on TSA media plates and cultured at 28 °C for 3 days. The colonies from each plate were selected and separated based on morphology and color, then were streaked on fresh plates to purify and finally stored at -80 °C in TSA broth containing 18% glycerol.

Antagonistic activity of endophytic bacterial isolates against *Magnaporthe oryzae* in vitro

All endophytic bacterial strains were screened for their ability to inhibit the growth of rice blast fungi by dual culture on the same TSA medium plate. The rice blast fungus, *Magnaporthe oryzae*, used in this experiment was provided by the laboratory of the Central Institute

for Natural resources and Environmental Studies, Vietnam National University Hanoi. Used sterile filter paper disks (6 mm diameter) were placed around the fungal strains to ensure that the mycelium grows over the paper disks. Then, transferred each paper disk containing the phytopathogenic fungi to the center of the TSA medium plate. After that, placed a sterile filter paper disk (6 mm diameter) at a distance of 2 cm from the fungal paper disks. A 10 µL of log-phase inoculum ($OD_{600}=1$) was added to each paper disk. Each disk was tested with a strain of endophytic bacteria. The experimental plates were incubated at 28 °C for 5 days, or until the fungus in the control plate (no bacteria) spread to the entire surface of the plate. The growth inhibition ability of the endophytic bacteria was calculated by the formula: $I = [(C - T)/C] \times 100$, where I is the percentage of growth inhibition (%), C and T are the diameters of fungi grown on control and experimental plates, respectively. Bacterial strains with an average inhibition rate of 50% or more will be selected for later in planta testing. The experiment was done in triplicates and was repeated 3 times.

Molecular strain identification

Selected strains were grown overnight in LB broth media. The culture was centrifuged to collect the bacterial cells that were used to extract genomic DNA by using the Rapid Bacteria Genomic DNA Isolation Kit (Biobasic, Canada) as per the kit instructions. The 16S rRNA gene fragment (1.5 kb) was amplified by PCR using primers 27 F (5'-AGA GTT TGA TCC TGG CTC AG-3'), and 1492 R (5'-TAC GGT TAC CTT GTT ACG ACT T-3') and sequenced by First Base Company (Singapore). The obtained nucleotide sequence was blasted on the BLAST server (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>). The sequence with high similarity (more than 99%) was used for multiple cluster alignment and phylogenetic analysis on MEGA software (v.7.2). The obtained sequences were deposited on GenBank.

Effect of ND06 and ND10 strains on rice blast development in pot experiments

The antagonistic effect of selected strains on rice blast development in pot experiments was evaluated as the method described by He et al. (2019). Briefly, the five-leaf seedlings were sprayed with 30 ml of conidial suspensions per pot, which were prepared as in (Table 1).

All treated rice seedlings were grown in the dark at 28 °C in a chamber with 100% relative humidity. After 30 h of incubation, the pots were kept under constant light for 3 d and finally were moved to the greenhouse with a temperature was 28 ± 3 °C. After 6 days grown greenhouse, the disease severity was observed and measured following Prabavathy et al. (2006) on a 0–9 scale.

In vitro assay for plant growth-promoting activities

The isolated strains were examined for plant growth promotion (PGP) traits in vitro conditions such as the ability to produce Indole-3-acetic acid (IAA), ammonia, siderophore, ACC deaminase, and the phosphate solubilization.

IAA production was qualitatively calculated by the coloring methodology as described by Wuryanto et al. (2018), with some modifications and additions. Briefly, a 20 µL of bacterial culture ($OD_{600}=1$) was pipetted and placed on of sterile filter paper disk (20 mm) located in the middle of the plates containing TSA medium supplemented with 1 g/L L-Tryptophan. After 72 h of incubation at 28 °C, the paper disks were stained with 100 µL of Salkowski solution and kept for 30 min at room temperature in the dark. The color change in the filter paper disk from pale pink to dark pink is the indicator of IAA production. The ability to produce ammonia was examined by qualitative and quantitative methods as described by Malleswari and Bagyanarayana (2013).

