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Research article

Plant growth-promoting bacteria for biological control of *Pyricularia oryzae* causing rice blast disease and biodegradation of organophosphate pesticide

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Abstract

Importance of the work: Rice blast disease causes yield losses exceeding 50–80%, while excessive pesticide use leads to chemical accumulation and environmental concerns.

Objectives: To evaluate rhizosphere bacteria multifunctional capabilities: antagonistic activity against rice blast disease, plant growth promotion and organophosphate pesticide degradation.

Materials and Methods: In total, 301 bacterial isolates from the rice rhizosphere were screened for antagonistic activity against *Pyricularia oryzae* using the dual culture method. The antagonistic mechanisms were investigated. Selected antagonistic isolates were evaluated for plant growth-promoting activities and organophosphate pesticide degradation efficiency. The isolates were identified using 16S rRNA gene sequencing.

Results: Among the 301 bacterial isolates, 13 had strong antagonistic activity, with over 90% inhibition of *P. oryzae* mycelial growth. The antagonistic mechanisms identified were the production of volatile organic compounds, antifungal substances and cell wall-degrading enzymes (protease and cellulase). All antagonistic isolates had plant growth-promoting activity, including indole-3-acetic acid production (5.75–74.70 µg/mL with tryptophan; 2.79–15.62 µg/mL without tryptophan), phosphate solubilization, biofilm formation, siderophore production, nitrogen fixation and ammonia production. The highest mean ± SD organophosphate pesticide degradation was achieved by isolate SR4KB4.9 (48.78 ± 7.61%), followed by SR5KB5.3 (38.47 ± 4.33%) and SR4KB4.6 (33.39 ± 2.56%). Molecular identification showed these isolates were closely related to the *Bacillus* and *Brevibacillus* genera.

Main finding: In total, 13 bacterial isolates had strong antagonistic mechanisms and demonstrated dual capabilities as plant growth promoters and significant degradation of organophosphate pesticide. These isolates, identified as *Bacillus* and *Brevibacillus species*, offer an integrated sustainable approach for rice cultivation.

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Introduction

Rice (*Oryza sativa* L.) serves as a primary carbohydrate source for the population of Thailand (Vejchasarn et al., 2021). It is an important food crop for over 50% of the global population, making a major contribution to food security and economic stability in numerous areas (Samal et al., 2022). However, the impact of phytopathogenic fungi, especially *Pyricularia oryzae* (teleomorph: *Magnaporthe oryzae*), which causes rice blast disease, can greatly reduce the rice yield by more than 60% in Thailand (Disthaporn, 1994).

Traditionally, the management of rice blast disease has relied on chemical fungicides and resistant cultivars. However, these approaches have limitations, including the evolution of fungicide-resistant pathogens and the breakdown of host resistance (Sharma et al., 2021). Additionally, the extensive application of agrochemicals, particularly organophosphate pesticides, has been detected in rice and soil across Thailand, raising concerns about environmental contamination and potential human health risks due to their accumulation in rice (Khammanee et al., 2020). Consequently, biological control using beneficial microorganisms has emerged as a promising alternative strategy, offering environmentally sustainable solutions for plant disease management. Antagonistic bacteria, including *Bacillus*, *Actinomycetes* and *Pseudomonas*, have been widely documented for their multifunctional capabilities such as plant growth promotion, elicitation of host-defense responses and degradation of chemical recalcitrance for methidathion, carbophenothion, chlorpyrifos and diazinon and triazophos (Gao et al., 2020; Nasrollahi et al., 2020; Zhou et al., 2021; Chakraborty et al., 2024).

The biodegradation of organophosphate insecticides has emerged as a crucial environmental remediation strategy (Mali et al., 2022). Numerous bacterial species have been recognized for their ability to degrade these substances via specialized enzymatic pathways (Yang et al., 2019). However, while extensive research exists on either the biological control of rice blast disease or pesticide degradation independently, there remains a large knowledge gap in developing integrated microbial solutions that address both challenges simultaneously (Wu et al., 2022).

The current study investigated the capabilities—blast disease suppression, plant growth promotion and organophosphate pesticide degradation—from previously isolated rhizospheric bacteria from rice. Typically, other biocontrol approaches have selected bacterial strains based solely on their antagonistic activity, overlooking other beneficial traits that could enhance overall agricultural sustainability. This limitation has reduced their practical effectiveness in field applications where multiple agricultural challenges exist simultaneously. The current study focused on screening antagonistic bacteria that had multifunctional properties for sustainable agriculture. The antagonistic bacteria were evaluated for their antagonistic mechanisms and plant growth-promoting abilities. Additionally, their organophosphate pesticide degradation efficiency was investigated. The results of the study of these three critical capabilities should provide a foundation for future investigations, where high-potential antagonistic isolates will be combined into consortia to control rice blast disease.

