International Journal of Agricultural Technology 2025 Vol. 21(2):443-458
DOI: https://doi.org/10.63369/ijat.2025.21.2.443-458
Available online http://www.ijat-aatsea.com
ISSN 2630-0192 (Online)

Biocontrol potential of *Bacillus* spp. against bacterial blight and bacterial leaf streak pathogens in rice

Banluehan, J. and Tunchai, M.*

Department of Plant Production Technology, School of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang, Bangkok 10520, Thailand.

Banluehan, J. and Tunchai, M. (2025). Biocontrol potential of *Bacillus* spp. against bacterial blight and bacterial leaf streak pathogens in rice. International Journal of Agricultural Technology 21(2):443-458.

Abstract Bacterial leaf blight and bacterial leaf streak are major rice diseases caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) and *X. oryzae* pv. *oryzicola* (*Xoc*). In this study, rhizosphere soil samples were collected from healthy rice in Suphanburi, Kanchanaburi, and Surin provinces of Thailand. Among 135 bacterial strains isolated from the soil samples, only six isolates were selected mainly based on their strong antagonistic activity against *Xoo*. By dual-culture test, isolated strain KRI2 provided the largest *Xoo* inhibition zone diameters of 30.16 mm, followed by SRN19 and SPB1_1 with diameters of 24.30 mm and 24.06 mm, respectively. The largest *Xoc* inhibition zone diameter was obtained from strain SRN19 (15.46 mm), followed by strain SPB1_1 (14.21 mm) and strain SPB1_10 (14.00 mm). In addition, all six selected strains were able to inhibit the growth of *Curvularia lunata*, the rice pathogenic fungus. Morphological characterization revealed that all six strains were Gram-positive bacteria with rod shape. Molecular characterization by 16S rDNA gene sequencing analysis exhibited that all six strains belong to the genus *Bacillus*. Four isolated strains, SPB1_1, SPB1_10, SRN19, and KRI6, were identified as *Bacillus velezensis*. KRI2 and KRI4 were identified as *Bacillus sonorensis* and *Bacillus subtilis*, respectively. Besides, antagonist-related lytic enzyme production ability and plant growth-promoting traits of the six strains were observed. It was found that all six strains were able to produce protease and cellulase but showed an ability to solubilize phosphate and nitrogen fixation slightly.

Keywords: *Xanthomonas oryzae*, Bacterial rice diseases, Antagonistic bacteria, Biocontrol

Introduction

Rice (*Oryza sativa*), an essential crop that serves as the main staple food of many people worldwide, is extensively grown in over 100 countries with almost 90% of the global supply originating from Asia (Azizi and Lau, 2022). In Thailand, rice is the most economically important crop, and the main areas of rice cultivation are the northeast and central regions. About 3.55 million households are rice farmers. More than fifty percent of rice production in

* **Corresponding Author**: Tunchai, M.; **Email:** mattana.tu@kmitl.ac.th

Thailand is produced for export. Therefore, Thailand is positioned as one of the top three rice-exporting nations worldwide. Although Thailand is among the world's top rice exporters, its competitiveness is declining due to poorer yields compared to other countries and higher production costs per area (Ngammuangtueng *et al.*, 2019). Rice diseases are considered the main problem in rice production, which causes both quality and yield losses.

Rice is susceptible to several plant pathogens, including fungi, bacteria, viruses, and nematodes, resulting in over 70 distinct rice diseases. Major rice diseases caused by bacteria are bacterial leaf blight (BLB) and bacterial leaf streak (BLS), both belonging to closely related pathovars of *Xanthomonas oryzae* species: *X. oryzae* pv. *oryzae* (*Xoo*) and *X. oryzae* pv. *oryzicola* (*Xoc*), respectively (Jiang *et al*., 2020). BLB was discovered by a farmer in Fukuoka, Japan, in 1884, so it is one of the earliest recorded rice diseases. BLB is a highly destructive rice disease that can result in yield reductions of up to 50%, depending on factors such as environmental conditions, rice variety, and growth stage. Losses from the kresek syndrome of BLB affecting rice-transplanted seedlings may have reached up to 75%. BLB is widespread in both tropical and temperate regions. Currently, rice BLB is extensively prevalent in almost all rice-producing countries (Niño-Liu *et al*., 2006). BLS came later, which was first discovered in the Philippines in 1918. However, it has increasingly drawn attention, particularly in Asia and Africa. Yield losses attributed to BLS vary between 8% to 32%. *Xoo* enters the xylem tissue via wounds or water pores, where it multiplies and moves throughout the plant, resulting in a systemic infection. On the contrary, *Xoc* primarily invades leaves through stomata and limitedly moves to the intervascular areas, leading to the leaf streaking symptom (Jiang *et al*., 2020).

In general, chemicals are frequently used to control rice diseases due to their convenience and effectiveness. However, concern about the toxicity of chemical pesticides and environmental pollution is rising. Biological control, a sustainable method for managing plant diseases, offers a promising alternative for preventing and controlling rice diseases. (Marin *et al*., 2019; Sanya *et al*., 2022). A majority of antagonistic bacteria used as biological control agents (BCAs) in rice cultivation are *Bacillus* sp., which is a group of Gram-positive, rod-shaped, and endospore-forming bacteria (Prasad *et al*., 2023). Members of this genus are omnipresent and widely found in diverse environments, such as in soil, water, and the rhizosphere. Many species of *Bacillus* have been reported as BCAs, for example, *B. subtilis*, *B. amyloliquefaciens, B. licheniformis*, *B. velezensis*, and *B. megaterium*, etc. According to Prasad *et al.* (2023), different *Bacillus* taxa, and even strains within the same species, may exhibit distinct biological roles against plant pathogens. Reports mainly describe the antagonistic

mechanisms of *Bacillus* sp. as competing for ecological niches with plant pathogens and producing antibiotics and extracellular lytic enzymes. The lipopeptide antibiotics from *Bacillus* spp., such as iturins, surfactins, and fengycins, showed a broad-spectrum of antagonistic activity against various plant pathogens (Zhou *et al*., 2022). In addition, many strains of *Bacillus* sp. were found to have the ability to promote plant growth with well-studied mechanisms, including phosphate-solubilizing, nitrogen-fixing, and plant hormone-producing (Prasad *et al*., 2023).