The siderophore production of isolated strains was determined by the method of using the chrome-azurol S reagent (CAS) (Arora and Verma 2017). A sterile inoculating loop was used to take a single colony. Then, placed a single colony on the CAS media plates and incubated at 28 °C for 7 days. After 7 days of incubation, bacteria

Table 1 Treatments applied in pot experiments

Treatments	Preparations
T1	A blank solution without fungus (<i>M. oryzae</i>) and endophytic bacteria
T2	A mixture of LB medium only and conidial suspension (5×10^5 conidia/mL) of <i>M. oryzae</i> . added with 0.025% Tween 20
T3	A mixture of the fungicide tricyclazole (750 µg/mL) and conidial suspension was added with 0.025% Tween 20
T4	A mixture of culture broth from 3-day-old cultures (10%, v/v) and conidial suspension amended with 0.025% Tween 20
T5	Rice seeds were germinated in a 3-day-old culture broth of selected strains for 24 h. After rising with autoclaved water, the germinated seeds were planted in a pot. The seedlings at the five-leaf stage were sprayed with conidial suspensions

Each treatment included three pots (20 × 25 × 30 cm) with 30 seedlings per pot. The experiments were done in triplicates

capable of producing siderophores would form a yellow-orange color around colonies. The diameter of the orange-yellow circle depended on the content of the produced siderophore.

The phosphate solubilization of isolates was investigated on the NBRIP (National Botanical Research Institute's Phosphate) growth medium. The plate of bacteria capable of solubilizing the insoluble phosphates would produce a clear zone surrounding the colonies. The Phosphate Solubilizing Index (PSI) was calculated according to the formula: $PSI = (\text{colony diameter} + \text{clear zone diameter}) / \text{colony diameter}$ (Pande et al. 2017). The ACC deaminase production of the isolates was also screened on minimal media containing ACC as their nitrogen source, as the method described by Penrose and Glick (2003).

All of the above experiments were designed in triplicate and were repeated three times.

Evaluation of the effect of endophytic bacteria strains on the growth of rice seeds

Promising strains of endophytic bacteria were selected based on their plant growth-promoting characteristics and were used to evaluate their effects on seed germination and seedling growth in a greenhouse.

The rice seeds (*Oryza sativa* Ngoc Chau) were used in this experiment. Rice seeds were surface sterilized as the method described by Trung et al. (2022). After sterilization, the sterilized seeds were inoculated with prepared bacterial suspensions (about 10^8 CFU ml⁻¹) for 24 h. The experiment was arranged in a completely randomized design with 3 replicates, each replicate corresponds to a plastic box containing 30 rice seeds inoculated with the bacterial strain. In the negative control, sterilized seeds were inoculated in sterile distilled water. The plastic boxes containing inoculated seeds were incubated in a dark at 28 °C for 7 days. The parameters such as germination rate, shoot, and root length were measured. After 7 days of dark incubation, 10 rice seedlings with synchronized development were transferred and planted in each pot (15 × 9 cm), filled up with sterile sand. The experiment was carried out in 3 replicates. The pots were kept in a growth room at 28 °C and 14 h light/10 h dark condition. To keep seedlings growing normally, the seedlings were watered every 2 days with a nutrient solution including macronutrients (g/l, KNO₃ 6.07, Ca(NO₃)₂·4H₂O 9.45, MgSO₄·7H₂O 4.93, NH₄H₂PO₄ 1.15 g) and micronutrients (mg/L; H₃BO₃ 2.86, MnCl₂·4H₂O 2.13, ZnSO₄·7H₂O 0.22, CuSO₄·5H₂O 0.08, H₂MoO₄·4H₂O 0.02, FeSO₄·7H₂O 2.78, Na₂-EDTA 3.72). The measurement of plant criteria (fresh and dry weight of shoot and root) was recorded after 15 days of planting.

The chlorophyll content of leaves was determined as the method described by Porra (2002). Briefly, about 100 mg of leaves was chopped and put in a 50-mL capped tube containing 25 ml of 80% acetone. The tube then was kept at 4 °C for 28 h. The extract solution was spectrophotometrically measured at 663 nm and 645 nm. The extracted chlorophyll amount was identified, using the following formula: total chlorophyll (mg g⁻¹) = $(20.2 \times A_{645} + 8.02 \times A_{663}) \times V \times 1000 / W$, where A_{645} and A_{663} are the absorbances at 645 and 663 nm, respectively, V is the volume of the extract and W is the leaf fresh weight.