Materials and Methods

Bacterial isolate and fungal pathogen

Bacterial samples, originally isolated from rice rhizosphere, were obtained from the Culture Collection of Microbiome Bacteria, Department of Plant Pathology, Faculty of Agriculture at Kamphaeng Saen, Kasetsart University Kamphaeng Saen Campus, Nakhon Pathom, Thailand. The bacteria were cultivated on nutrient agar. *P. oryzae* NST040101, isolated from a leaf of infected rice, was obtained from the Molecular Plant Pathogenic Fungi Laboratory, Department of Plant Pathology, Faculty of Agriculture at Kamphaeng Saen, Kasetsart University Kamphaeng Saen Campus, Nakhon Pathom, Thailand. The pathogen was inoculated on potato-dextrose agar (PDA; Himedia; India) and cultivated at room temperature (RT; 30–35 °C) under a full spectrum light emitting diode (Grow Light; Lampton; Thailand) set at a 12 hr photoperiod for 14 d. The sporulation followed the method originally developed by Chen et al. (2021), with modifications as described in Khongkhaluong et al. (2024).

In vitro screening of antagonistic bacteria against P. oryzae

In total, 301 bacterial isolates were evaluated for antagonistic activity using the dual culture method. Each bacterial sample was streaked on PDA medium; then, a *P. oryzae* NST040101 agar plug was placed 3 cm away on the opposite side of the plate and incubated at room temperature (RT) for 7 d. The inhibition percentage of mycelial growth was calculated using Equation 1 (Khongkhaluong et al., 2024):

Inhibition ratio (%) = [(A1 - A2) / A1] × 100 (1)

where A1 and A2 are the radii of *P. oryzae* in the control and the treatment plates, respectively.

Any bacterium producing an inhibition greater than 90% was selected for subsequent experiments.

Evaluation of antagonistic mechanisms

Cell wall-degrading enzyme production

The antagonistic isolates were investigated for the production of cell wall-degrading enzymes (protease, chitinase, cellulase). An agar piece from the inhibition zone between the antagonistic bacterium and *P. oryzae* NST040101 from the dual culture experiments was excised using a cork borer (6 mm in diameter); then, it was placed onto skim milk agar, chitin agar and carboxyl-methyl cellulose agar, to evaluate the production of protease, chitinase and cellulase, respectively (Khongkhaluong et al., 2024). The assay plates of protease and chitinase were incubated at RT for 24 hr. The mechanisms of protease and chitinase production were indicated by clear zones around the agar plugs. In addition, cellulase activity was observed based on flooding with Congo red dye, with any clear zones around antagonistic agar plugs indicating the production of cellulase.

Antifungal activity assay

The antifungal activity assay was evaluated using the agar well diffusion method (Magaldi et al., 2004). Briefly, each antagonistic isolate was inoculated in a test tube containing 5 mL of potato dextrose broth (PDB; HiMedia, India) and cultivated at RT under shaking conditions at 200 revolutions per minute (rpm) for 7 d. The supernatant was centrifuged at 12,000 rpm for 5 min (Kubota 3700; Japan) and sterilized using a 0.2 µm Puradisc™ syringe filter (Whatman; Fontenay Sous Bois; France). A *P. oryzae* NST040101 agar plug was placed at the center of the PDA. Then, agar wells using a 5 mm diameter cork borer were cut 2 cm away from the pathogen. The wells were filled with 100 µL of sterilized bacterial culture supernatant. PDB was used as a negative control. All experiments were conducted in triplicate and incubated at RT for 14 d. The production of antibiotics as an antagonistic mechanism was indicated by mycelial retraction between the pathogen and the agar well.

Inhibitory effect of volatile organic compounds

The inhibitory effect of volatile organic compound (VOC) production by selected antagonistic bacteria was determined following the method of Liu et al. (2022), with some modification. A mycelial agar plug of *P. oryzae* NST040101 (0.6 cm in diameter) was placed on PDA. Another PDA plate was spread with 100 µL of selected antagonistic isolate cell suspension (final concentration at 10^6 colony forming units (CFU)/mL) and then placed upside down on top of the plate containing the fungal agar plug. Next, the two inoculated plates were sealed with parafilm to prevent any loss of VOCs and the plates were incubated at RT for 14 d. The control plate used an uninoculated pathogen agar plate. The inhibition of mycelial growth by the VOCs produced by the selected antagonistic isolates was calculated using Equation 2:

$$\text{Fungal inhibition by VOCs (\%)} = \left(\frac{D_c - D_{vocs}}{D_c}\right) \times 100 \quad (2)$$

where D_c and D_{VOCs} are the average diameters of *P. oryzae* NST040101 colonies grown on PDA without or with the VOC treatment, respectively.

Evaluation of plant growth promotion abilities

The plant growth promotion capabilities of selected antagonistic isolates were evaluated, using indole-3-acetic acid (IAA), siderophore production, biofilm formation, phosphate and potassium solubilization, and nitrogen fixation, as proposed by Khongkhaluong et al. (2024), and well as ammonia production. IAA production was examined using nutrient broth (NB) supplemented with or without 0.15% (w/v) tryptophan as a precursor. Siderophore production was assessed using chrome azurol S agar (Schwyn and Neilands, 1987). Biofilm production was determined using the resazurin microtiter plate assay (Martin et al., 2003). Phosphate and potassium solubilization were evaluated using Pikovskaya's agar and Alexandrov agar, respectively (Kumar et al., 2012). The nitrogen fixation was investigated using Okon's nitrogen-free medium (Bashan and Levanony, 1985). Ammonia production was investigated in peptone water, according to the method of Laslo et al. (2012).