This study aimed to obtain BCAs with the potential to control bacterial leaf blight and bacterial leaf streak disease in rice caused by *X. oryzae* pv. *oryzae* and *X. oryzae* pv. *oryzicola*. We, therefore, isolated and screened rhizosphere bacteria associated with rice plants that have antagonistic activity against both pathovars of *X. oryzae.* The obtained antagonists were also tested for their spectrum on the pathogenic fungi, *Curvularia lunata*, which causes leaf spot, root rot, and seed discoloration diseases in rice. The antagonistic isolates were further detected in their ability to produce lytic enzymes, promote plant growth, and determine the presence of genes involved in the synthesis of antimicrobial peptides.

Materials and methods

Pathogens and cultural conditions

Xanthomonas oryzae pv. *oryzae* (*Xoo*) DOA-BCC2568 and *X. oryzae* pv. *oryzicola* (*Xoc*) DOA-BCC2562 were generously given by the Plant Protection Research and Development Office, Department of Agriculture (DOA), Thailand. *Xoo* and *Xoc* were grown at 28 °C with shaking at 150 rpm in nutrient glucose broth (NGB, peptone 5 g, beef extract 3 g, and glucose 10 g per l) for a duration of 24 hours. The pathogens were stored on nutrient glucose agar (NGA, NGB adding 15 g of agar per l) and kept at 4 °C for future use. *Curvularia lunata* was obtained from the Plant Pathology Laboratory, Department of Plant Production Technology, King Mongkut's Institute of Technology Ladkrabang. *C. lunata* was cultured and maintained on Potato Dextrose Agar (PDA, Himedia™, India).

Isolation of bacterial strains from rice rhizosphere soil

Rice rhizosphere soil samples were randomly collected from healthy rice fields across central, western, and northeastern Thailand. Each soil sample (1 g) was mixed thoroughly with 10 ml of sterilized distilled water, followed by serial dilution up to 10^{-6}. A 100 µl aliquot of the diluted suspension, ranging from 10^{-4} to 10^{-6}, was spread onto nutrient agar (NA) plate. After incubation at 28 °C for

two days, colonies exhibiting distinct morphologies were picked and cross-streaked onto fresh NA plates for purification. The isolated bacterial strains were then cultured in nutrient broth (NB) at 28 °C with shaking at 150 rpm for 24 hours. The strains were maintained on NA plates and kept for subsequent use at 4 °C. For long-term storage, the bacterial cultures were preserved in 20% (v/v) glycerol at -80 °C.

Selection of antagonistic bacteria against X. oryzae

A dual-culture method was carried out to screen the isolated bacteria in order to obtain antagonists against *Xoo* and *Xoc.* The same protocols employed in the antagonistic test conducted on *Xoo* were used for *Xoc*. Briefly, the pathogen cell suspension was adjusted to an optical density of 0.2 at 600 nm (OD_{600}) and evenly swabbed across the surface of an NGA medium using a sterile cotton swab. A sterile paper disc (5 mm in diameter) was then placed on the NGA surface, followed by the application of 10 µl of each isolated bacterial suspension, also adjusted to an OD_{600} of 0.2. For the negative control, the same volume of NB medium was utilized instead. After incubation at 28 °C for two days, the diameters of the *Xoo* and *Xoc* inhibitory zones were measured. The values were reported as the diameter of the inhibition zones for *Xoo* and *Xoc* subtracting the paper disc diameter. Each experiment was conducted at least three times, with four replicates per bacterial isolate.

Antagonistic activity against Curvularia lunata

The six isolated bacteria showing antagonistic activity against *Xoo* and *Xoc* were further investigated in their spectrum against *C. lunata* using a dual-culture assay. The experiments were conducted in triplicate. The agar plug of *C. lunata* hyphal tip was placed 2 cm from the edge of a PDA plate and allowed the fungi to grow for 4 days. Then, each bacterial strain was linearly streaked 2 cm away from another side of the plate border. After 7 days of incubation at 28 °C, the mycelium radius was measured, and the percentage inhibition of radial growth (PIRG) was calculated as shown following (Chaiharn *et al*., 2020):

$$PIRG = R1 - R2/R1 \times 100$$

Where R1 = Radial growth of *C. lunata* in the control plates

R2 = Radial growth of *C. lunata* interacting with the bacteria

Identification of the antagonistic bacteria

The isolates exhibiting antagonistic activity against *Xoo* and *Xoc* were initially identified through morphological inspection of their single colonies and

Gram staining. For molecular identification, the 16S rDNA gene of each antagonistic bacterium was amplified using the universal primer pair, 27F and 1492R (Table 1). The PCR-amplified products were analyzed by agarose gel electrophoresis, purified, and subsequently sequenced by the commercial service of Macrogen Inc. (South Korea). The obtained DNA sequences were then compared with the known sequences in the National Center for Biotechnology Information (NCBI) with nucleotide BLAST tool.