Data analysis

All experiments were repeated 3 times, and the results were presented as mean values with \pm SD. Tukey's honestly significant difference (HSD) method in SPSS (version 17) was applied to compare the means in all experiments.

Results

Isolation of rice root endophytic bacteria

The surface sterilization of roots was sufficient to eliminate the epiphytic bacteria because no colonies were observed on the TSA agar plate that was incubated with washing water during the sterilization procedure. Endophytic bacteria were isolated from the roots of the Ngoc Chau rice variety at the branching stage. There were 14 endophytic bacteria strains (ND01–ND14) that were isolated from the rice root samples. Colonies presented different types of shapes and colors (Table 2). These isolated strains grew very well on the TSA media after 24–48 h of incubation at 28 °C (colony diameter ranged from 1–5 mm).

Screening for antagonism of EB strains on *Magnaporthe oryzae* fungal strains

All 14 isolates were screened for antagonistic activity against the fungi *M. oryzae*, a causal of rice blast disease. The results showed that 6 out of 14 strains showed antagonism against the fungus. The percentage of growth inhibition ranged from 2.07 ± 1.45 to 64.25 ± 1.24 . Of which, 2 strains were ND06 and ND10, accounting for 33.33%, belonged to the strong group with a percentage of inhibition of approximately 60% or more (Table 3, Fig. 1). The molecular identification indicated that the ND06 and ND10 strains were close to *Bacillus velezensis* (accession number MN704466.1) and *Pseudomonas putida* (accession number FJ976605.1), respectively. These 16S rDNA fragment sequences of strains ND06 and ND10 were deposited on GenBank with accession numbers ON386277 and ON386278, respectively. This group of EB strains that are considered a potential group for further

Table 2 Number and morphology characteristics of endophytic bacteria recovered on the TSA media from rice root

No	Bacterial strains	Colonies characteristics		
		Shape, surface, margin shape	Color	Size (mm)
1	ND01	Round, raised, smooth	Light yellow	2.4
2	ND02	Round, flat, smooth	Light yellow	1.8
3	ND03	Irregular, flat, saw	Light yellow	3.5
4	ND04	Irregular, convex, undulate	Light yellow	2.9
5	ND05	Round, raised, smooth	Dark yellow	3.7
6	ND06	Irregular, flat, saw	Dark yellow	4.5
7	ND07	Irregular, convex, undulate	Dark yellow	1.7
8	ND08	Irregular, convex, undulate	Opaque white	4.8
9	ND09	Irregular, flat, saw	Opaque white	2.6
10	ND10	Irregular, convex, undulate	Opaque white	2.4
11	ND11	Irregular, flat, saw	Opaque white	2.3
12	ND12	Round, raised, smooth	Brown	1.8
13	ND13	Round, raised, smooth	Light pink	1.5
14	ND14	Round, raised, smooth	Transparent white	1.6

Table 3 In vitro inhibition of growth of *Magnaporthe oryzae* by single bacterial antagonists on TSA medium

Strains	Growth inhibition (%)	Degree of antagonism
ND03	14.75 ± 1.45 ^c	+
ND06	62.87 ± 1.43 ^a	++
ND07	27.36 ± 2.64 ^b	++
ND09	2.07 ± 1.72 ^d	+
ND10	64.25 ± 1.24 ^a	++
ND11	19.15 ± 2.23 ^{bc}	+
<i>M. oryzae</i> + sterile-distilled water		–

Values in the same column with the same letter(s) are not significantly different as determined by the HSD test ($P < 0.05$). Weak '+' (1–5.99 mm), moderate '+' (6–10.99 mm), strong '+' (11–20 mm), inhibition effects on the growth of the pathogen

research to select the most desired strains to apply in the biocontrol of the disease in rice.