Determination of Triazophos™ degradation by selected antagonistic bacteria

The degradation of Triazophos™ (TAP) by selected antagonistic isolates was studied in carbon free mineral medium (CFMM), according to Naloka et al. (2021).

Acclimatization was conducted using CFMM supplemented with 100 mg/L of TAP at RT and 200 rpm for 7 d. The acclimatized antagonistic isolates were used to screen TAP-degrading capability. The inoculum preparation involved inoculating 1 mL of acclimatized antagonistic isolates aged 7 d in 0.1× tryptic soy broth (Himedia; India) and cultivating at RT and 200 rpm for 24 hr. The cell pellets were harvested using centrifugation at 8,000 rpm and 4°C for 15 min and washed twice with 0.85% (w/v) NaCl solution. Then, the cells were resuspended in 0.85% (weight per volume, w/v) NaCl solution and the optical density at 600 nm was adjusted to 1.0 using a spectrophotometer (SP-V1000; ONiLAB; USA), which corresponded to 10^8 CFU/mL. The inoculum was rested overnight before use. TAP degradation was performed in tubes containing CFMM (4.5 mL) supplemented with 100 mg/L of TAP. The inoculum (0.5 mL) was transferred into the tube to obtain a cell count of approximately 10^7 CFU/mL. Abiotic losses were assessed using only media supplemented with TAP that served as a control. The experiments were conducted in triplicate. The investigations were performed at RT and the samples were shaken in a rotary shaker at 200 rpm for 7 d. Sample collections were done on days 0 and 7. The solvent extraction of TAP was conducted three times using a two-fold volume of dichloromethane and dried in a laminar flow and then resuspended with acetonitrile. The efficiency of TAP degradation was evaluated using high performance liquid chromatography (Ultimate™ 3000; Thermo Scientific; USA) according to the method of Ambreen and Yasmin (2020). The percentage degradation of TAP was calculated using Equation 3 (Bai et al., 2020):

$$\text{Degradation of TAP (\%)} = \left[\frac{C_0 - C_t}{C_0}\right] \times 100 \quad (3)$$

where C_0 is the concentration (measured in milligrams per liter) at time 0 (minutes) and C_t is the concentration (in milligrams per liter) remaining at the time of sample collection (minutes).

Molecular identification using 16S rRNA gene analysis of selected antagonistic isolates

Bacterial isolates were grown in NB medium for 24 hr at RT before extracting the genomic DNA using the phenol/chloroform technique, described by Kheirandish and Harighi (2015). 16S rRNA gene amplification was performed with the universal primer pairs of 20f (5′- AGAGTTTGATCATGGCTCAG -3′) and 1500r (5′- GGTTACCTTGTTACGACTT -3′) (Weisburg et al., 1991). The amplification conditions were: initial denaturation at 95°C for 15 min, followed by 29 cycles of denaturation at 95°C for 20 s, annealing at 55°C for 30 s and then extension at 72°C for 1 min, followed by a final extension at 72°C for 10 min. The polymerase chain reaction (PCR) products were purified using a Universal DNA Purification Kit (Tiangen; China), following the manufacturer's instruction. The purified PCR product was sent to ATGC Co. Ltd (Pathum Thani, Thailand) for Sanger sequencing. The sequence data of each isolate were quality filtered and assembled using the Bioedit 7.2 software program (http://www.mbio.ncsu.edu/BioEdit/bioedit.html) and then compared with other closely related bacterial species in the EzTaxon database (http://eztaxon-e.ezbiocloud.net/), according to Kim et al. (2012). Each assembled sequence was aligned with related species in the ClustalW program (Thompson et al., 1994) using Bioedit 7.2. Phylogenetic trees were reconstructed using the MEGA X sequence analysis software (Kumar et al., 2018) based on neighbor-joining approaches (Saitou and Nei, 1987). The neighbor-joining method was performed according to the Kimura two-parameter model (Kimura, 1980). The confidence values of nodes were evaluated using bootstrap analysis based on 1,000 resamplings (Felsenstein, 1985). The sequences were submitted to the National Center for Biotechnology Information (https://www.ncbi.nlm.nih.gov/) and the accession numbers are listed in Table 2.

Statistical analysis

All statistical analyses were performed using the R software (version 4.2.0; R Core Team, 2021) and one-way analysis of variance (ANOVA). Differences between treatments were analyzed using one-way ANOVA followed by Duncan's multiple range test. All values were expressed as mean ± SD values of at least three independent experiments.

