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RESEARCH

Diversity of *Bacillaceae* on Rice Grown in Acid Sulfate Soils in Vietnam: Taxonomy, Specialized Metabolites, and Inhibitory Effects on Fungal Pathogens

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ABSTRACT

Acid sulfate soils pose significant challenges to rice production due to their negative impact on root development and nutrient uptake, reducing rice yield and quality. The excessive use of fungicides by farmers to control rice diseases has exacerbated the issue, as it contributes to environmental pollution and poses health risks. Our study aimed to isolate indigenous bacteria from rice grown in acid sulfate soils with potential biocontrol activity against common fungal pathogens in Vietnam. Using *16S rRNA* amplicon sequencing, a total of 91 bacterial strains were identified up to the genus level. *Bacillaceae* were predominant on healthy rice plants, whereas *Pseudomonas* spp. also occurred on plants infested with the rice blast pathogen *Pyricularia oryzae*. Genome sequencing, protein orthology, and multilocus sequence analyses revealed the presence of six taxonomic groups of *Bacillaceae*: *Rossellomorea marisflavi* (basionym: *Bacillus marisflavi*), *Priestia megaterium* (basionym: *Bacillus megaterium*), *Priestia koreensis* (basionym: *Bacillus koreensis*), *Bacillus thuringiensis*, *Bacillus altitudinis*, and *Bacillus siamensis*. Dual-culture assays showed that most strains had antifungal activity against *Pyricularia oryzae*, *Rhizoctonia solani* AG2-1, and *Bipolaris oryzae*, whereas only *B. siamensis* and two *B. thuringiensis* strains were active against *Rhizoctonia solani* AG1-IA. *R. marisflavi* strains were in general inactive. Genome mining identified various biosynthetic gene clusters associated with specialized metabolite production, some of which were linked to potential antimicrobial activity. Production of these metabolites was confirmed by chemical analysis. These results suggest that indigenous *Bacillaceae* strains from acid sulfate soils could be used as biocontrol agents for the sustainable management of rice diseases under acidic conditions.

Keywords: acid sulfate soil, *Bacillus siamensis*, biocontrol agent, fungal pathogen, indigenous bacteria, rice cultivation, secondary metabolite

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Author contributions: V.B.L. performed most of the practical experiments and wrote the first draft of the manuscript. H.M.M.I. conducted bioinformatics analyses, including genome assemblies and protein orthology analysis; deposited sequencing reads and genome assemblies to GenBank; and revised and edited the manuscript. F.E.O. provided guidance for rice plant sample collection, bacterial isolation and purification, setting up in vitro antagonism assays, and revised and edited the manuscript. A.A.-A. and M.O. performed the chemical analyses. B.M. and L.Z. helped with the antifungal assays and data analysis. E.F. facilitated the genome sequencing. B.D.C. provided critical feedback and revised and edited the manuscript. M.H. designed and supervised the study, provided critical feedback, and revised and edited the manuscript. All authors have read and approved the final version of the manuscript.

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Acid sulfate soils (ASSs) pose several limitations to plant growth, including chemical, biological, and physical challenges. Chemically, ASSs are characterized by severe acidity (usually pH < 4), leading to increased solubility and toxicity of aluminum and other ions (Marschner 1991). Other chemical problems may include reduced phosphate availability, salinity, low base status, and nutrient deficiencies (Dent and Pons 1995; Marschner 1991). Additionally, crops suffer physical limitations due to water stress and delayed soil ripening, resulting in poorly structured and inadequately drained soils.

Rice (*Oryza sativa* L.) plays a crucial role in food security for the world's population (Laborte et al. 2017). In Vietnam, rice is the most important crop and is indispensable for the meals of Vietnamese individuals (Nhẫn et al. 2016). To meet the increasing rice demand while agricultural land is increasingly being used for industrialization, intensification of rice cultivation is necessary. In Vietnam, ASSs are situated in the Mekong Delta, accounting for more than 1.6 million hectares, distributed mainly in the Long Xuyen Quadrangle. In this area, a large portion of ASSs has been ameliorated for rice cultivation (Husson et al. 2000a, b; Ren et al. 2004). However, rice monoculture has existed for many decades, resulting in increased disease pressure. Rice diseases such as blast caused by *Pyricularia oryzae* (Asibi et al. 2019), sheath blight caused by *Rhizoctonia solani* (Molla et al. 2020), and brown spot caused by *Bipolaris oryzae* (Barnwal et al. 2013) can cause high rice yield losses. To control rice pathogens, rice growers use an excessive amount of fungicides, which leads to pathogen resistance and environmental pollution (Aktar et al. 2009; Bourguet and Guillemaud 2016; Nguyen 2017).

Most of the previous studies on rice cultivated in ASSs focused on soil amendment measures to improve rice yields, such as the application of phosphate fertilizers, ground magnesium limestone, basalt, and biochar (Abdul Halim et al. 2018; Diallo 2016; Panhwar et al. 2016; Ren et al. 2004; Shamshuddin et al. 2014, 2017). However, research on microorganisms in ASSs is very limited (Högfors-Rönnholm et al. 2018). Several acid-resistant purple non-sulfur bacteria were characterized and successfully applied as bioremediation for soil detoxification in rice fields (Khuong et al. 2017, 2018). Furthermore, *Burkholderia vietnamiensis* TVV75, isolated from the rice rhizosphere in ASSs in the south of Vietnam, could increase several rice yield components (Trân Van et al. 2000). Therefore, our research aimed to answer the following questions: (i) What is the diversity of indigenous bacteria associated with rice grown in ASSs; (ii) which culturable bacteria related to rice are dominant and could have potential as biocontrol agents against fungal pathogens; and (iii) which specialized metabolite gene clusters with antimicrobial potential can be found in these isolates?

MATERIALS AND METHODS

Sampling sites. To assess the culturable bacterial diversity in rice grown in ASSs in Vietnam, we collected rice samples from five different fields (Fig. 1) at the same reproductive stage (52 days after sowing). The fields were located in the Hon Dat district, Kien Giang province, and represent ASSs belonging to the Long Xuyen Quadrangle area (Khuong et al. 2018). The selected fields were larger than two hectares and contained areas of healthy plants and areas of rice blast-infected plants. Five rice plants were randomly sampled from the healthy areas of each field. In the rice blast-diseased areas of each field, two rice plants with fewer rice blast disease symptoms in comparison with the rest of the area were collected. Each plant was separately stored in a disposable bag, transported to the Laboratory of Phytopathology at Ghent University, Belgium, and stored at 4°C until further use for bacterial isolation.

Bacterial isolation. Bacteria were isolated from the rhizosphere (rhizobacteria), leaves, and root endorhizosphere (endophytes). To isolate bacteria from the roots and leaves, the samples were washed under running tap water and briefly dried on sterile filter paper. Then, weighed roots and leaves were cut into 2-cm pieces. To isolate endophytes, root pieces were surface-sterilized in 70% (vol/vol) ethanol for 2 min, followed by 2% (wt/vol) sodium hypochlorite for 3 min, rinsed three times with sterile water, and dried on sterile filter paper. Subsequently, each sample was crushed in a 0.85% sterile saline solution and autoclaved sand. Serial 10-fold dilutions (up to 10^{-6}) of the crushed suspension were prepared, and 0.1 ml of each dilution was spread on lysogeny broth (Luria Bertani [LB]; ingredients from Becton Dickinson, Sparks, MD, U.S.A.) medium or R2A agar (Fischer Scientific, Merelbeke, Belgium) for root endophytes. Plates were incubated in the dark at 28°C for 24 to 48 h until colony development was observed. Then, based on morphology, including color and shape, different colonies were selected and separately cultured on the same medium, individually purified, and stored at −80°C in 20% glycerol until used for bacterial identification.

Molecular identification. To preliminarily identify bacteria isolated from rice grown in ASSs, purified colonies of each strain were tested by using colony PCR with the universal *16S rRNA* primers 16F27 (5′-AGAGTTTGATCMTGGCTCAG-3′) and 16R1492 (5′-TACGGYTACCTTGTTACGACTT-3′) (Frank et al. 2008). Briefly, a single colony was gently mixed into a 150-µl reaction tube containing 25 µl of PCR reaction comprising 15.15 µl of sterile water, 5 µl of 5× Green GoTaq buffer, 0.25 µl of dNTP mixture (10 mM; Promega Benelux, Leiden, the Netherlands), 1 µl of each primer (10 µM), and 0.1 µl of DNA polymerase (5 U μl^{-1}; Promega Benelux). The thermal profile of the PCR program consisted of a 10-min initial denaturation at 94°C followed by 30 cycles at 94°C for 30 s, an annealing stage at 55°C for 1 min, an extension at 72°C for 1 min, and a final extension cycle at 72°C for 10 min. Gel electrophoresis was used to detect the amplicons on 1.5% agarose gels in TAE buffer run at 100 V for 25 min; ethidium bromide was used to visualize amplicons. The amplified products were purified using ExoSAP-IT (Thermo Fisher Scientific) before being sent for DNA sequencing. The purified PCR products were sequenced using Sanger DNA sequencing by LGC Genomics GmbH (Berlin, Germany). BioEdit Sequence Alignment Editor software (version 7.2.5) was used to generate the consensus sequences. These sequences were queried against GenBank using BLASTn to search for the species most closely related to the bacterial strains in this study.