Effect of ND06 and ND10 on rice blast development in pot experiments

The two selected strains were evaluated for their ability in controlling the development of rice blast in the greenhouse by 2 approaches, spraying and seed treatments. The results were shown in Table 4. As can be seen, the plants inoculated with *M. oryzae* by spraying with T2 solution presented the highest value of disease index after 6 d at 28 °C (Table 4). When the fungicide tricyclazole was applied (T3 treatment), the disease index was dramatically decreased (Table 4). Interestingly, when applied

T4 treatment, which was a combination of 10% bacterial culture broth and conidial suspensions, a significant decrease in disease index was observed for strains ND06 (14.8 ± 1.5) and ND10 (15.1 ± 1.3) (Table 4). Especially, when the rice seeds were bacterized by submerging in a 3-day-old culture broth of strain ND06 or ND10 for 24 h before planting, the disease index was the lowest value with 74.7 (for ND06 inoculation) and 756 (for ND10 inoculation). Notably, the inoculation with endophytic bacteria ND06 or ND10 also led to the highest efficacy in controlling the rice blast on the tested seedlings.

In vitro screening of endophytic bacteria strains for PGP traits

Endophytic bacteria have been shown the ability to stimulate plant growth by direct and indirect mechanisms. All isolates were used to evaluate their plant growth promotion by screening for production of IAA, NH_3 , siderophore, ACC deaminase activity, and organic phosphate solubilization. Screening results for growth-stimulating traits were shown in (Table 5).

The results showed that there were 11 isolates (78.57%) capable of producing IAA at different efficiencies under in vitro conditions. As shown in (Table 5), the ND10 isolate produced the highest levels of IAA ($89.69 \pm 1.61 \mu\text{g ml}^{-1}$). In addition, the ability to convert air nitrogen into ammonia of potential isolates was also investigated. The results indicated that among 14 isolates, only 12 isolated endophytic bacteria formed colonies in an N-free medium which was the indicator of their ability for N-fixation. The data presented a wide range of ammonia production efficiency of isolates from 17.83 ± 0.47 to

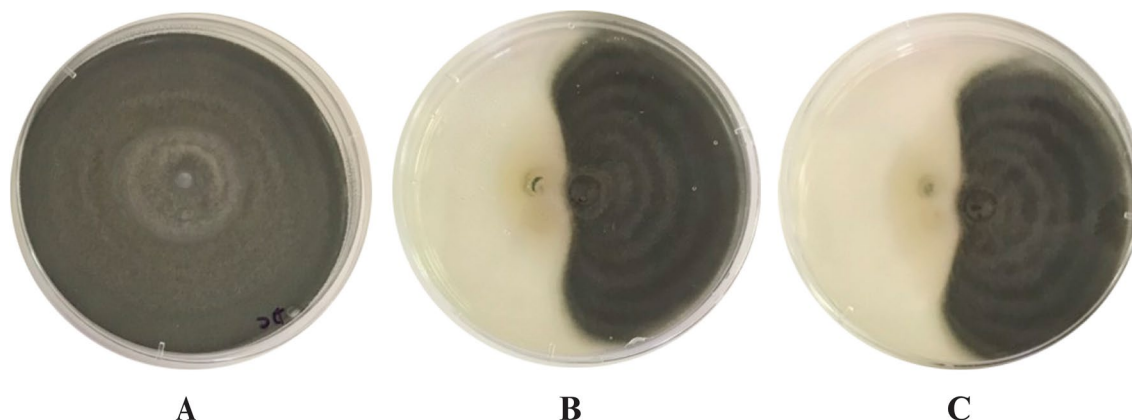


Fig. 1 Endophytic bacteria inhibited the mycelial growth of *Magnaporthe oryzae*. **(A)** *A. alternata* YZU growth alone. **(B)** The antagonistic effect of ND06 against *M. oryzae* and **(C)** Antagonistic activity of ND10 isolates against *M. oryzae*

Table 4 Control efficacy of culture broth of strains ND06 and ND10 against rice blast in greenhouse conditions