Results and Discussion

Screening antagonistic bacteria against P. oryzae

From the screening of the 301 rice rhizosphere bacteria against *P. oryzae*-caused rice blast disease using the dual culture method, 142 isolates (41.17%) had antagonistic activity against *P. oryzae*. Among these, 13 isolates had strong antagonistic activity exceeding 90%, namely SR1TT2.1, SR2TT5.4, SR3KB3.6, SR4KB4.6, SR4KB4.9, SR5KB5.3, EnSR2KB3.4, PCNA5.1,

PCNA5.2, PCNA2.6, PCNA2.4, PCNA5.8 and PCNA3.2 (Fig. 1). Notably, the high proportion of antagonistic isolates suggested that the rice rhizosphere might be a rich source of potential biocontrol agents, which may have been due to the natural selection of beneficial microorganisms in this competitive environment (Ding et al., 2019; Nabila and Kasiamdari, 2021). Therefore, these 13 isolates were selected for the evaluation of the antagonistic mechanism in subsequent experiments.

Antagonistic mechanisms of selected antagonistic isolates

The investigation of the biocontrol mechanisms applied by the selected antagonistic isolates revealed multiple modes of action. The agar plugs from the antagonistic zones on the dual-culture plates were assessed for cellulase, protease and chitinase activity in the production of cell wall-degrading enzymes. This experiment revealed context-specific enzyme production by the antagonistic bacteria, which was induced by the pathogen, suggesting that the enzyme production may be specifically triggered as part of the antagonistic response against *P. oryzae*. Based on these results, all 13 isolates produced cellulase, whereas 12 isolates had protease activity, excluding SR4KB4.9. None of the selected isolates produced chitinase (Table S1). Antagonistic bacteria, such as *Bacillus, Pseudomonas and Streptomyces*, are known to produce cellulases (Khan et al., 2023; Zhang et al., 2023). Both different types of cellulase and protease play important roles in the biocontrol of plant pathogenic microorganisms (Panchal, 2022). Cell wall-degrading enzymes target the fungal cell wall structure, which consists of a protein-glycoprotein outer layer linked with structural polymers such as chitin and glucan (Ajuna et al., 2023). These enzymes are responsible for breaking down these structural components, leading to major alterations in the integrity of the cell wall and potentially causing cytoplasmic leakage when the wall is compromised (Jadhav et al., 2017). Additionally, the production of these enzymes has been reported to be upregulated during bacterial interaction with host plants. This upregulation is involved in the recognition, attachment and movement of beneficial bacteria through plant tissues, leading to the induction of the plant immune system against biotic stresses (Riseh et al., 2024). However, notably, cellulase is also capable of degrading plant cell walls. Therefore, further studies should evaluate the enzyme specificity and mode of action of the 13 identified antagonistic bacteria against plant cell walls to ensure their safety and efficacy as biocontrol agents.