DNA extraction. To obtain bacterial suspensions for DNA extraction, a single colony was grown in 5 ml of LB broth and incubated for 24 h at 28°C at 150 rpm. Cells were collected by centrifuging 1 ml of the bacterial culture at 13,000 × *g* for 2 min. Bacterial genomic DNA was extracted using the Wizard* Genomic DNA purification kit (Promega, Madison, WI, U.S.A.) according to the manufacturer's instructions. The final extract was treated with RNaseA (2 mg ml^{-1}) and incubated at 65°C for 1 h. DNA quality was examined using 1% agarose gel electrophoresis. DNA was quantified using the QuantiFluor ONE dsDNA system and the Quantus fluorometer (Promega). Subsequently, DNA was stored at −20°C prior to further analysis.

Genome sequencing, assembly, and annotation. DNA sequencing was performed using the Illumina Hiseq4000 platform (PE150 reads) at the Oxford Genomics Centre (London, U.K.). The raw reads were quality- and adapter-trimmed using Trimmomatic (version 0.39; Bolger et al. 2014). Quality control on the Illumina paired-end raw and trimmed reads was done using FastQC (version 0.11.8; Babraham Bioinformatics, Cambridge, U.K.). Genomes were assembled using SPAdes (v3.14.1; Bankevich et al. 2012)

with arrays of k-mer sizes (21, 33, 55, 77, 121, and 127). The assembled genomes were quality-checked using blobtools (Laetsch and Blaxter 2017), BWA (version 0.7.17; Li and Durbin 2009), and BLASTn against the NCBI GenBank nucleotide database (updated 11/2020) as described before (Kusch et al. 2020). Completeness of the assembled genomes was assessed using BUSCO (version 4.1.2; Simão et al. 2015) against the database Bacillales-odb9.

Phylogenetic analysis. For the phylogenetic tree based on multilocus sequences, the representative housekeeping genes *rpoB* (3,581 bp), *gyrA* (2,522 bp), *gyrB* (1,916 bp), and *recA* (1,040 bp) were extracted from the whole-genome sequences. Multiple sequence alignment was performed using MUSCLE (Edgar 2004) in the software package MEGA6 (Tamura et al. 2013). A concatenated phylogenetic tree was constructed using the maximum-likelihood method, and confidence analysis was conducted using 1,000 bootstrap replicates.

Phylogenetic orthology inference analysis was performed with the total proteomes of the final assembled genomes. A high-accuracy ortholog inference tree was generated using the STAG algorithm based on the ortholog relationship of the gene trees generated by OrthoFinder (Emms and Kelly 2019).

In vitro antagonism assays against fungal pathogens. Dual culture assays were performed to screen for the inhibitory effect of *Bacillaceae* isolated from healthy plants on fungal pathogens, including *P. oryzae* strain VT5m1 (Thuan et al. 2006), *Rhizoctonia solani* AG1-IA strain STMX04-3 (Hua et al. 2014), *Rhizoctonia solani* AG2-1 strain BK001-1-1 (Pannecoucque et al. 2008), and *Bipolaris oryzae* strain Cm988 (De Vleesschauwer et al. 2010). Bacterial suspensions were prepared as previously described. The tested fungal isolates were grown on potato dextrose agar (PDA) plates at 28°C for 4 to 7 days depending on each fungus. A cork borer (diameter 7.5 mm) was used to make a fungus plug from the edge of growing mycelia, then placed at the center of each PDA plate, or complete medium in the case of *R. marisflavi* because these isolates did not grow on PDA medium. Following this, 5 µl of each bacterial strain grown overnight in LB broth was streaked at both sides at a 2-cm distance from the fungus plug, whereas untreated control treatments received the same amount of LB broth instead of the bacterial suspension. Inoculated plates were incubated in the dark at 28°C. After a 5-day incubation period for *R. solani* AG2-1 (BK001-1-1), *R. solani* AG1-IA (STMX04-3), and *B. oryzae* Cm988, and a 7-day incubation for *P. oryzae* VT5m1,

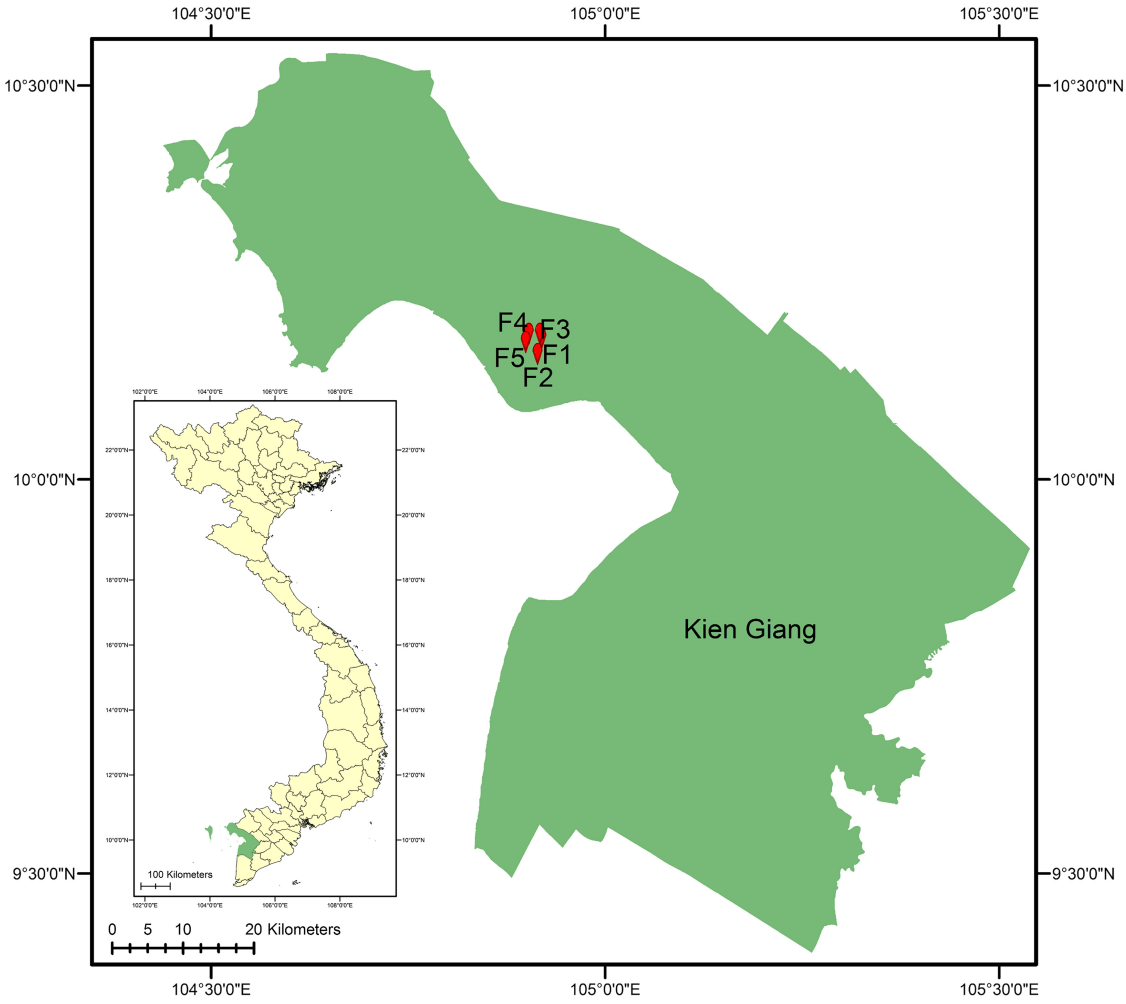


Fig. 1. Sampling sites of rice plants cultivated in acid sulfate soils in Hon Dat district, Kien Giang province, Vietnam. Samples were collected in five different fields at the reproductive stage (52 days after sowing). In all fields, the local rice variety OM 6976 was cultivated. Locations: F1 (Field 1): 10°10′44.0″N, 104°55′10.9″E; F2 (Field 2): 10°10′46.0″N, 104°54′59.0″E; F3 (Field 3): 10°10′46.0″N, 104°54′59.0″E; F4 (Field 4): 10°11′05.0″N, 104°54′12.0″E; F5 (Field 5): 10°10′28.0″N, 104°53′55.0″E.

the area of mycelial growth was determined using ImageJ. Subsequently, the mycelial growth area of each fungus was measured and converted to relative inhibition (%) according to the following equation:

$$\frac{\left(\begin{array}{l}\text{Growth area of untreated control}\\ -\ \text{Growth area of treated control}\end{array}\right)\times 100}{\text{Growth area of untreated control}}$$

Genome mining and ultra-performance liquid chromatography-mass spectrometry (UPLC-MS) analysis of specialized metabolites produced by *Bacillus* spp. For genome mining, antiSMASH version 5.0 (Blin et al. 2019) and the RAST Annotation Server (Aziz et al. 2008) were used to search for biosynthetic gene clusters (BGCs) encoding specialized metabolites. All *Bacillaceae* genomic sequences were queried to identify BGCs encoding non-ribosomal peptide synthetases (NRPSs), polyketides, siderophores, terpenes, and other BGCs. Subsequently, the detected BGCs were compared with other protein sequences in the GenBank database using BLASTp. The percentage identity to known BGCs was set at approximately 90%.