Treatments	Incidence rate (%)		Disease index		Control efficacy (%)	
	ND06	ND10	ND06	ND10	ND06	ND10
T1	–	–	–	–	–	–
T2	92.3 ^a	95.1 ^a	65.2 ^a	66.1 ^a	–	–
T3	77.8 ^b	78.1 ^b	31.2 ^b	32.3 ^b	49.7 ^b	50.1 ^c
T4	39.8 ^c	40.7 ^c	14.8 ^c	15.1 ^c	73.2 ^a	73.9 ^b
T5	32.5 ^d	34.9 ^d	13.7 ^c	12.8 ^d	74.7 ^a	75.6 ^a

T1: A blank solution without fungus (*M. oryzae*) and endophytic bacteria; T2: A mixture of LB medium only and conidial suspension (5×10^5 conidia/ml) of *M. oryzae*, added with 0.025% Tween 20; T3: A mixture of the fungicide tricyclazole (750 µg/mL) and conidial suspension added with 0.025% Tween 20; T4: A mixture of culture broth from 3-day-old cultures (10%, v/v) and conidial suspension amended with 0.025% Tween 20; T5: Rice seeds were germinated in the 3-day-old culture broth of selected strains for 24 h. After rising with autoclaved water, the germinated seeds were planted in a pot. The seedlings at the five-leaf stage were sprayed with conidial suspensions. Means \pm standard errors within each column, followed by the same letter were non-significantly ($P < 0.05$) different according to the HSD test

Table 5 Characterization of in vitro plant growth-promoting traits of endophytic bacteria isolated from rice root

Bacterial strain	IAA ($\mu\text{g ml}^{-1}$)	NH ₃ ($\mu\text{g/ml}$)	ACC deaminase	Siderophore	Phosphate solubilizing index
ND01	31.22 \pm 1.01	42.34 \pm 0.22	–	+	2.34
ND02	13.77 \pm 0.85	82.45 \pm 0.25	6.32 \pm 0.3	+	–
ND03	34.73 \pm 0.81	–	–	–	1.56
ND04	21.29 \pm 1.31	80.32 \pm 1.19	7.65 \pm 0.82	+	–
ND05	32.25 \pm 1.34	105.82 \pm 4.25	–	–	2.25
ND06	43.71 \pm 1.37	84.66 \pm 0.45	16.15 \pm 0.86	+	2.87
ND07	37.43 \pm 0.87	73.52 \pm 0.67	–	+	1.31
ND08	33.73 \pm 1.02	–	13.32 \pm 0.54	+	–
ND09	63.78 \pm 1.11	112.34 \pm 1.23	–	+	1.78
ND10	89.69 \pm 1.61	98.87 \pm 0.56	19.58 \pm 0.34	+	2.67
ND11	51.67 \pm 1.57	32.16 \pm 1.25	12.47 \pm 0.86	–	–
ND12	25.19 \pm 0.96	–	–	–	2.34
ND13	13.28 \pm 1.36	102.77 \pm 0.63	–	+	1.46
ND14	–	17.83 \pm 0.47	–	–	–

+ Presence of trait; – absence of trait; The data represent the mean \pm standard error (SE) based on three replicates. The ACC deaminase activity was measured spectrophotometrically at 590 nm and expressed as $\mu\text{mol mg}^{-1}$ protein h^{-1}

112.34 ± 1.23 µg/ml. Moreover, 9 of 14 endophytic bacteria isolates showed positive results for siderophore production. The diameter of the orange circle around the colonies ranged from 1 to over 50 mm.

Furthermore, the data in Table 3 also presented the screening result for endophytic strains of bacteria capable of solubilizing calcium phosphate. The results showed that only 9 strains were able to solubilize the insoluble phosphates, with the PSI from 1.31 to 2.87 (Table 5). Together with phosphate solubilization, the activity of ACC deaminase was observed in 6 cultures of isolates. The range of enzyme activity was from 6.32 ± 0.3 µmol mg⁻¹ protein h⁻¹ (ND02) to 19.58 ± 0.34 µmol mg⁻¹ protein h⁻¹ (ND10). These properties were very useful in promoting plant development under stress conditions. Overall, the endophytic bacteria potentially used for biocontrol and biofertilizer in sustainable agriculture should have multiple plant growth-promoting traits. Hence, 2 strains (ND06 and ND10) were chosen for further experiments.