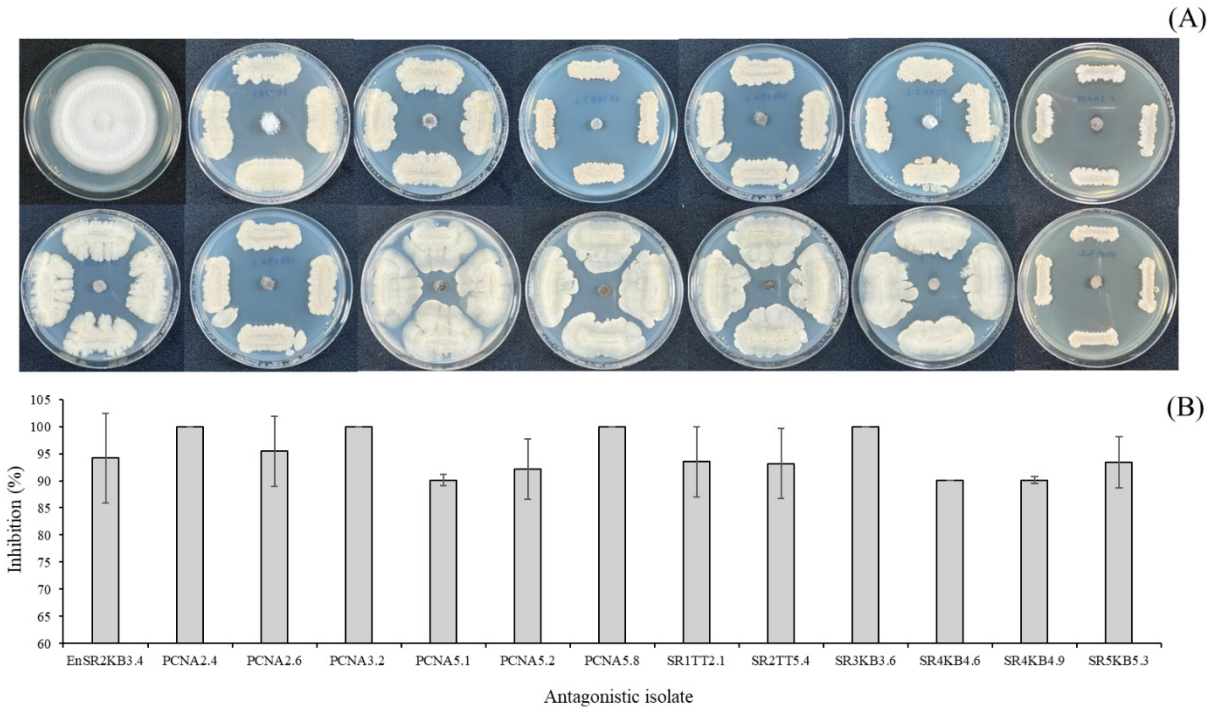

Fig. 1 Antagonistic activity against *Pyricularia oryzae*: (A) Dual culture assay on potato dextrose agar after 7 d at room temperature, with control plate (top left) and antagonistic plates showing isolates SR1TT2.1, SR2TT5.4, SR3KB3.6, SR4KB4.6, PCNA2.6, PCNA2.4 (top, left to right) and SR4KB4.9, SR5KB5.3, ENSR2KB3.4, PCNA5.1, PCNA5.2, PCNA5.8 and PCNA3.2 (bottom, left to right); (B) Growth inhibition percentage of *P. oryzae* by 13 antagonistic isolates. Data represent mean ± SD from three independent experiments. No significant differences ($p > 0.05$) in inhibition percentage were observed among all antagonistic isolates.

Antifungal compound production is recognized as one of the important mechanisms in managing plant pathogens (Ayaz et al., 2023). In the current study, only isolate SR3KB3.6 had inhibitory activity against *P. oryzae* (Fig. S1.). The PDB medium was specifically selected for antifungal compound assessment to maintain consistency with the dual culture assay conditions, allowing direct comparisons of antagonistic mechanisms. However, the biosynthesis of antimicrobial compounds is dependent on various nutritional and environmental factors, such as nitrogen, carbon and microelements, as well as the agitation rate, temperature and pH (Raaijmakers et al., 2002; Evangelista-Martínez et al., 2023). The absence of observable antifungal activity in the other isolates may be attributed to the specific composition of the production medium and cultivation conditions, which are known to trigger secondary metabolite production (Baptista et al., 2022). Notably, the agar well diffusion method relies on the diffusion capability and concentration gradient of the antimicrobial agent in the agar medium. Therefore, it is possible that antifungal compounds were produced but either diffused poorly through the agar or were present at concentrations below the detection threshold of the assay, while still maintaining efficacy in direct interactions with the pathogen (Bubonja-Šonje et al., 2020).

VOCs provide considerable plant protection through a synergistic combination of mechanisms. These compounds effectively inhibit plant pathogen growth while simultaneously triggering induced resistance responses in host plants, creating a comprehensive defense strategy (Poulaki and Tjamos, 2023). Determination of the inhibitory effect of VOCs produced by antagonistic bacteria revealed major antifungal activity across all isolates, with inhibition rates in the range 51.21–85.98%. The highest percentage of VOC-mediated inhibition was with isolate SR2TT5.4 (85.98 ± 1.40%), followed by PCNA3.2 (83.88 ± 0.70%) and SR1TT2.1 (83.18 ± 0.00%), as shown in Fig. S2. Bacteria produce a wide variety of VOCs, including alcohol, esters, ketones and phenolic compounds (He et al., 2020). Bacterial VOC producers in other studies have been reported to cause substantial suppression of plant pathogenic fungi such as *Phytophthora*, *Pythium*, *Fusarium* and *Pyricularia* (Surovy et al., 2023; Ávila-Oviedo et al., 2024). Notably, VOC-producing bacteria do not require direct colonization, as these compounds can diffuse through soil or the phyllosphere and subsequently interact with plants and other microbes at a distance (Ortega et al., 2016).

Plant growth-promoting activities

Indole-3-acetic acid production

Beneficial bacteria can enhance plant growth through both direct interactions with their host plants and antagonistic activities against plant pathogens. IAA is an auxin phytohormone that plays crucial roles in plant growth and development, including cell elongation, division and differentiation (Sosnowski et al., 2023). The biosynthesis of IAA by bacteria occurs through two distinct pathways: tryptophan-dependent and tryptophan-independent (Tang et al., 2023). *In vitro* assay of the 13 antagonistic isolates was evaluated under conditions with or without tryptophan supplementation as a precursor. The production of IAA was detected using Salkowski's reagent, where a color change from pale yellow to pink after 72 hr of incubation at 37°C indicates positive IAA production. All 13 isolates demonstrated IAA production capability, with varying concentrations observed under conditions with and without tryptophan supplementation (1.0% w/v), as shown in Table 1 and Fig. S3. In the presence of tryptophan, isolate SR4KB4.6 had the highest IAA production (74.70 ± 3.66 µg/mL), followed by SR2TT5.4 (16.60 ± 0.43 µg/mL) and SR5KB5.3 (13.21 ± 1.09 µg/mL). Similarly, in the tryptophan-independent conditions, SR4KB4.6 maintained its position as the highest producer (15.62 ± 1.43 µg/mL), followed by SR2TT5.4 (9.23 ± 1.11 µg/mL) and SR1TT2.1 (8.92 ± 0.66 µg/mL). The antagonistic bacteria used both tryptophan-dependent and tryptophan-independent pathways, indicating metabolic flexibility that could provide advantages in diverse rhizosphere environments (Spaepen et al., 2007).