Table 1. List of primers used in this study

Targeted gene	Primer's sequence (5'-3')	Annealing temp (°C)	Product size (bp)	Reference
16S rDNA	F: AGAGTTTGATCCTGGCTCAG R: GGTTACCTTGTTACGACTT	50	1,465	Tan et al., 2013
ituA	F: ATGAAAATTTACGGAGTATATATG R: TTATAACAGCTCTTCATACGTT	53	675	Zhou et al., 2022
bmyC	F: AGTAAATGAACGCGCCAATC R: CCCTCTCCTGCCACATAGAG	55	957	Zhou et al., 2022
srfAA	F: TCGGGACAGGAAGACATCAT R: CCACTCAAACGGATAATCCTGA	55	201	Zhou et al., 2022
fenD	F: GGCCCGTTCTCTAAATCCAT R: GTCATGCTGACGAGAGCAAA	55	269	Zhou et al., 2022
bacA	F: CAGCTCATGGGAATGCTTTT R: CTCGGTCCTGAAGGGACAAG	55	469	Zhou et al., 2022

Detection of antagonist-related lytic enzymes

The ability of antagonistic strains to produce and cellulase was assessed. Cell suspension of the isolated strain was adjusted to an OD_{600} of 0.2 prior to testing. Protease activity was detected using skim milk agar medium, while cellulase activity was measured with carboxymethyl cellulose medium (Zhou *et al.*, 2022). A sterile paper disc was positioned on the surface of the testing medium, and 10 µl of the bacterial suspension was applied to the disc. After incubating the agar plates for 2 to 4 days at 28 °C, the diameters of the hydrolytic zone surrounding the disc were measured. The hydrolytic zone was dyed with Congo red for cellulase detection.

Detection of plant growth promotion (PGP) traits

The antagonistic strains were tested for three PGP traits, which were phosphate solubilization, nitrogen fixation, and indole-3-acetic acid (IAA) production. Before testing, the bacterial suspension was adjusted to an OD_{600} of 0.2 prior to testing. Pikovskaya's agar (Patel *et al.*, 2017) was utilized for

phosphate solubilization testing, whereas Burk's nitrogen-free agar (Thamkhongdee *et al*., 2019) was employed for nitrogen fixation tests. A sterile paper disc was placed on the medium surface, followed by the application of 10 µl of the bacterial suspension. The agar plates were incubated at 28 °C for a duration of 3 to 7 days. Thereafter, the hydrolytic zone diameter surrounding the disc was recorded for the phosphate solubilization test. For nitrogen fixation test, the diameter of the bacterial colony surrounding the disc was measured.

The IAA production was evaluated following the method of Godon and Weber (1951). Bacterial isolates were cultured in NB supplemented with 1 mg/ml L-tryptophan and incubated at 28 °C under agitation for 48 hours. The supernatant was then collected and mixed with Salkowski's reagent in a 1:2 ratio, followed by 30 minutes of incubation in the dark. The production of IAA is evidenced by the purple to red color development.

Detection of antimicrobial peptide synthetic genes

The lipopeptide synthetic genes of the antagonistic strains were detected by PCR technique. Specific primers targeting five antimicrobial peptide synthetic genes (*ituA, bmyC, srfAA, fenD,* and *bacA*) are listed in Table 1. The PCR reaction mixture consisted of 12.5 µl of Green PCR Master Mix Direct-Load, 2X (Biotechrabbit, Germany), 1 µl of each primer (10 µM), 1 µl of genomic DNA, and ultrapure water, bringing the total volume to 25 µl. The amplification conditions were set according to the manufacturer's recommendation with annealing temperatures specified in Table 1. The obtained PCR products were visualized using electrophoresis on a 1.5% agarose gel.

Results

In vitro antagonistic efficacy of isolated bacteria against X. oryzae

Rhizosphere soil from healthy rice, as a source of rice rhizobacteria, was collected across several rice fields in the central, western, and northeastern regions of Thailand. Isolation of rhizobacteria were carried out on NA medium. From these isolates, a random selection was made to evaluate their ability to inhibit the growth of *Xoo* strain DOA-BCC2568, a highly virulent strain affecting rice. Among 135 bacterial isolates, only 6 strains exhibited high growth inhibition of *Xoo* on NGA medium by dual-culture assay (Table 2 and Figure 2A). The colony morphology of all 6 strains was quite similar, with milky white, opaque, flat, rough, and wrinkled colonies on NA medium. All six strains are Gram-positive bacteria with bacilli-shaped (Figure 1).

These six strains were further tested on their antagonistic activity against the *Xoc* strain DOA-BCC2562 (Figure 2B). By comparing the inhibition zone diameters of *Xoo* and *Xoc*, the results showed that the three largest *Xoo* inhibition zone diameters were found to be 30.16 mm for strain KRI2, 24.30 mm for strain SRN19, and 24.06 mm for strain SPB1_1 (Figure 3). The largest *Xoc* inhibition zone diameter was obtained from strain SRN19 (15.46 mm), followed by strain SPB1_1 (14.21 mm) and strain SPB1_10 (14.00 mm).

Table 2. Potential antagonistic bacterial strains isolated from rhizosphere soil of rice plants

Isolated strain	Gram-staining	Shape	Source of rhizosphere soil
SPB1_1	+	bacilli	Suphan Buri
SPB1_10	+	bacilli	Suphan Buri
SRN19	+	bacilli	Surin
KRI2	+	bacilli	Kanchanaburi
KRI4	+	bacilli	Kanchanaburi
KRI6	+	bacilli	Kanchanaburi

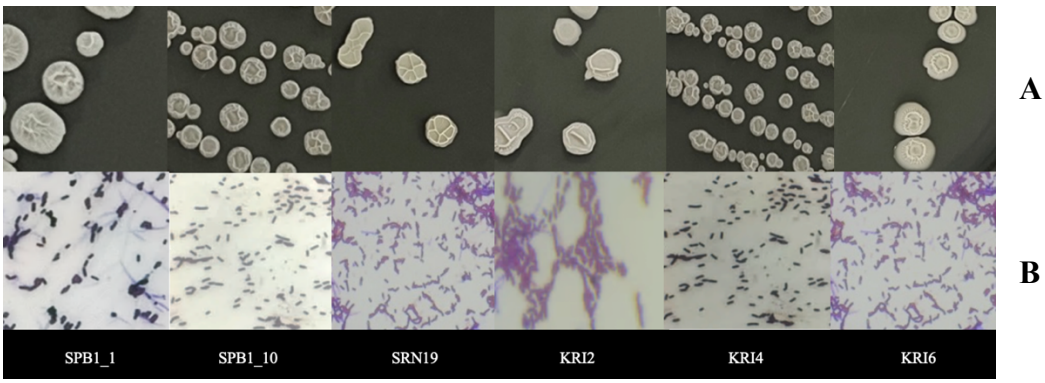

Figure 1. Morphological characteristics of the six potential antagonistic strains: SPB1_1, SPB1_10, SRN19, KRI2, KRI4, and KRI6. A) Single colony morphology and B) Gram staining under the microscope at a total magnification of 1000