Rice growth was enhanced under inoculation with selected endophytic bacteria

The above results of in vitro experiments indicated that both ND06 and ND10 had multiple PGP traits and were chosen to explore their effects on rice seed germination and rice growth under the growth chamber. The results were presented in (Table 6). As shown in the table, rice seeds bacterized with ND06 or ND10 had a higher germination rate than those one inoculated with water (control). The highest germination rate was recorded

for seeds treated with ND10 strain. A similar trend was observed for the shoot and root length of the inoculated seed.

After 15 days of growing in a pot, the plant performance had significant differences between the inoculated and un-inoculated seedlings (Table 7, Fig. 2). The results showed that the rice seedlings inoculated with ND06 or ND10 strains significantly increase the plant parameters compared to the un-inoculated ones. Among the 2 strains applied, the ND10 inoculation produced the strongest improvements in shoot length (27.37 cm), dry weight (110.12 mg), and chlorophyll content (1.86 mg/g). Presumably, inoculation with ND10 strain most effectively enhanced the rice root, shoot, dry weight, and chlorophyll content.

Discussions

In Vietnam, the rice blast disease is one of the main factors causing serious yield loss. Currently, the farmers often used chemical pesticides, which are often deposited in soils and accumulated in plants to control the disease (De Hita et al. 2020). Currently, the alternative method being applied widely is using biological agents such as bacteria, fungi, and natural resources to suppress or inhibit phytopathogenic growth (Trung et al. 2022). In this study, endophytic bacteria were isolated from the rice (*Oryza sativa* Ngoc Chau) to explore their potential to biocontrol blast disease caused by the fungus *M. oryzae*.

The results showed that there were 14 endophytic bacteria isolated from rice root, but only 6 isolates had

Table 6 Effects of endophytic bacteria inoculation on rice seed germination

Treatments	3 days	5 days	7 days		
	Germination rate (%)	Germination rate (%)	Germination rate (%)	Shoot length (cm)	Root length (cm)
Control	43.21 ^c	71.34 ^c	95.21 ^b	1.38 ± 0.18 ^b	1.23 ± 0.15 ^a
ND06	51.53 ^b	80.27 ^b	95.89 ^b	2.09 ± 0.23 ^a	2.05 ± 0.53 ^b
ND10	55.65 ^a	86.78 ^a	97.61 ^a	2.17 ± 0.24 ^a	2.49 ± 0.27 ^b

The data represent the mean ± standard error (SE) (n = 20); Values in the same column with the same letter(s) are not significantly different as determined by the HSD test (P < 0.05)

Table 7 Effects of inoculation with two PGPE strains on different growth parameters in rice after transplantation

Treatment	Shoot length (cm)	Root length (cm)	Dry weight (mg)	Chlorophyll content (mg g ⁻¹)
Control	21.61 ± 0.24 ^a	8.21 ± 0.24 ^a	60.82 ± 1.21 ^a	1.15 ± 0.12 ^a
ND06	25.14 ± 0.32 ^c	9.36 ± 1.37 ^a	90.65 ± 2.12 ^b	1.65 ± 0.31 ^b
ND10	27.37 ± 1.36 ^b	11.31 ± 1.19 ^b	110.12 ± 4.12 ^c	1.86 ± 0.12 ^c

Data represent averages based on 15 seedlings (n = 15). Chlorophyll contents represent averages based on three replicates (n = 3). Mean values with the same letter(s) in the column do not differ significantly according to HSD test (p < 0.05)



Fig. 2 Effects of inoculating with PGPE bacteria on the growth of shoots and roots in rice seedlings after treatment for 15 days. **A** Control (non-inoculated), **B** Rice seedlings inoculated with strain ND06, **C** Rice seedlings inoculated with strain ND10

antifungal activity over the *M. oryzae* fungus under in vitro conditions. Consistency with some previous studies, which demonstrated the bacteria inhibited the fungal growth by some mechanisms such as the production of bioactive compounds like extracellular lytic enzymes, siderophores, salicylic acid, antibiotics, and volatile metabolites (De Hita et al. 2020). In addition to direct antagonism, the result of the pot experiment also showed that the inoculation of ND06 or ND10 significantly suppressed rice blast in tested seedlings. These results might explain due to the production of bioactive compounds that induced systemic resistance by potentially regulating defense-related genes in salicylate (SA)- or jasmonate (JA)-dependent defense signaling pathways (He et al. 2019). In this study, the results indicated that fungal antagonists produced IAA and siderophore that could play roles in inhibiting fungal growth.