Biofilm and siderophore production

The formation of surface-associated biofilm structures enables bacteria to function more effectively in agricultural field conditions compared to planktonic cells (Backer et al., 2018). The current investigation revealed that all antagonistic isolates produced biofilm formation (Table 1), which is particularly important for phyllosphere colonization (Legein et al., 2020). Biofilm formation on leaf surfaces enhances bacterial survival against phyllosphere stresses, including UV exposure, desiccation and temperature fluctuations (Fessia et al., 2022). Additionally, six isolates produced siderophores (Table 1). The antagonistic isolate PCNA5.2 had the highest siderophore-producing index (3.48 ± 0.15). The production of siderophores and biofilm formation suggested their potential role in plant protection through iron competition with pathogens and the triggering of induced systemic resistance.

Table 1 Plant growth-promoting attributes of 13 antagonistic isolates

Isolate	Plant growth promotion ability attribute							
	Indole-3-acetic acid (µg/mL)		Biofilm	P	K	Siderophores	NH_3 production	N_2 fixation
	With tryptophan	Without tryptophan						
SR1TT-2.1	12.32±0.94^{cd}	8.92±0.66^{b}	0.564±0.15^{e}	+	–	0.00±0.00^{d}	+	+
SR2TT-5.4	16.60±0.43^{b}	9.23±1.11^{b}	0.168±0.07^{g}	+	–	2.00±0.00^{c}	+	+
SR3KB-3.6	7.40±1.08^{efg}	2.79±0.43^{e}	2.529±0.21^{a}	+	–	0.00±0.00^{d}	+	+
SR4KB-4.6	74.70±3.66^{a}	15.62±1.43^{a}	0.022±0.04^{g}	–	–	2.00±0.00^{c}	+	+
SR4KB-4.9	5.75±0.97^{g}	3.21±0.21^{e}	0.094±0.04^{g}	+	–	2.00±0.00^{c}	+	+
SR5KB-5.3	13.21±1.09^{c}	8.60±0.43^{c}	0.506±0.22^{ef}	–	–	3.31±0.56^{a}	+	+
ENSR2KB-3.4	9.52±0.98^{def}	6.38±0.56^{c}	0.399±0.11^{ef}	–	–	0.00±0.00^{d}	+	+
PCNA 2.4	6.48±0.76^{fg}	2.92±0.54^{e}	1.325±0.12^{b}	–	–	2.69±0.17^{b}	+	+
PCNA 2.6	6.67±1.17^{efg}	2.83±0.43^{e}	1.081±0.07^{c}	–	–	2.64±0.18^{b}	+	+
PCNA 3.2	7.97±0.41^{efg}	5.14±0.65^{cd}	0.353±0.05^{f}	–	–	0.00±0.00^{d}	+	+
PCNA 5.1	8.19±1.19^{efg}	5.84±0.36^{cd}	0.047±0.05^{g}	–	–	0.00±0.00^{d}	+	+
PCNA 5.2	9.65±0.48^{de}	8.03±0.39^{b}	0.023±0.04^{g}	–	–	3.48±0.15^{a}	+	+
PCNA 5.8	8.16±1.17^{efg}	4.86±0.20^{d}	0.82±0.13^{d}	–	–	2.89±0.05^{b}	+	+

Values (mean ± SE) in a column with different lowercase superscripts are significantly ($p < 0.05$) different.

P and K solubilization

The solubilization of P and K was assessed using Pikovskaya's medium and Alexandrov medium, respectively. Four isolates—SR1TT2.1, SR2TT5.4, SR3KB3.6 and SR4KB4.9—produced clear halos around spot inoculation on Pikovskaya's medium, indicating their ability to solubilize phosphorus (Table 1 and Fig. S4). The ability to solubilize phosphate is particularly valuable in rice cultivation, as approximately 80% of phosphorus in agricultural soils exists in insoluble forms unavailable to plants (Prathap et al., 2023). The phosphate-solubilizing capability of these isolates could enhance phosphorus availability in the rice rhizosphere, potentially improving plant growth and disease resistance (Cao et al., 2024). However, none of the selected antagonistic isolates had K solubilization activity.

Nitrogen fixation and ammonia production

All 13 of the antagonistic isolates were capable of nitrogen fixation and ammonia production, an important trait for potential agricultural applications (Table 1). The ability of these antagonistic isolates to fix nitrogen and produce ammonia correlated with another report mentioning *Bacillus pumilus* and *B. subtilis* (da Costa Neto et al., 2024), suggesting their potential role in supporting soil fertility and offering a possible alternative to reduce chemical fertilizer inputs in plant cultivation (Jain et al., 2021).

Degradation capability of Triazophos™

TAP is an organophosphate pesticide widely used in rice cultivation (Liao et al., 2002). It has considerable effects on living organisms, leading to both acute and chronic toxicity (Chowdhary et al., 2014). These substances can accumulate in the environment, cereal grains, rice, vegetables and fruits (Fatunsin et al., 2020). Additionally, TAP accumulation has been detected in agricultural soils across Thailand (Kroeksakul et al., 2023).