For UPLC-MS analysis, bacteria were grown in casamino acid liquid medium at 30°C for 48 h and then centrifuged at 5,000 rpm. The supernatant was filter-sterilized (0.22 µm pore size filters) and analyzed to detect specialized metabolite production by UPLC-MS as follows. Samples were analyzed in positive mode by UPLC-ESI-qTOF MS (Jet Stream ESI-Q-TOF 6530; Agilent, Santa Clara, CA, U.S.A.) using parameters set up as follows and previously described elsewhere (Andrić et al. 2021): capillary voltage 3.5 kV, drying gas 8 liters/min, drying gas temperature 300°C, and fragmentor voltage of 175 V. Accurate mass spectra were recorded in a mass-to-charge range of 100 to 1,700. A C18 UPLC column (Acquity BEH 2.1 × 50 mm, 1.7 µm; Agilent) was used to resolve the metabolites by applying a gradient of acidified acetonitrile (0.1% formic acid) (solvent B) into water (0.1% formic acid; solvent A) and at a constant flow rate of 0.6 ml/min starting at 10% B and rising to 100% B in 20 min. The identification of the metabolites was confirmed by comparing chromatograms with an in-house database (validated with knockout mutants) that takes accurate mass and retention times into account. Identification of kurstakins, petrobactin, and schizokinen was based on the mass-to-charge value of the corresponding molecular ions according to published data (Dimkić et al. 2017; Manck et al. 2022; Storey et al. 2006).

Statistical analysis. The statistical software GraphPad Prism 9.0. was employed to compare means among treatments and perform one-way analysis of variance followed by Tukey's post-hoc tests. A chi-squared test was used to analyze the frequency distribution of isolates in healthy and diseased fields. The results were considered statistically different at a P value less than 0.05.

RESULTS

Sample collection, bacterial isolation, and molecular identification. Bacterial isolates were obtained from rice grown in ASSs located in five different areas in Vietnam (Fig. 1). Samples were collected from healthy and infected plants in rice blast-free and -infected areas. Bacterial isolation was performed from the roots, endorhizosphere, and leaves of the collected plants. Initially, 92 bacteria isolated from rice grown in ASSs in Vietnam and selected based on morphotype were identified to the genus level based on sequence analysis of the *16S rRNA* gene (Table 1; Supplementary Table S1). *Bacillaceae* were the most abundant (34.8%), followed by *Pseudomonas* (23.9%). In particular, 32 of 92 isolates clustered in the *Bacillaceae* group, and the majority of these isolates (31) were found in the rhizosphere (27) or leaves (4) of healthy plants, with only one *Bacillus* strain detected in the roots of diseased rice plants. In contrast, *Pseudomonas* could be isolated from both healthy and infected rice plants (Table 1), with 8 isolates obtained from the roots of diseased plants and 14 isolates from the roots (4), endorhizosphere (5), and leaves (5) of healthy plants. In addition to *Bacillus* and *Pseudomonas*, various other bacterial genera were identified, including *Acinetobacter*, *Pantoea*, *Klebsiella*, *Paenibacillus*, *Exiguobacterium*, *Cronobacter*, *Chryseobacterium*, *Flavobacterium*, *Rahnella*, *Aquitalea*, *Chromobacterium*, and *Arthrobacter* (Supplementary Table S1).

Phylogenetic analysis of *Bacillaceae* isolates. Because *Bacillaceae* were the most dominant bacteria obtained from the rhizosphere of healthy rice plants, the genomes of the 27 rhizosphere isolates were sequenced to further study their exact phylogenetic position and metabolite spectrum. Phylogenetic trees were constructed based on multilocus sequence analysis using *rpoB*, *gyrA*, *gyrB*, *recA* (Fig. 2A), and orthologous protein sequences (Fig. 2B). Both approaches resulted in very similar phylogenetic trees. The *Bacillaceae* strains clustered into five major groups, represented by *B. altitudinis* (5 isolates), *B. velezensis* (2 isolates), *B. thuringiensis* (8 isolates), *Priestia megaterium* (basionym *Bacillus megaterium*, 7 isolates), and *Rossellomorea marisflavi* (basionym *Bacillus marisflavi*, 5 isolates) (Fig. 2; Supplementary Tables S2 and S3). The two isolates in the *B. velezensis* group, RHF1.1-3 and RHHF4.1-25, are most closely related to *B. siamensis* (Fig. 2B). *B. siamensis* forms, together with the closely related species *B. velezensis* and *B. amyloliquefaciens*, the "operational group *B. amyloliquefaciens*" (Fan et al. 2017). The *Priestia* isolate RHF5.5-4 is most closely related to *P. koreensis*.

Antagonistic activity of *Bacillaceae* strains in dual-culture assays. All identified *Bacillaceae* strains were screened for their in vitro inhibitory capacity against various common rice pathogens. Co-inoculation assays were performed between the fungal pathogens and *Bacillaceae* strains to test their potential as

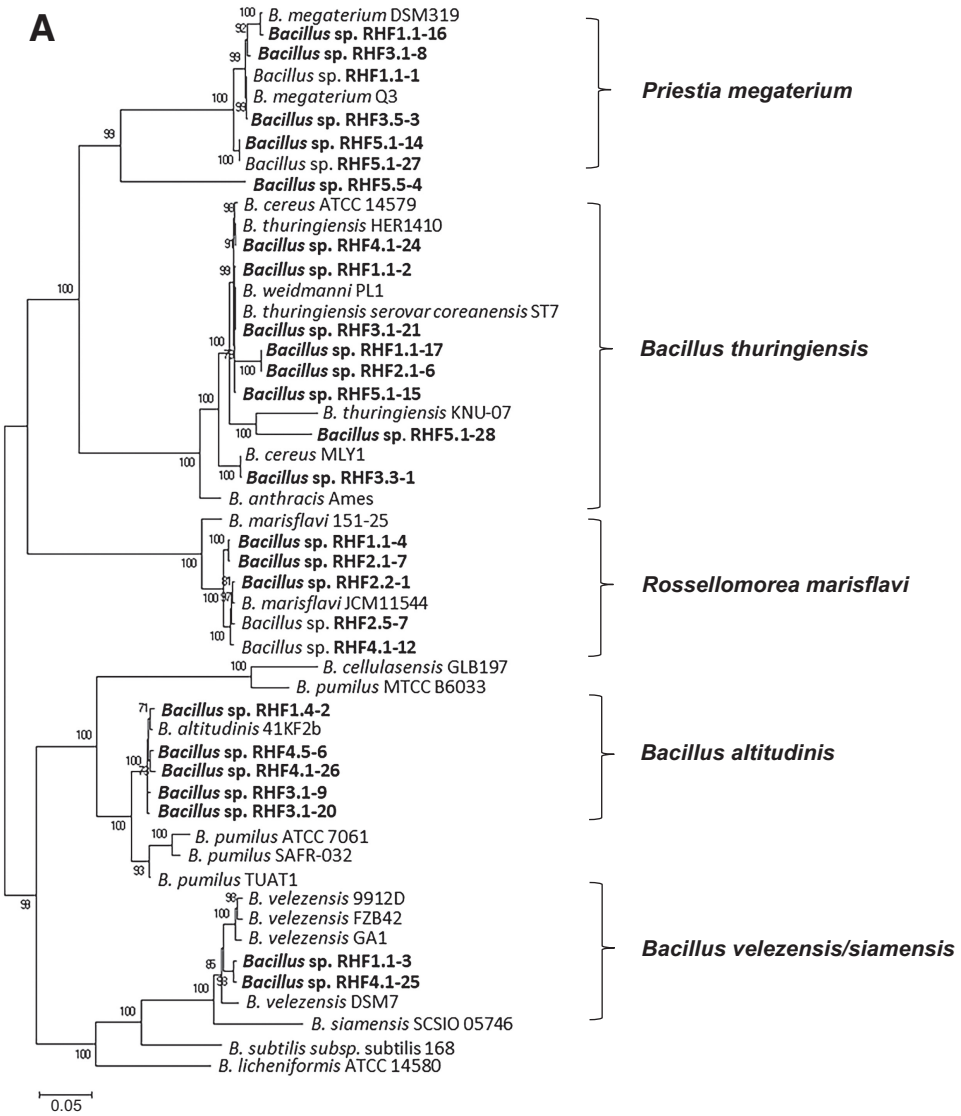
TABLE 1
Distribution of *Bacillaceae*, *Pseudomonas*, and other bacteria isolated from rice grown in acid sulfate soils in Hon Dat district, Kien Giang province, Vietnam[a]

	Bacillaceae	*Pseudomonas*	Other	Row totals
Healthy fields	31 (26.78) [0.66]	14 (18.41) [1.06]	32 (32.00) [0.00]	77
Diseased fields	1 (5.22) [3.41]	8 (3.59) [5.43]	6 (6.20) [0.01]	15
Column totals	32	22	38	92

[a] The chi-square statistic is 10.5676. The P value is 0.005073. The result is significant at $P < 0.05$. Values between brackets report the expected distribution, values between square brackets report the chi-square statistics.

biocontrol agents. Following incubation in environmental conditions favorable for fungal growth, the relative inhibition compared with the control treatments was recorded after 5 days of incubation in the case of *R. solani* AG2-1, *R. solani* AG1-IA, and *B. oryzae* Cm988, and 7 days of incubation for *P. oryzae* VT5m1. *B. thuringiensis*, *B. altitudinis*, and *B. siamensis* isolates showed the most pronounced inhibition of mycelial growth of the tested fungal pathogens, whereas *P. megaterium* showed variable activity, and *R. marisflavi* isolates were in general not active (Fig. 3). The two *B. siamensis* strains (RHF1.1-3 and RHF4.1-25) demonstrated the highest effectiveness in suppressing the mycelial growth of all tested fungal pathogens with strong swarming activity (Fig. 3). Bacteria of the *B. altitudinis* group also effectively suppressed the mycelial growth of *P. oryzae*, *B. oryzae*, and *R. solani* AG2-1 but were not active against *R. solani* AG1-IA. Most *B. thuringiensis* strains effectively suppressed the mycelial growth of *P. oryzae*, *B. oryzae*, and R. solani AG2-1, except for *B. thuringiensis* strain RHF3.3-1, which was ineffective against *R. solani* and less effective against *B. oryzae*. Two of the *B. thuringiensis* strains, RHF5.1-15 and RHF5.1-28, had a slight effect against *R. solani* AG1-IA. *P. megaterium* strains showed a variable effect against the different pathogens, with strains RHF1.1-1 and RHF1.1-16 being the most active, comparable to the activity of *P. koreensis* RHF5.5-4. Strains belonging to the *R. marisflavi* groups had no effect on the mycelium of tested pathogens (Fig. 3).