Previous studies indicated that plant was the host of a wide range of endophytes, which showed multiple plant growth promotion abilities and could be potentially exploited as biofertilizers. It is reported that the endophytic community in each plant mainly depended on both the plant genotype and the type of soil used to cultivate a plant species (Li et al. 2018). In terms of plant growth promotion, an important characteristic of endophytes was the production of IAA, a regulator of root

development (Gilbert et al. 2018) and a factor promoting the plant–microbe interaction (Duca et al. 2014). Notably, there are 6 different metabolic pathways to produce IAA in bacteria and 5 of them used tryptophan precursors (Duca et al. 2014). Hence, the tryptophan was added to the media to screen for microbial IAA production. Obtained results showed that almost all the isolates (92.86%) could produce IAA with the highest value ($89.69 \pm 1.61 \mu\text{g ml}^{-1}$) observed for the ND10 strain. These were consistent with previous studies on endophytic bacteria isolated from rice (Gilbert et al. 2018) who demonstrated that the bacterial IAA accumulation in plants led to the enhancement of ethylene synthesis in plants and subsequently caused the abscission of leaf and fruit and inhibition of stem growth. These results suggested that the IAA produced by endophytic bacteria has a direct effect on the development of the plant.

It was demonstrated that one of the main factors inhibiting plant growth was a low bioavailability of *P* in soil (Gamalero and Glick 2019), which could be reduced by using PGPB with the ability in solubilizing insoluble phosphates (Trung et al. 2022). One of the main groups of PGPB is endophytic bacteria, which has shown the capacity to release *P* from immobilized mineral phosphates (Trung et al. 2022). The results in this study indicated that there was about 64% of isolated endophytic

bacteria presented a trait of CaP-Solubilization, with the PSI from 1.31 to 2.87. These results are in agreement with the report of Hameed et al. (2015). The author isolated bacterial endophytes from different tissue of rice cultivars grown in acidic P-limited soil and documented that majority of isolates were phosphate solubilizing bacteria (PSB). According to Khan et al. (2007), the PGP endophytic bacteria could solubilize the inorganic P due to their ability to the production of low molecular weight organic acids leading to soil pH < 7 and subsequently enhancing the inorganic phosphate solubilization. Furthermore, Chabot et al. (1996) demonstrated a linear relationship between the increase of P amount in PSB-inoculated plants and the phosphate solubilization activity of applied PSB. All in all, the results indicated the variation of phosphate solubilization ability of endophytic bacteria isolated from rice root that might be due to the selection of plant, in which many types of bacteria were chosen to utilize a diverse nutrient source.

Another plant growth-promoting trait of isolates was the ammoniac production, in which the atmospheric nitrogen was converted into NH_4^+ form by the plant-microbe symbiosis (Nag et al. 2020). Obtained results showed a large number of isolates (11 out of 14 isolates) could form colonies on N-free media suggesting their functions in fixing air nitrogen (Nag et al. 2020). Presumably, the results indicated the isolated PGP endophytic bacteria in this study offered great potential as biofertilizers.

Furthermore, it was documented that the siderophore production capacity of endophytic bacteria played important role in stimulating the bacteria-plant interactions and plant growth (Ferreira et al. 2019). There were several studies on rice that demonstrated the isolation of siderophore-producing bacteria belonging to different genera (Loaces et al. 2011). It was in agreement with our results that presented 05 bacterial endophytes could produce siderophores. Vansuyt et al. (2007) reported positive effects on plant development of siderophores produced by bacteria, which are efficiently taken up by the plants increasing the iron content inside plant tissues and finally enhancing plant development. On the other hand, siderophores produced by PGPB also played roles in the biocontrol of phytopathogenic fungi by preventing them to form iron chelators, hence limiting their iron uptake (Marschner et al. 1986) or triggering induced systemic resistance (ISR) in plants (Aznar and Dellagi 2015). Hence, the endophytic bacteria isolated in this study with a high siderophore production capacity could be exploited as a promising biocontrol agent.