Recent research has addressed these concerns by increasingly focusing on biological approaches for both pesticide degradation and plant disease control. Current approaches not only emphasize biological control methods but also extensively investigate chemical degradation mechanisms. For example, one study demonstrated the efficiency of *Pseudomonas putida* strain T7 in degrading paclobutrazol, while promoting plant growth through mineral solubilization capabilities for zinc, potassium and phosphorus, as well as producing IAA, HCN and ammonia. In addition, this strain has shown inhibitory effects against *Fusarium oxysporum* (MTCC 284), *Colletotrichum gloeosporioides* (MTCC 2190) and *Pythium aphanidermatum* (Kumar et al., 2021). Similarly, in the current investigation of TAP degradation, the 100 mg/L concentration over 7 d of antagonistic bacterial strain SR4KB4.9 achieved the highest degradation efficiency (48.78 ± 7.61%), which was significantly different to the other bacterial isolates. The subsequent TAP degradation efficiencies were 38.47 ± 4.33%, 33.39 ± 2.56%, 25.89 ± 0.94%, 17.90 ± 7.29% and 12.99 ± 7.75% for SR5KB5.3, SR4KB4.6, SR3KB3.6, SR2TT5.4 and PCNA5.1, respectively (Fig. 2). Furthermore, the cell viability of all isolates decreased from the initial 10^7 CFU/ml to 10^3–10^4 CFU/mL (3–4 log CFU/ml) by day 7 of TAP degradation. This significant reduction suggested TAP toxicity on bacterial populations (Yang et al., 2019). Despite this decline, several isolates (particularly SR4KB4.9) maintained effective degradation activity, indicating that surviving cells adapted to the toxic environment and developed mechanisms to metabolize TAP.

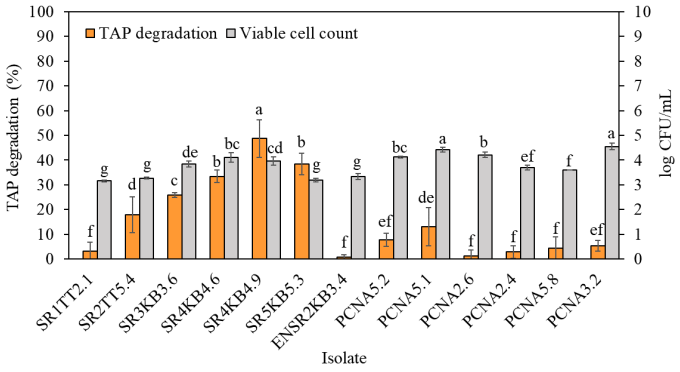

Fig. 2 Degradation efficiency of Triazophos™ (TAP) by 13 antagonistic isolates, cultivated in carbon free mineral medium supplemented with 100 mg/L of TAP at room temperature for 7 d. Different lowercase letters above columns indicate significant differences ($p < 0.05$) among means within each trait. Error bars = ± SD.

Molecular identification of antagonistic isolates

The identification of the 13 antagonistic bacterial isolates was performed using 16S rRNA gene sequence analysis. The nucleotide sequences were compared with those in the EzTaxon database using reference type strains. Based on this analysis, among the 13 isolates, 12 were classified within the genus *Bacillus*, while one isolate belonged to the genus *Brevibacillus* (Fig. 3 and Table 2). The rice rhizosphere harbors numerous diverse bacteria, with the Firmicutes representing a predominant phylum in this microenvironment (Rasul et al., 2024). Within this phylum, members of the genera *Bacillus* and *Brevibacillus* have been widely documented in association with rice roots and are known for their ecological importance (Kakar et al., 2018). These genera constitute a well-known group of antimicrobial producers (Fira et al., 2018; Yang et al., 2023) with considerable plant growth-promoting abilities (Costa-Santos et al., 2021). The predominance of *Bacillus* species among the 13 antagonistic isolates reflected their ecological adaptability and competitiveness in the rice rhizosphere. *As* endospore-forming bacteria they can survive under unfavorable environmental conditions, including the fluctuating conditions typical of paddy fields, with alternating flooding and drying cycles (Radhakrishnan et al., 2017). The current findings aligned with other studies that have highlighted the potential of *both these genera* as biocontrol agents against various plant pathogens (Khan et al., 2023; Kim et al., 2024).