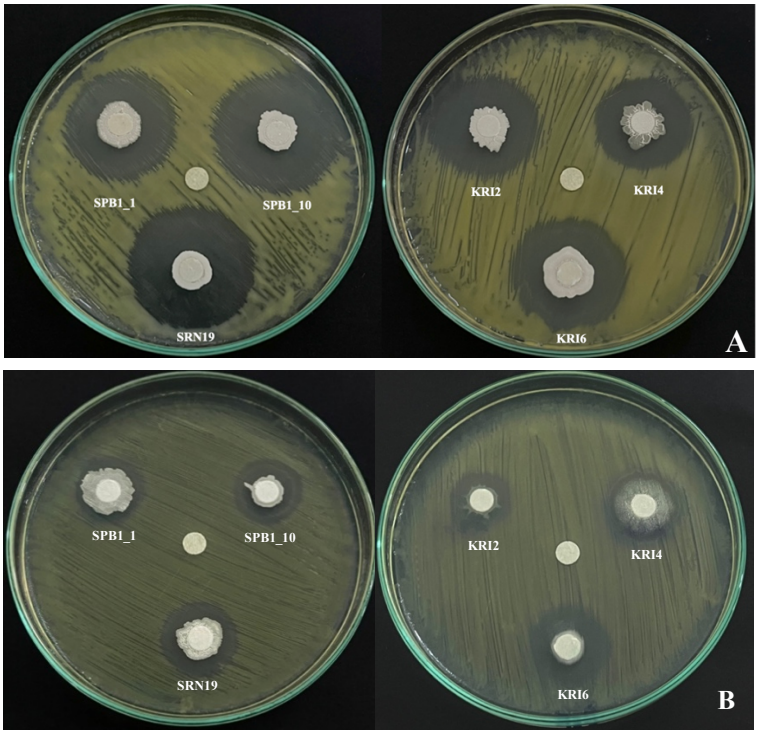

Figure 2. Antagonistic activity of the six isolated bacteria (SPB1_1, SPB1_10, SRN19, KRI2, KRI4, and KRI6) against A) *X. oryzae* pv. *oryzae* and B) *X. oryzae* pv. *oryzicola* obtained from the dual-culture assay

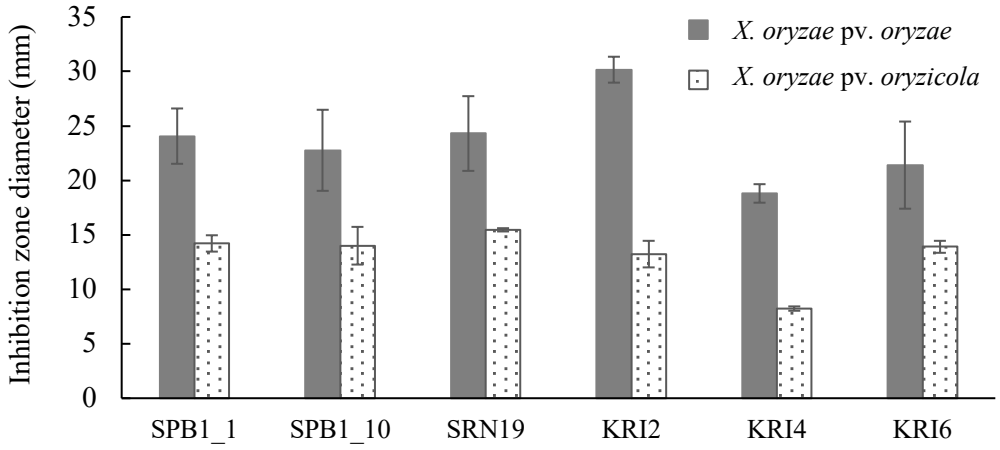

Figure 3. Comparison of inhibition zone sizes produced by the six isolated bacteria (SPB1_1, SPB1_10, SRN19, KRI2, KRI4, and KRI6) against *X. oryzae* pv. *oryzae* and *X. oryzae* pv. *oryzicola*. Error bars represent the SD values from the means of measurements done in at least triplicate experiments

Antagonistic activity against Curvularia lunata

The six strains that showed antagonistic activity against *Xoo* and *Xoc* were further investigated for their antagonistic ability on rice phytopathogenic fungi. *Curvularia lunata,* the causal agent of rice brown leaf spot, was tested. The dual-culture test showed that all six strains could inhibit the growth of *C. lunata* (Figure 4). Mycelium radians of *C. lunata* co-cultured with the six antagonistic bacteria displayed significantly smaller than the fungus radians in the control plate (Table 3). Strain SPB1_10 exhibited the highest PIRG at 33.52% on the 7th day of co-culture (Table 3). Afterward, the mycelium and spores of *C. lunata* confronting antagonistic bacteria were observed under a light microscope at a total magnification of 400. The noticeable morphological change of *C. lunata* hyphae was observed from co-cultured *C. lunata* with strain SRN19 (Figure 4C), which showed hyphal swelling and bulb formation compared with the normal hyphae of *C. lunata* in the control plate (Figure 4G).