In terms of plant growth and development, plant growth-promoting bacteria could interact with plants to alleviate the effects of stress on the plant by producing

the enzyme ACC deaminase, which can degrade ACC, an ethylene precursor (Murali et al. 2021). According to Bal et al. (2013), a bacterium with ACC deaminase activity ranging from 0.062 to 2.664 $\mu\text{mol } \alpha\text{-ketobutyrate mg}^{-1}$ protein h^{-1} or higher could act as PGPB. In this study, there were 6 cultures of isolates that could produce ACC deaminase activity of 6.32–19.58 $\mu\text{mol mg}^{-1}$ protein h^{-1} , and the highest activity was observed for ND06 and ND10 strains. Therefore, the ND06 and ND10 may be used as efficient PGPB to increase plant stress tolerance.

The results of seed germination and greenhouse experiments used 2 selected strains, ND06 and ND10, presented the improvement of germination rate and plant development parameters. These could be explained by the ability of ND06 and ND10 in generating PGP properties such as IAA production, siderophore-producing, NH_3 production, phosphorus solubilization, and ACC deaminase activities. Notably, the inoculation of endophytic bacteria, ND06 and ND10, increased the chlorophyll content of the leaves of rice seedlings compared to control seedlings. It was reported that chlorophyll synthesis strongly depends on some main factors such as light intensity, nitrogen, magnesium, iron, temperature, water, and trace elements (Mn, Cu, and Zn) [33]. Hence, the increase in chlorophyll content in seedlings inoculated with strains ND06 and ND10 could be due to their abilities in nitrogen fixation and siderophore production, which positively affected to amount of nitrogen and iron in the leaves of inoculated rice seedlings. These explanations were consistent with some previous reports, in which a lack of nitrogen or iron in leaves caused a loss of green pigment, and subsequently a reduction of photosynthesis activity (Gholizadeh et al. 2017). For example, Li et al. (2021) demonstrated that the iron deficiency led to chloroplast degeneration and reduction in chlorophyll synthesis, while excessive iron treatment increased the chlorophyll contents, chloroplast sizes, and inflated starch granules. Consistently, several other reports of the positive effect of endophytic bacteria on plant development and the improvement of the photosynthetic efficiency of crops were also reported (Trung et al. 2022). These results suggest the two endophytic bacteria, ND06 and ND10, isolated from rice roots could potentially be used as biological agents to stimulate tolerance to environmental stress and promote plant growth.

Conclusions

It was concluded that endophytic bacteria could inhibit the fungus *M. oryzae* that causes the blast disease on rice under in vitro and greenhouse conditions. Also, it was showed many growth-stimulating traits such as IAA production, ammonia production, siderophore production, ACC deaminase production, and phosphate

solubilization. In addition, positive effects of 2 selected strains, ND06 and ND10, which were, respectively, identified as *B. velezensis* (accession number MN704466.1) and *P. putida* (accession number FJ976605.1) were demonstrated, in improving the seed germination rate and plant growth of rice. More research should be studied to contribute to the collection of beneficial microbial strains for crops in Vietnam, particularly in northern Vietnam. The potential application of ND06 and ND10 to develop into probiotics was significantly reduced the use of chemical fertilizers and pesticides in sustainable and environmentally friendly agricultural production.

Abbreviations

ACC: 1-Aminocyclopropane-1-carboxylic acid; ISR: Induced systemic resistance; IAA: Indole-3-acetic acid; NBRIP: National botanical research institute's phosphate; PSI: Phosphate solubilizing index; PSB: Phosphate solubilizing bacteria; PGP: Plant growth promoting; TSA: Tryptic soy agar.

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Author contributions

DQT was involved in conceptualization, supervision, the final interpretation of data, and editing of the manuscript. The author read and approved the final manuscript.

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