Specialized metabolites produced by *Bacillaceae* originating from rice plants. BGCs in the genomes of *Bacillaceae* strains isolated from the healthy rice rhizosphere were detected using antiSMASH 5.0 and the RAST Server. A diverse set of BGCs was found in the genomes of *Bacillus* spp. encoding NRPSs, antibiotics, and siderophores (Fig. 4). Specifically, NRPS gene clusters encoding enzymes for the synthesis of cyclic lipopeptides (CLiPs) were identified in *B. thuringiensis* (kurstakin) (Fig. 5A), *B. altitudinis* (pumilacidin) (Fig. 5B), and *B. siamensis* (surfactin, fengycin,



(*Continued*)

Fig. 2. A, Concatenated unrooted phylogenetic tree of *Bacillaceae* isolates using four representative housekeeping genes *rpoB* (3,581 bp), *gyrA* (2,522 bp), *gyrB* (1,916 bp), and *recA* (1,040 bp). The tree was constructed with MEGA6 using the maximum-likelihood method with 1,000 bootstrap replicates. Only bootstrap values higher than 70% are indicated. Strains from this study are indicated in bold. **B,** Phylogenetic tree of *Bacillaceae* strains constructed based on the total proteome of the strains with the STAG algorithm based on the ortholog relationship of the gene trees generated by OrthoFinder (Emms and Kelly 2019). Only bootstrap values above 70% are indicated. Strains in bold were obtained in this study.

and iturin-type CLiPs; Fig. 5C and D) but were not found in the *P. megaterium* and *R. marisflavi* groups.

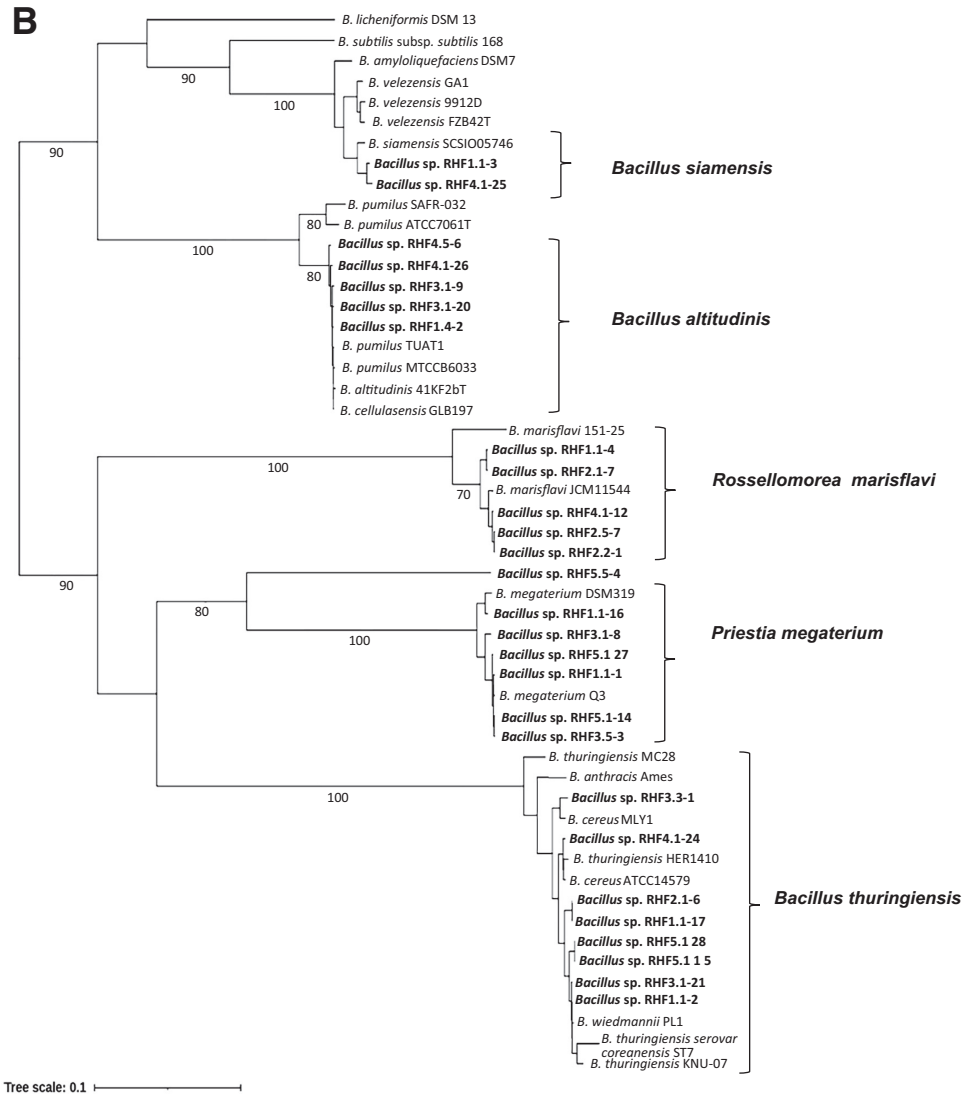
The kurstakin gene cluster was similar in all *B. thuringiensis* genomes (Fig. 5A). The three core genes *krs-ABC* are involved in the biosynthesis of kurstakin, followed by the genes encoding 4′-phosphopantetheinyl transferases (*sfp*) and thioesterase (*krsD*). The gene *krsE*, which is located upstream of the biosynthetic genes, encodes a protein responsible for kurstakin secretion (Béchet et al. 2012). Chemical analysis revealed that the five *B. thuringiensis* strains analyzed were able to produce two to five kurstakin homologues that differ in the length of the fatty acid (Table 2; Supplementary Fig. S1).

The pumilacidin gene cluster (Fig. 5B) in the *B. altitudinis* genome is similar to the surfactin gene cluster but is composed of six core genes (*srfAA*, *srfAB*, *srfAC*, *orfX*, *orfY*, and *srfAD*) involved in pumilacidin biosynthesis. The two additional uncharacterized *orf-XY* genes are not present in surfactin gene clusters (Saggese et al. 2018). Moreover, unlike the other *B. altitudinis* strains, the *B. altitudinis* strains RHF3.1-20 and RHF4.5-6 had two additional open reading frames downstream of the BGC (Fig. 5B). The *ycxC* gene encodes a carrier protein, whereas the *ycxD* gene encodes a transcriptional regulator. Pumilacidin production was confirmed by chemical analysis in the two *B. altitudinis* isolates tested, revealing the presence of four homologues differing in the length of the fatty acid chain (Table 2; Supplementary Fig. S2).

The surfactin operon in the genomes of the *B. siamensis* isolates comprises three main polycistronic genes (*srfAA-sfrAB-sfrAC*) involved in the biosynthesis of the surfactin compound (Fig. 5C) (Geissler et al. 2019). The core biosynthetic genes of the iturin operons, organized in four genes (*itu-ABCD*), were found in both genomes of the *B. siamensis* strains (Fig. 5D). The fengycin gene cluster, which consists of five genes (*fen-ABCDE*), is responsible for fengycin biosynthesis (Fig. 6E) (Geissler et al. 2019). Chemical analysis confirmed that the *B. siamensis* strains were able to produce four different homologues of surfactin and three to four homologues of fengycin, but the two strains differed in their iturin spectrum. *B. siamensis* strain RHF1.1-3 produced five different homologues of bacillomycin D with a C13 to C17 fatty acid tail, whereas *B. siamensis* RHF4.1-25 produced four variants of iturin A with a C13 to C16 fatty acid tail (Table 2; Supplementary Fig. S3).

BGCs for the polyketides difficidin and bacillaene were uniquely found in the genomes of the *B. siamensis* strains (Fig. 5). The difficidin (*dfn*; formerly *dif*) and bacillaene (*bae*) gene clusters, encoding the genes *dfn-AYXBCDEFGHIJKLM* (analogous to

Fig. 2. (*Continued from previous page*)



dif-ABCDEFGHIJKLMNO) (Chen et al. 2006; Fan et al. 2017) (Fig. 6A) and *bae-BCDE*, *acpK*, and *bae-GHIMNRS* (Fig. 6B), respectively, are responsible for the biosynthesis of the polyketides difficidin and bacilleane (Aleti et al. 2015). Production of difficidin, bacillaene, and dihydroxybacillaene was confirmed by chemical analysis in both *B. siamensis* RHF4.1-25 and RHF1.1-3 (Table 2; Supplementary Fig. S4).