Molecular-based identification of the antagonistic bacteria

To identify the genus and species of the six antagonistic strains, their 16S rDNA genes were amplified. The PCR products were revealed using agarose gel electrophoresis, which showed the product's size of about 1,500 base pairs (data not shown). The PCR products were purified, sequenced, and analyzed by the nucleotide BLAST tool in NCBI. The results showed that all six strains belong to the genus *Bacillus* (Table 4). Four strains, SPB1_1, SPB1_10, SRN19, and KRI6, were identified as *Bacillus velezensis*. While KRI2 and KRI4 showed high nucleotide homology with *Bacillus sonorensis* and *Bacillus subtilis*, respectively.

Table 3. Percentage of *Curvularia lunata* radial growth inhibition by the six antagonistic bacteria at 7 days of the dual-culture on PDA medium

Isolated strain	*Curvularia lunata* colony radian (cm)[/1]	Percent inhibition radial growth (%)
SPB1_1	4.03 ± 0.10^{bc}	32.24 ± 1.52
SPB1_10	3.95 ± 0.03^{c}	33.52 ± 0.44
SRN19	4.00 ± 0.03^{bc}	32.72 ± 0.64
KRI2	3.99 ± 0.10^{bc}	32.88 ± 1.45
KRI4	4.12 ± 0.19^{b}	30.63 ± 3.31
KRI6	3.98 ± 0.03^{bc}	33.04 ± 0.61
Control	5.95 ± 0.02^{a}	-

[1]/ Values are the means ± SD, which are followed by different letters indicating a significant difference between isolated strains by the least significant difference (LSD) test at $P \leq 0.05$.

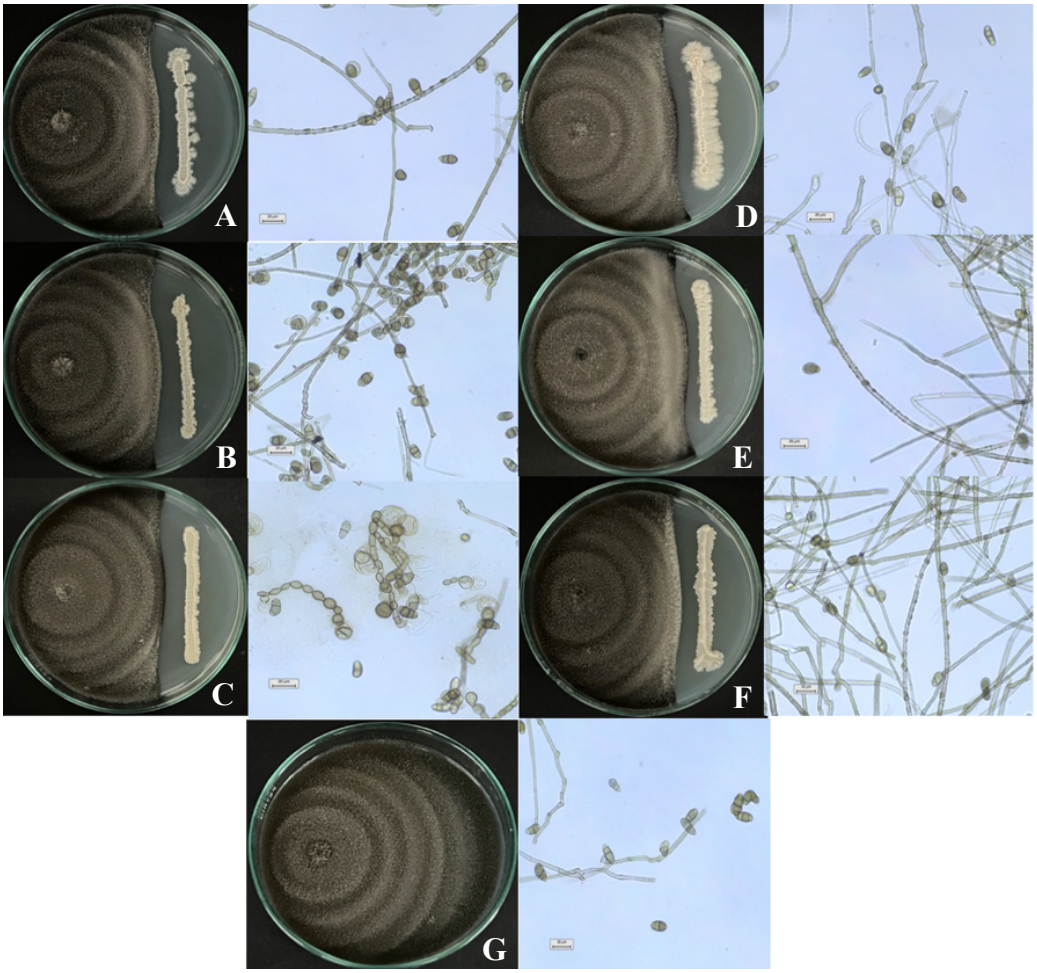

Figure 4. Antagonistic effect of the six obtained antagonistic bacteria against *Curvularia lunata* by the dual-culture method on PDA medium and morphology of *C. lunata* mycelium and spores under the microscope at a total magnification of 400. A) strain SPB1_1, B) strain SPB1_10, C) strain SRN19, D) strain KRI2, E) strain KRI4, F) strain KRI6, and G) control (without bacterial antagonists)

Detection of antagonist-related lytic enzymes and PGP traits

The six antagonistic strains were evaluated for their production of two lytic enzymes associated with antagonistic activity and three plant growth-promoting (PGP) traits, as part of a preliminary investigation into their mechanisms of antagonism and potential to promote plant growth. Hydrolysis circles of skim milk and CMC medium were formed around the colonies (Figure 5), indicating

that all six strains of *Bacillus* sp. were able to produce protease and cellulase. The six antagonistic strains also showed an ability to solubilize phosphate and nitrogen fixation slightly (Table 5). On the contrary, none of the isolates exhibited positive coloration for indole-3-acetic acid (IAA) production tests (Table 5).