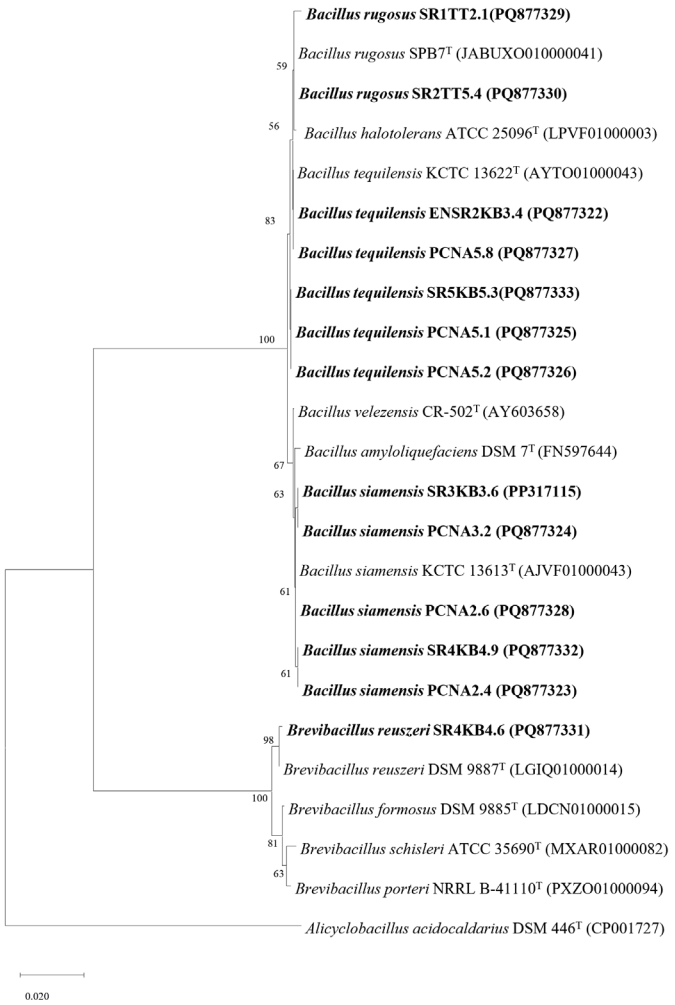

Fig. 3 Phylogenetic relationships based on 16S rRNA gene sequences between 13 antagonistic isolates (in bold) and representative members of the families *Bacillaceae* and *Brevibacillaceae*. The phylogenetic tree was reconstructed using the neighbor-joining method, with *Alicyclobacillus acidocaldarius* DSM446T as the outgroup. Bootstrap values (> 50%) from 1,000 replications are shown at branch nodes. Scale bar represents 0.020 nucleotide substitutions per site.

Table 2 Closest phylogenetic affiliations of bacterial isolates based on 16S rRNA gene sequences, showing GenBank accession numbers, related species, percentage similarity, and the number of nucleotide differences over aligned positions.

Isolate	Accession number	Related closest species	16S rRNA gene similarity (%)	No. nucleotide differences
SR1TT-2.1	PQ877329	"Bacillus rugosus" SPB7^{T} (JABUXO01000041)	99.86	2/1,418
SR2TT-5.4	PQ877330	"Bacillus rugosus" SPB7^{T} (JABUXO01000041)	100.00	0/1,400
SR3KB-3.6	PP317115	*Bacillus siamensis* KCTC 13613^{T} (AJVF01000043)	99.72	4/1,413
SR4KB-4.6	PQ877331	*Brevibacillus reuszeri* DSM 9887^{T} (LGIQ01000014)	100.00	0/1,398
SR4KB-4.9	PQ877332	*Bacillus siamensis* KCTC 13613^{T} (AJVF01000043)	99.86	2/1,419
SR5KB-5.3	PQ877333	*Bacillus tequilensis* KCTC 13622^{T} (AJVF01000043)	99.93	1/1,415
ENSR2KB-3.4	PQ877322	*Bacillus tequilensis* KCTC 13622^{T} (AJVF01000043)	99.93	1/1,425
PCNA 2.4	PQ877323	*Bacillus siamensis* KCTC 13613^{T} (AJVF01000043)	99.79	3/1,421
PCNA 2.6	PQ877328	*Bacillus siamensis* KCTC 13613^{T} (AJVF01000043)	100.00	0/1,406
PCNA 3.2	PQ877324	*Bacillus siamensis* KCTC 13613^{T} (AJVF01000043)	99.86	2/1,416
PCNA 5.1	PQ877325	*Bacillus tequilensis* KCTC 13622^{T} (AYTO01000043)	99.86	2/1,414
PCNA 5.2	PQ877326	*Bacillus tequilensis* KCTC 13622^{T} (AYTO01000043)	99.93	1/1,400
PCNA 5.8	PQ877327	*Bacillus tequilensis* KCTC 13622^{T} (AYTO01000043)	99.93	1/1,421

Conclusion

In total, 13 antagonistic bacteria belonging to the *Bacillus* and *Brevibacillus* genera that had been isolated from the rice rhizosphere had strong inhibition against *P. oryzae* through multiple mechanisms consisting of VOCs, antifungal substances and cell wall-degrading enzymes (cellulase and protease). In addition, these isolates had plant growth-promoting ability and organophosphate pesticide degradation capability, offering potential benefits for plant health and reduced pesticide accumulation in paddy soil. Among them, SR3KB3.6, SR4KB4.6 and SR4KB4.9 had complementary advantageous characteristics that position them as excellent candidates for multifunctional applications. For example, isolate SR3KB3.6 had the most potent antagonistic activity, achieving complete inhibition of *P. oryzae* and notable biofilm formation. Isolate SR4KB4.6 had notable potential for promoting plant growth, achieving the highest levels of IAA, while also having effective pathogen inhibition. Isolate SR4KB4.9 had exceptional performance in degrading TAP, a widely used organophosphate pesticide in rice farming, while also having major antagonistic properties. Future work will focus on developing bacterial consortia and evaluating their combined antagonistic, plant growth-promoting and pesticide degradation efficiencies under greenhouse conditions. The multiple beneficial properties of these isolates suggest their potential as eco-friendly alternatives in sustainable rice cultivation, though field validation studies are still required.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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