Siderophore BGCs encoding bacillibactin, petrobactin, and schizokinen were detected in the genomes of the various *Bacillaceae*. Bacillibactin gene clusters consist of five *dhb* genes (*dhbACEBF*) and encode the enzymes involved in bacillibactin synthesis (May et al. 2001). The gene cluster was detected in the genomes of all *B. thuringiensis* isolates, the two *B. siamensis* strains, and *B. altitudinis* RHF4.1-26 (Fig. 7A). Remarkably, *B. thuringiensis* RHF3.3-1 had an insertion of five nucleotides in the first part of the *dhbA* gene, leading to a premature stop codon and a subsequent deletion of 44 nucleotides in the second part (Fig. 7A). The petrobactin gene cluster, found only in the genomes of *B. thuringiensis* group, contains an operon of six genes, *asb-ABCDEF*, responsible for the petrobactin biosynthesis (Fig. 7B) (Nusca et al. 2012). The production of bacillibactin and petrobactin was confirmed by chemical analysis for most of the *B. thuringiensis* isolates, except for

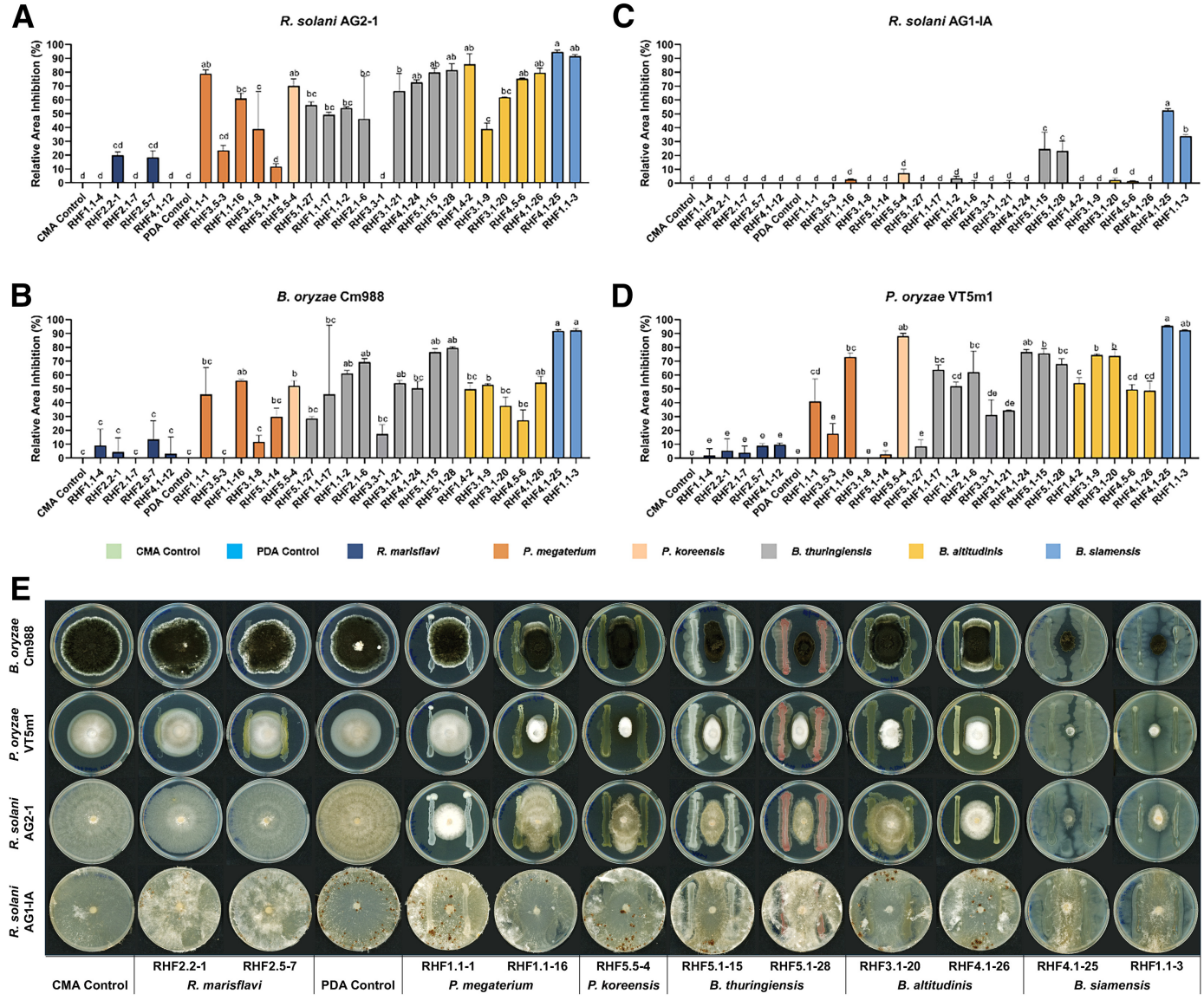


Fig. 3. Inhibitory effect of *Bacillaceae* strains isolated from the rice rhizosphere in acid sulfate soils on the mycelial growth of fungal pathogens, including **A,** *Rhizoctonia solani* AG2-1; **B,** *Bipolaris oryzae* Cm988; **C,** *R. solani* AG1-IA; and **D,** *Pyricularia oryzae* VT5m1. A dual-culture assay was performed between the pathogens and bacterial strains. Potato dextrose agar (PDA) plates were used for *Bacillus altitudinis*, *Priestia megaterium*, *P. koreensis*, *B. thuringiensis*, and *B. siamensis* groups, and complete medium agar (CMA) plates were used for *R. marisflavi* strains. Data were recorded after 5 days of co-incubation in the case of *R. solani* AG2-1, *R. solani* AG1-IA, and *B. oryzae* Cm988 and 7 days of co-incubation for *P. oryzae* VT5m1. Results are expressed as relative area of inhibition compared with the untreated control. Error bars show the standard deviation ($n = 3$). One-way analysis of variance followed by Tukey's post-hoc tests were used, and different letters among these treatments indicate statistically significant differences ($P < 0.05$). **E,** Representative pictures of dual-culture plate assay showing the inhibitory capacity of *Bacillaceae* strains on the mycelial growth of the fungi *Pyricularia oryzae* VT5m1, *Rhizoctonia solani* AG2-1, *R. solani* AG1-IA, and *B. oryzae* Cm988. The pictures were taken after 5 days of co-incubation in the case of *R. solani* AG2-1, *R. solani* AG1-IA, and *B. oryzae* Cm988 and 7 days of co-incubation in case of *P. oryzae* VT5m1. The experiment was carried out with three repetitions and repeated in time with very similar results.

B. thuringiensis RHF3.3-1, which was unable to produce bacillibactin, and RHF1.1-17, unable to produce pseudobactin (Table 2; Supplementary Fig. S5). We found that the *B. thuringiensis* strain RHF1.1-17 lacks the *fpuAf-fpuB* gene cluster that encodes the petrobactin receptor protein FpuA and the petrobactin permease FpuB (Dixon et al. 2012), whereas these genes are present in all other *B. thuringiensis* strains. The inability to bind and import the iron-petrobactin complex may explain the absence of petrobactin production in strain RHF1.1-17. Six genes (*rhb-ABCDEF*) make up an operon encoding a siderophore, most likely schizokinen (Mullis et al. 1971), an intermediary in the rhizobactin 1021 synthesis (Lynch et al. 2001). The *rhb-ABCDEF* operon was found in the genomes of *P. megaterium*, *P. koreensis*, and *B. altitudinis* strains and in three strains belonging to the *R. marisflavi* group (Fig. 7C). An additional gene (the corresponding protein is annotated as an MSF transporter in GenBank) is present in front of *rhbF* in the genomes of the *P. megaterium* and *P. koreensis* strains but absent from the *rhb* operon in the *B. altitudinis* and *R. marisflavi* genomes. Schizokinen production was confirmed by chemical analysis for all tested *Bacillaceae* isolates possessing the schizokinen BGC (Table 2; Supplementary Fig. S6).

In addition to the NRPSs, polyketides, and siderophores, two other well-known antibiotic gene clusters, encoding bacilysin and zwittermicin, were recognized in the genomes of all *B. altitudinis* strains and the *B. thuringiensis* strains RHF5.1-15 and RHF5.1-28, respectively (Fig. 8). The genes *zmaA* through *zmaV* are responsible for the production of zwittermicin (Kevany et al. 2009) (Fig. 8A). The production of zwittermicin was confirmed by chemical analysis of the *B. thuringiensis* RHF5.1-15 strain (Table 2; Supplementary Fig. S7). The *bac* operon encoding bacilysin comprises *bac-ABCDE* genes, which are involved in bacilysin biosynthesis (Özcengiz and Öğülür 2015) (Fig. 8B). The production of bacilysin was confirmed by chemical analysis of both *B. altitudinis* strains tested (Table 2; Supplementary Fig. S8). Most *Bacillaceae* genomes contained additional BGCs encoding terpenes and bacteriocins (data not shown).