Table 4. Molecular identification results of the six antagonistic strains by the nucleotide BLAST tool in NCBI

Antagonistic strain	Reference strain	Query cover (%)	Identity (%)	Accession no.
SPB1_1	*Bacillus velezensis* strain EB14	100%	99.93%	CP065473.1
SPB1_10	*Bacillus velezensis* strain SRCM102744	100%	99.70%	CP028208.1
SRN19	*Bacillus velezensis* strain B14	100%	99.41%	OL706755.1
KRI2	*Bacillus sonorensis* strain SL-2	100%	99.85%	MF977361.1
KRI4	*Bacillus subtilis* strain BDU/BMS/KS/JE-RM4	100%	99.93%	KU166865.1
KRI6	*Bacillus velezensis* strain V 3.14	100%	99.85%	CP097784.1

Table 5. Detection of antagonist-related lytic enzymes and plant growth promotion traits in six antagonistic bacterial strains

Antagonistic strain	Protease[/1]	Cellulase[/1]	Phosphate-solubilizing[/1]	Nitrogen-fixing[/2]	IAA[/3]
SPB1_1	+++	+++	+	+	-
SPB1_10	+++	+++	+	+	-
SRN19	+++	++	+	+	-
KRI2	+++	+++	+	+	-
KRI4	++	++	+	+	-
KRI6	++	++	+	+	-

[1]/ Diameter of hydrolytic zone: + < 10 mm; ++ = 10 - 20 mm; +++ > 20 mm.
[2]/ Diameter of colony: + < 10 mm; ++ = 10 - 20 mm; +++ > 20 mm.
[3]/ Purple to red color development: - = no color development.

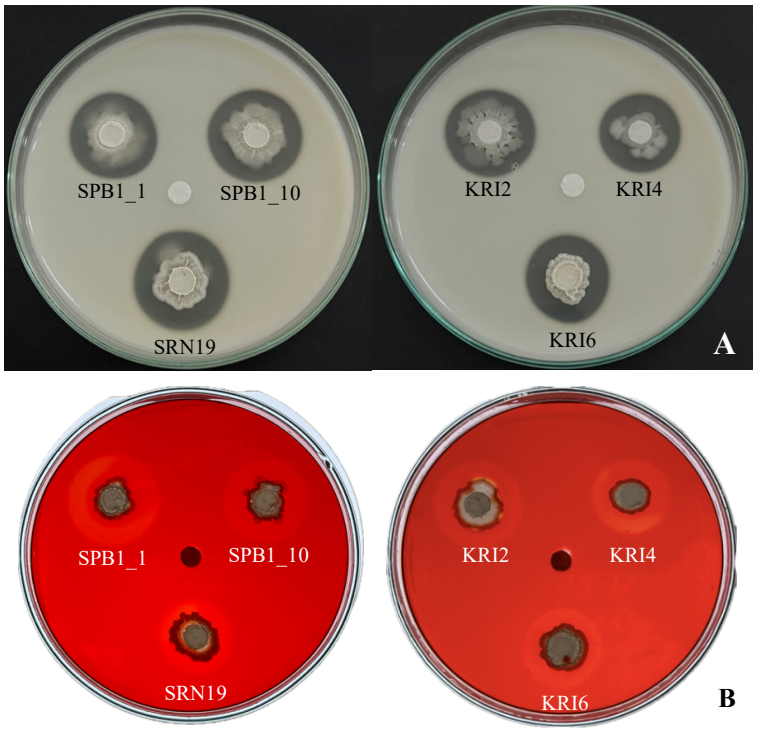

Figure 5. Hydrolytic zone developed by the six antagonistic bacterial strains on the test media for A) protease and B) cellulase detection. The paper disc at the center of the plates served as a negative control

Detection of genes encoding antimicrobial peptides

Many types of peptides, especially lipopeptides, produced by *Bacillus* spp. were reported as antimicrobial agents against plant pathogens (Zhou *et al*., 2022; Jin *et al*., 2020). To determine whether the six isolated strains of *Bacillus* sp. produce antimicrobial peptides, the genes responsible for the synthesis of iturin (*ituA*), bacillomycin (*bmyC*), surfactin (*srfAA*), fengycins (*fenD*), and bacilysin (*bacA*) were amplified by the specific primer pair for each gene. Genomic DNA from each *Bacillus* strain served as the template for PCR amplification. The results showed that genomic DNA of *B. velezensis* SPB1_1, SPB1_10, SRN19, and KRI6 yielded the right size of the PCR products from the five gene-specific primers, suggesting that these four strains might contain all five genes (Figure 6 and Table 6). While *B. sonorensis* KRI2 and *B. subtilis* KRI4 were found to potentially harbor *bmyC*, *srfAA*, and *bacA* genes (Table 6).

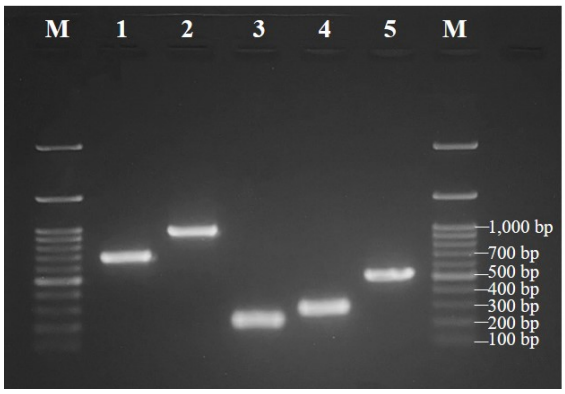

Figure 6. Agarose gel electrophoresis of PCR-amplified lipopeptide biosynthetic genes, using genomic DNA of *B. velezensis* SRN19 as a template. Lane M: 100 bp ladder, Lane 1: *ituA*, Lane 2: *bmyC*, Lane 3: *srfAA*, Lane 4: *fenD*, and Lane 5: *bacA*

Table 6. Detection of lipopeptide synthetic genes in the six antagonistic bacteria strains by PCR

Antagonistic strain	PCR product detection[1]				
	ituA	***bmyC***	***srfAA***	***fenD***	***bacA***
SPB1_1	+	+	+	+	+
SPB1_10	+	+	+	+	+
SRN19	+	+	+	+	+
KRI2	ND	+	+	ND	+
KRI4	ND	+	+	ND	+
KRI6	+	+	+	+	+

[1]/ + represents the expected size of the PCR product obtained, and ND represents non-detection.