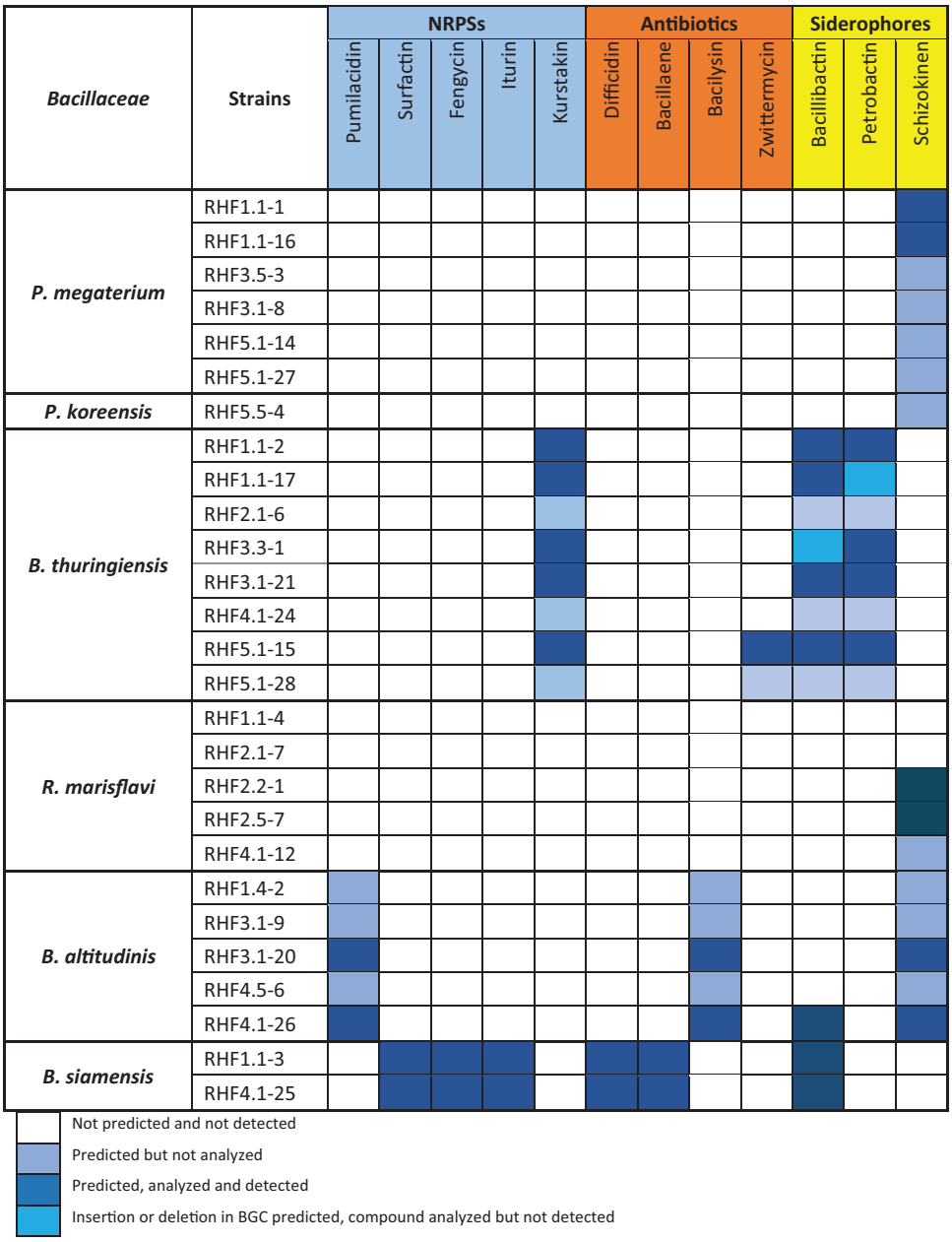


Fig. 4. Diversity of biosynthetic gene clusters across the *Bacillaceae* strains isolated from the rice rhizosphere in acid sulfate soils in Vietnam. The rows indicate the bacterial species (left column) and the strain designation (second column); columns display the biosynthetic gene clusters for non-ribosomal peptide synthetases (NRPSs) (blue), antibiotics (orange), and siderophores (yellow). The shade of blue indicates presence of the gene cluster and chemical detection of the metabolite in the respective strain, as indicated in the legend.

DISCUSSION

In this study, we explored indigenous bacteria associated with rice grown in ASSs in Vietnam to obtain information about microorganisms that can thrive in this environment and to investigate their biocontrol potential as direct antagonists against several fungal rice pathogens. Based on the amplification of the *16S rRNA* gene, we identified various bacteria isolated from rice plants in ASSs. ASSs are characterized by low pH, phosphate deficiency, and high concentrations of aluminum, iron, and other potentially toxic elements (Michael 2013). Nevertheless, our findings indicate that various bacterial genera could colonize rice cultivated in ASSs, such as *Bacillus*, *Priestia*, *Rossellomorea*, *Pseudomonas*, *Acinetobacter*, *Pantoea*, *Klebsiella*, *Paenibacillus*, *Exiguobacterium*, *Cronobacter*, *Chryseobacterium*, *Flavobacterium*, *Rahnella*, *Aquitalea*, *Chromobacterium*, and *Arthrobacter.* Previously, *Bacillus* spp., *Paenibacillus* spp., *Klebsiella* spp., and *Burkholderia vietnamiensis* TVV75 were found in the rice rhizosphere of ASSs in Vietnam (Trân Van et al. 2000; Xuan et al. 2016). Our findings indicate that the most dominant rhizobacteria belong to the *Bacillaceae* family, which agrees with previous studies on cultivated rice in other soils from different geographic locations (Joshi et al. 2011; Susilowati et al. 2015; Zhou et al. 2020). *Bacillaceae* can grow in adverse conditions, such as acidic grassland soils (Felske et al. 1998), and are highly resistant to extreme environmental conditions due to their capacity to form endospores. Moreover, swarming motility and biofilm formation result in an efficient root colonization (Gopal et al. 2015). It can thus be assumed that the *Bacillaceae* obtained are competent in the rice rhizosphere, but this requires further investigation.

Because *Bacillaceae* were predominantly found in the rice rhizosphere, the genomes of these dominant bacteria were sequenced to obtain a more comprehensive understanding of their precise taxonomic position, specialized metabolite spectrum, and antagonistic activity. Phylogenetic analysis based on multilocus sequence analysis using the housekeeping genes *rpoB*, *gyrA*, *gyrB*, and *recA*, and orthologous protein sequences clustered our *Bacillaceae* iso-