Discussion

The utilization of antagonistic bacteria for biological control is considered an eco-friendly strategy and a sustainable alternative method for managing plant diseases (Marin *et al.,* 2019). Rice, one of the most economically important crops worldwide, has been threatened by many diseases affecting rice production in both quantity and quality. The biological control of rice diseases, therefore, is gaining more and more attention from both economic and ecological viewpoints in today's globe.

In this study, the screened antagonistic bacteria from the rice rhizosphere for biological control of bacterial leaf blight (BLB) and bacterial leaf streak (BLS). Rice rhizosphere soil was collected from healthy rice fields in various areas in central, western, and northeastern Thailand. The six isolated bacteria

showed strong antagonistic activity toward *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), the BLB pathogen. These six strains also exhibited moderate antagonistic effects against the BLS pathogen, *X. oryzae* pv. *oryzicola* (*Xoc*). Moreover, all six obtained antagonists were able to inhibit the mycelium growth of rice pathogenic fungi, *Curvularia lunata.* By morphological characterization, all six strains were Gram-positive bacteria with rod shape. Molecular characterization through 16S rDNA gene sequencing analysis exhibited that all six strains belong to the genus *Bacillus*. The four strains, which are SPB1_1, SPB1_10, SRN19, and KRI6, were identified as *B. velezensis*. KRI2 and KRI4 were identified as *B. sonorensis* and *B. subtilis*, respectively. To date, many reports have revealed that many strains of *Bacillus* spp. were effective antagonistic bacteria on rice pathogens (Marin *et al*., 2019; Sanya *et al*., 2022; Zhou *et al*., 2022; Doni *et al.,* 2022; Prasad *et al.,* 2023). *B. subtilis* is a well-studied antagonistic bacterium that has many reports to control various rice diseases such as sheath blight, blast, and bacterial leaf blight. *B. subtilis* can produce antibiotics, organic acids, and chitinases that inhibit pathogens directly or indirectly by enhancing plant defense mechanisms. *B. velezensis* and *B. sonorensis*, however, are recently reclassified species within the *Bacillus* genus. For *B. velezensis,* there is evidence that many strains of this species can suppress the rice pathogen's growth and promote plant growth. Zhou *et al.* (2022) reported that *B. velezensis* BR-01 isolated from the Chinese herb, *Bolbostemmatis rhizoma*, exhibited broad-spectrum antagonistic activities against various rice pathogens, including pathogenic fungi (*Fusarium fujikuroi*, *Magnaporthe oryzae*, *Ustilaginoidea virens*) and bacteria (*Xoo* and *Xoc*). Strain BR-01 was found to produce a range of bioactive compounds, including protease, cellulase, β-1,3-glucanase, chitinase, IAA, siderophore, 1-aminocyclopropane-1-carboxylate deaminase, and lipopeptide antibiotics (surfactin, iturin, and fengycin). Moreover, *B. velezensis* strain 504 isolated from rhizosphere soil of water spinach (Zhou *et al*., 2022) and strain HN-2 isolated from soil (Jin *et al*., 2020) were reported to exhibit inhibitory activity against *X. oryzae*. On the other hand, to the best of our knowledge, the biocontrol activity of *B. sonorensis* against rice diseases has not yet been investigated.

All six antagonistic strains could produce protease and cellulase. Both enzymes were reported to degrade the cell wall of many phytopathogenic fungi (Prasad *et al*., 2023). The lipopeptides, iturin, fengycin, and surfactin, synthesized by *Bacillus* ssp. strains, have antibacterial efficacy against several phytopathogens (Zhou *et al*., 2022; Jin *et al*., 2020; Prasad *et al*., 2023). Surfactin is a powerful biosurfactant that interacts with lipid bilayers by disrupting and solubilizing them. Fengycin and iturin directly impact fungal cell membranes, ultimately resulting in cell death. Bacillomycin, belonging to the iturin family, shows antifungal and hemolytic activities (Penha *et al*., 2020; Saiyam *et al*.,

2024). Bacilysin, a dipeptide antibiotic, was reported as an antimicrobial agent against *X. oryzae* (Wu *et al*., 2015). By detection of antimicrobial peptide synthetic genes, *B. velezensis* SPB1_1, SPB1_10, SRN19, and KRI6 might contain *ituA*, *bmyC*, *srfAA*, *fenD*, and *bacA* genes, which have the potential to produce iturin (*ituA*), bacillomycin (*bmyC*), surfactin (*srfAA*), fengycin (*fenD*), and bacilysin (*bacA*). *B. sonorensis* KRI2 and *B. subtilis* KRI4 might produce bacillomycin, surfactin, and bacilysin. Besides, the swollen and bulbous mycelia of *C. lunata* were obviously observed from co-culture with *B. velezensis* SRN19. The abnormal hyphae, including swelling and bulb formation of *C. lunata* hyphae, have been reported in the presence of iturin and surfactin (Saechow *et al*., 2016). Taken together, these results suggest that the antibiosis activity exhibited by the six isolated *Bacillus* strains against *X. oryzae* pathovars and *C. lunata* is likely mediated through the production of lytic enzymes and antimicrobial peptides.