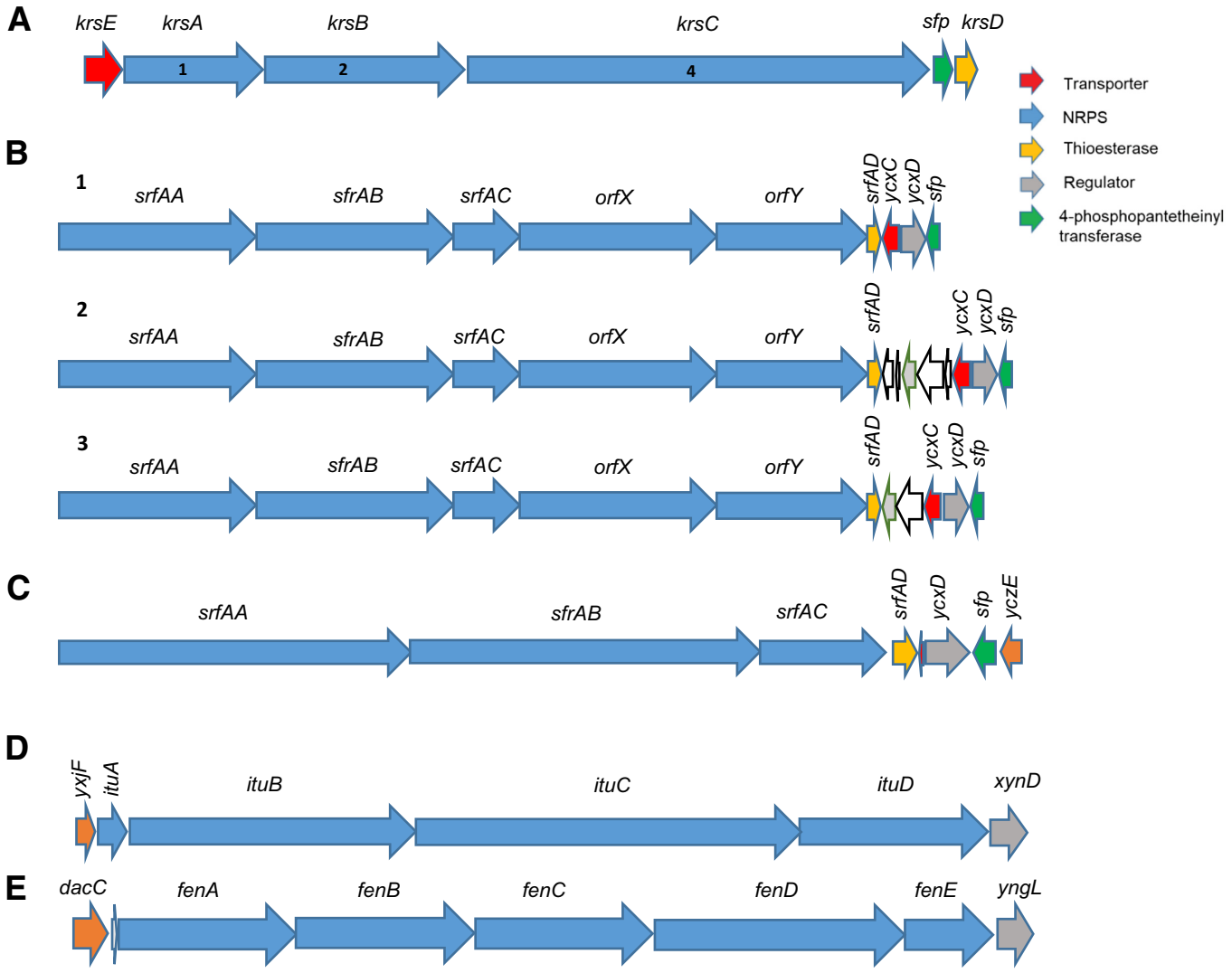


Fig. 5. Non-ribosomal peptide synthetases (NRPS) gene clusters, including **A,** kurstakin gene cluster encoded by *krs* genes in the genome of *Bacillus thuringiensis* strains; **B,** variants of the pumilacidin gene cluster encoded by *srfA* genes with two additional *orfX* and *orfY* genes in the genomes of *B. altitudinis* strains (**1:** the pumilacidin gene cluster found in the genomes of *B. altitudinis* strains RF4.1-26, RHF3.1-9, and RHF1.4-2; **2** and **3:** the pumilacidin gene cluster with two insertion elements in downstream region found in the genomes of *B. altitudinis* strains RHF3.1-20 and RHF4.5-6, respectively); **C,** the *srf* operon encoding the surfactin gene cluster in the genomes of *B. siamensis* strains; **D and E,** the iturin and fengycin gene clusters encoded by *itu* and *fen* genes, respectively, in the genomes of *B. siamensis* strains.

lates into six different species (i.e., *P. megaterium*, *P. koreensis*, *B. thuringiensis*, *R. marisflavi*, *B. altitudinis*, and *B. siamensis*). Within the *Bacillus* genus, *B. altitudinis* and *B. siamensis* belong to the Subtilis clade, whereas *B. thuringiensis* is a member of the Cereus clade. *Priestia* and *Rossellomorea* are recently established new genera that group former *Bacillus* bacteria belonging to two distinct clades, the Megaterium clade and Aquimaris clade, respectively (Gupta et al. 2020). A high diversity of *Bacillaceae* was previously found in the rice rhizosphere grown in coastal soils in Indonesia, but they were only tentatively identified based on sequencing of the *16S rRNA* gene (Susilowati et al. 2015).

We investigated the inhibitory effect of these rhizobacteria on phytopathogenic fungi and searched for BGCs via genome mining to shed light on their biocontrol potential. First, we revealed that most of the *Bacillaceae* strains have biocontrol potential, with the *B. siamensis* strains being the most effective. Second, we also showed that the majority of *Bacillaceae* strains clustering in the same phylogenetic group had a similar inhibitory capacity on the mycelial growth of the tested pathogens. Previous studies have indicated the potential of *Bacillus* species to suppress different phytopathogens (Fan et al. 2018; Sha et al. 2020; Shafi et al. 2017). Moreover, these *Bacillus* groups produce crucial special-

TABLE 2
Diversity of specialized metabolites produced by *Bacillaceae* isolated from acid sulfate soils in Vietnam (see Fig. 4 for producing strains and Supplementary Figs. S1 to S8 for details)

	Observed mass peaks observed			
Metabolite	$(M + H)^+$	$(M + Na)^+$	Assignment	Reference
Kurstakin		872.4	C9-kurstakin	Dimkić et al. 2017
	864.5	886.4	C10-kurstakin	
	878.5	900.5	C11-kurstakin	
	892.5	914.5	C12-kurstakin	
	906.5	928.5	C13-kurstakin	
Pumilacidin	1036.7	1058.7	C15[Val7], C14[Leu/Ile7]	Saggese et al. 2018
	1050.7	1072.7	C16[Val7], C15[Leu/Ile7]	
	1064.7	1086.7	C17[Val7], C16[Leu/Ile7]	
	1078.7			
Surfactin	994.7	1016.6	C12-surfactin	Arguelles-Arias et al. 2009
	1008.7	1030.6	C13-surfactin	
	1022.7	1044.7	C14-surfactin	
	1036.7	1058.7	C15-surfactin	
Fengycin	1449.8	1471.8	Ala-6-C15 fengycin	Arguelles-Arias et al. 2009
	1463.8	1485.8	Ala-6-C16 fengycin	
	1477.8	1499.8	Ala-6-C17 fengycin	
	1491.8		Ala-6-C18 fengycin	Dimkić et al. 2017
Iturin	1029.5	1051.5	C13-iturin A	Arguelles-Arias et al. 2009
	1043.6	1065.5	C14-iturin A	
	1057.6	1079.6	C15-iturin A	
	1071.6	1093.6	C16-iturin A	
Bacillomycin	1017.5	1039.5	C13-bacillomycin D	Koumoutsi et al. 2004
	1031.5	1053.5	C14-bacillomycin D	
	1045.6	1067.5	C15-bacillomycin D	
	1059.6	1081.6	C16-bacillomycin D	
	1073.6	1095.6	C17-bacillomycin D	
Difficidin	561.3	583.3	Oxydifficidin	Wilson et al. 1987
Bacillaene	581.4	603.3	Bacillaene	Chen et al. 2006
	583.4	605.4	Dihydrobacillaene	
Bacillibactin	883.3	905.2	Bacillibactin	Chen et al. 2006
Petrobactin	719.4	741.3	Petrobactin	Manck et al. 2022
Schizokinen	421.2	443.2	Schizokinen	Storey et al. 2006
	403.2	425.2	Schizokinen A	
Zwittermicin	397.2	419.2	Zwittermicin A	He et al. 1994
Bacilysin	271.1		Bacilysin	Walker and Abraham 1970

ized metabolites that play a role in biocontrol via direct antagonism and induced systemic resistance (Cochrane and Vederas 2016; Fira et al. 2018; Geissler et al. 2019; Sansinenea and Ortiz 2011; Zhao et al. 2017).

Isolates belonging to the *B. thuringiensis* group inhibited the mycelial growth of three of the fungal pathogens tested. BGCs encoding the CLiP kurstakin and two siderophores, bacillibactin and petrobactin, are present in all genomes of this *Bacillus* group. Kurstakin, a lipoheptapeptide comprising seven amino acids linked to a C9 to C13 fatty acid chain (Table 2), is a biomarker to identify *B. thuringiensis* species (Hathout et al. 2000). Purified kurstakin showed inhibitory activity against the fungus *Stachybotrys char-*

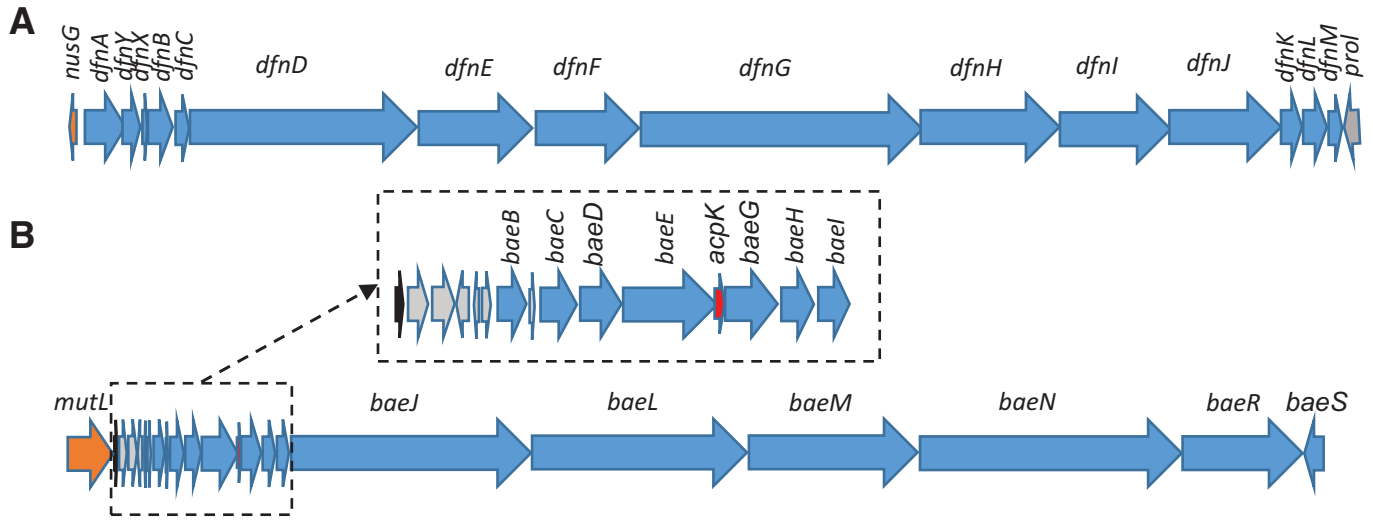


Fig. 6. Polyketide gene clusters comprising **A,** *dfn* operon encoding the difficidin gene cluster and **B,** the bacillaene gene cluster encoded by *bae* genes in the genomes of the *Bacillus siamensis* strains.