In conclusion, the present study showed that all six strains of *Bacillus* sp. isolated from the rhizosphere of rice plants from various fields in Thailand had strong *in vitro* antagonistic activity against rice pathogenic bacteria, *Xoo* and *Xoc*. Furthermore, these six isolated strains could inhibit the mycelium growth of *C. lunata* on the PDA plate. In future studies, they should be further explored their antagonistic potential against other rice phytopathogens. To be used as BCA, *in vivo* biocontrol efficacy assays in both greenhouse and field experiments are needed to be conducted. Moreover, it is interesting to further characterize the biocontrol activity of *B. sonorensis* KRI2, which showed the highest *Xoo* suppression in this study.

Acknowledgements

We extend our sincere gratitude to the Plant Protection Research and Development Office, Department of Agriculture, Thailand, for generously providing *X. oryzae* pv. *oryzae* DOA-BCC2568 and *X. oryzae* pv. *oryzicola* DOA-BCC2562. This study was financially supported by King Mongkut's Institute of Technology Ladkrabang (Grant no. 2566-02-04-012).

References

Azizi, M. M. F. and Lau, H. Y. (2022). Advanced diagnostic approaches developed for the global menace of rice diseases: a review, Canadian Journal of Plant Pathology, 44:627-651.

Chaiharn, M., Theantana, T. and Pathom-aree, W. (2020). Evaluation of biocontrol activities of *Streptomyces* spp. against rice blast disease fungi. Pathogens, 9:126.

Doni, F., Suhaimi, N. S. M., Mispan, M. S., Fathurrahman, F., Marzuki, B. M., Kusmoro, J. and Uphoff, N. (2022). Microbial contributions for rice production: from conventional crop management to the use of 'omics' technologies. International Journal of Molecular Sciences, 23:737.

Godon, S. A. and Weber, R. P. (1951). Colorimetric estimation of indole acetic acid. Plant Physiology, 26:192-195.

Jiang, N., Yan, J., Liang, Y., Shi, Y., He, Z., Wu, Y., Zeng, Q., Liu, X. and Peng, J. (2020). Resistance genes and their interactions with bacterial blight/leaf streak pathogens (*Xanthomonas oryzae*) in rice (*Oryza sativa* L.)-an updated review. Rice, 13:3.

Jin, P., Wang, Y., Tan, Z., Liu, W. and Miao, W. (2020). Antibacterial activity and rice-induced resistance mediated by C_{15} surfactin A, in controlling rice disease caused by *Xanthomonas oryzae* pv. *oryzae*. Pesticide Biochemistry and Physiology, 169:104669.

Marin, V. R., Ferrarezi, J. H., Vieira, G. and Sass, D. C. (2019). Recent advances in the biocontrol of *Xanthomonas* spp. World Journal of Microbiology and Biotechnology, 35:72.

Ngammuangtueng, P., Jakrawatana, N., Nilsalab, P. and Gheewala, S. H. (2019). Water, energy and food nexus in rice production in Thailand. Sustainability, 11:5852.

Niño-Liu, D. O., Ronald, P. C and Bogdanove, A. J. (2006). *Xanthomonas oryzae* pathovars: model pathogens of a model crop. Molecular Plant Pathology, 7:303-324.

Patel, S., Jinal, H. N. and Amaresan, N. (2017). Isolation and characterization of drought resistance bacteria for plant growth promoting properties and their effect on chilli (*Capsicum annuum*) seedling under salt stress. Biocatalysis and Agricultural Biotechnology, 12:85-89.

Penha, R. O., Vandenberghe, L. P. S., Faulds, C., Soccol, V. T. and Soccol, C. R. (2020). *Bacillus* lipopeptides as powerful pest control agents for a more sustainable and healthy agriculture: recent studies and innovations. Planta, 251:70.

Prasad, B., Sharma, D., Kumar, P. and Dubey, R. C. (2023). Biocontrol potential of *Bacillus* spp. for resilient and sustainable agricultural systems. Physiological and Molecular Plant Pathology, 128:102173.

Saechow, S., Thammasittirong, A. and Thammasittirong, S. N. (2016). In vitro inhibitory effect of *Bacillus subtilis* BAS114 against *Curvularia lunata*. Advances in Environmental Biology, 10:176-183.

Saiyam, D., Dubey, A., Malla, M. A. and Kumar, A. (2024). Lipopeptides from *Bacillus*: unveiling biotechnological prospects-sources, properties, and diverse applications. Brazilian Journal of Microbiology, 55:281-295.

Sanya, D. R. A., Syed-ab-rahman, S. F., Jia, A., Onésime, D., Kim, K. M., Ahohuendo, B. C. and Rohr, J. R. (2022). A review of approaches to control bacterial leaf blight in rice. World Journal of Microbiology and Biotechnology, 38:113.

Tan, S., Jiang, Y., Song, S., Huang, J., Ling, N., Xu, Y. and Shen, Q. (2013). Two *Bacillus amyloliquefaciens* strains isolated using the competitive tomato root enrichment method and their effects on suppressing *Ralstonia solanace*arum and promoting tomato plant growth. Crop Protection, 43:134-140.

Thamkhongdee, A., Chinachanta, W., Chromkaew, Y., Chaiwan, F. and Shutsrirung, A. (2019). Abilities of nitrogen fixing bacteria in enhancing growth of arabica coffee seedling. Journal of Agriculture, 36:79-91.

Wu, L., Wu, H., Chen, L., Yu, X., Borriss, R. and Gao, X. (2015). Difficidin and bacilysin from *Bacillus amyloliquefaciens* FZB42 have antibacterial activity against *Xanthomonas oryzae* rice pathogens. Scientific Reports, 5:12975.

Zhou, J., Xie, Y., Liao, Y., Li, X., Li, Y., Li, S., Ma, X., Lei, S., Lin, F., Jiang, W. and He, Y-Q. (2022). Characterization of a *Bacillus velezensis* strain isolated from *Bolbostemmatis Rhizoma* displaying strong antagonistic activities against a variety of rice pathogens. Frontiers in Microbiology, 13:983781.

(Received: 30 September 2024, Revised: 6 March 2025, Accepted: 12 March 2025)