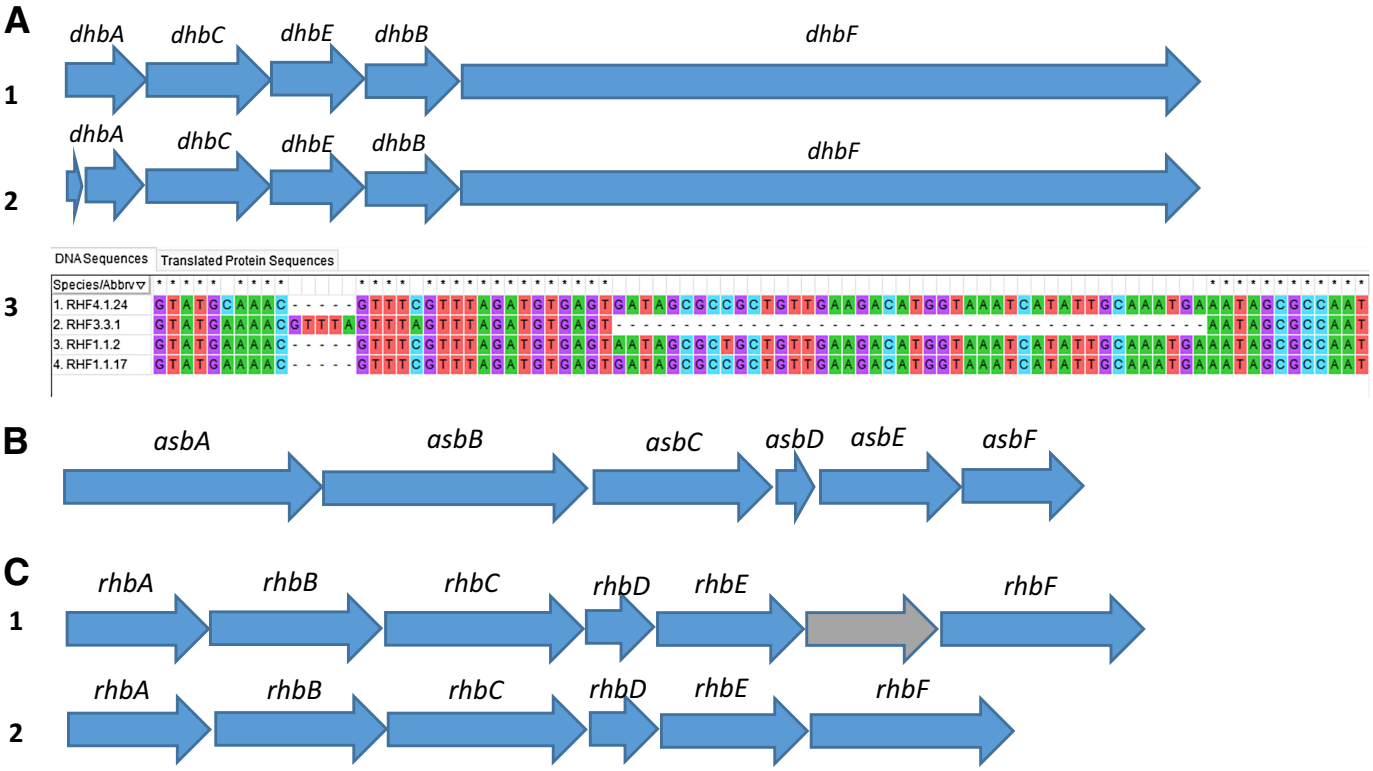


Fig. 7. Siderophore gene clusters consisting of **A,** the *dhb* operon encoding the bacillibactin gene cluster present in the genomes of all *Bacillus thuringiensis* isolates (except for *B. thuringiensis* RHF3.3-1), the two *B. siamensis* strains, and *B. altitudinis* RHF4.1-26 **(1),** and unusual truncations in the genes *dhbA* and *dhbF* presenting in the *dhb* operon in the genome of *B. thuringiensis* RHF3.3-1 **(2),** and part of the nucleotide sequence of the *dhbA* gen in various *B. thuringiensis* strains showing the insertion of 5 nucleotides leading to a stop codon (TAG) and the deletion of 44 nucleotides in the *dhbA* gene of *B. thuringiensis* RHF3.3-1 **(3)**; **B,** petrobactin gene cluster encoded by *asb* genes in the genomes of the *B. thuringiensis* group; **C,** *rhb* operon encoding the schizokinen gene cluster in the genomes of the *Priestia* strains with an additional gene in front of *rhbF* gene **(1),** not present in the *rhb* operon of the genomes of the *B. altitudinis* and *R. marisflavi* group **(2)**.

tarum (Hathout et al. 2000), whereas a kurstakin mutant of *B. cereus* AR156 lost its in vitro antagonistic activity against *P. oryzae*, *R. solani*, *Fusarium graminearum*, and *Fusarium oxysporum* f. sp. *cubense* and was impaired in biocontrol of the rice sheath blight pathogen *R. solani* on rice plants (Yu et al. 2023). Unlike the other *B. thuringiensis* strains, strain RHF3.3-1 was inactive against the fungus *Rhizoctonia solani* AG2-1 and less active against *B. oryzae*. *B. thuringiensis* strain RHF3.3-1 only produced a C11 and C13 homologue of kurstakin, whereas the other *B. thuringiensis* strains analyzed produced four to five different kurstakin homologues (Supplementary Fig. S1). Whether this lack of activity against *R. solani* is due to a different kurstakin spectrum remains to be investigated.

Bacillibactin and petrobactin are catecholate siderophores produced by several *Bacillus* spp., including *B. thuringiensis* (Miljaković et al. 2020). It is not entirely clear whether these compounds have antifungal activity. In pepper, the bacillibactin-producing *B. subtilis* CAS15 strain can control the fungus *Fusarium oxysporum* f. sp. *capsici* via direct and indirect antagonism. Suppression was significantly reduced after treatment with iron (Yu et al. 2011). Genome mining revealed an abnormal structure of the bacillibactin BGC in *B. thuringiensis* 3.3-1, with an insertion and a deletion in the *dhbA* gene (Fig. 6A). As a result, this strain did not produce bacillibactin (Supplementary Fig. S5).

Interestingly, the two strains, *B. thuringiensis* RHF5.1-15 and RHF5.1-28, that possess the zwittermicin gene cluster were persistently more effective in inhibiting the mycelial growth of the pathogens tested than the other *B. thuringiensis* strains in our analysis and also had a slight effect against *R. solani* AG1-IA. Zwittermicin A is a linear amino acid chain containing multiple hydroxyl groups (Rogers and Molinski 2007). In vitro, zwittermicin A shows antagonistic activity toward various phytopathogens, such as *Phytophthora megasperma*, causing damping-off of alfalfa seedlings (Silo-Suh et al. 1994) and fungal pathogens, including *Fusarium* spp., *Rhizoctonia solani* AG1/AG4, and *Alternaria* spp. (Silo-Suh et al. 1998). Therefore, zwittermicin may play a crucial role in the antifungal potential of these isolates.

Strains belonging to the *B. altitudinis* group had antagonistic activity against three of the fungal pathogens tested in vitro. These isolates encode BGCs for the CLiP pumilacidin and the antibiotic bacilysin. Pumilacidin belongs to the surfactin family and differs in amino acid composition at positions 4 and 7 compared with surfactin (Naruse et al. 1990). A pumilacidin-producing *B. pumilus* shows strong direct antagonism against the fungi *R. solani* and *Sclerotium rolfsii* and the oomycete *Pythium aphanidermatum* in vitro, but whether that activity is due to pumilacidin has not been shown (de Melo et al. 2009). Bacilysin is a non-ribosomally synthesized dipeptide consisting of L-alanine and the unusual glutamine analog L-anticapsin (Kenig et al. 1976). The compound has antibacterial, antifungal, and anti-oomycete activity (Caulier et al. 2019). Bacilysin exhibits antagonistic action against two main bacterial rice diseases caused by *Xanthomonas oryzae* pv. *oryzae* and *Xanthomonas oryzae* pv. *oryzicola* (Wu et al. 2015).

The two *B. siamensis* isolates were the most effective in inhibiting the fungal pathogens (Fig. 3B; Table 2). These strains contain BCGs encoding NRPSs (surfactin, iturin A, bacillomycin D, and fengycin), polyketides (difficidin and bacilleane), and the siderophore bacillibactin. The NRPSs surfactin, iturin A, and bacillomycin D are CLiPs composed of seven amino acids linked to a fatty acid tail with 12 to 16 carbons (Bonmatin et al. 1995), 13 to 17 carbons (Yang et al. 2015), and 13 to 19 carbons, respectively. However, a different point in the cyclization of iturin compared with surfactin is the amide bond between the first and last amino acids (Geissler et al. 2019). Fengycin is also a cyclic lipopeptide with 10 amino acids attached to a fatty acid chain between C_{12} and C_{19} (Yang et al. 2015). Fengycin and CLiPs from the iturin family, such as iturin A and bacillomycin D, are active against a wide spectrum of filamentous fungi (Caulier et al. 2019; Fira et al. 2018). We previously showed that *B. siamensis* RHF4.1-25 is very effective in controlling rice blast by direct antagonism and induced systemic resistance and that both iturin and fengycin can suppress the mycelial growth of the fungus *P. oryzae* VT5m1 (Lam et al. 2021). The polyketide difficidin has no reported antifungal activity but displays strong antagonism against phytopathogenic bacteria, such as *Xanthomonas oryzae* in rice (Wu et al. 2015) and *Ralstonia solanacearum* in tomato plants (Im et al. 2020). The polyketide bacillaene can effectively inhibit the growth of various bacteria but also shows antifungal activity against *Penicillium* (Chen et al. 2018). Moreover, *Bacillus* strains from termite colonies that live in symbiosis with the basidiomycete *Termitomyces* produce bacillaene A, which selectively inhibits antagonistic fungi of *Termitomyces* (Um et al. 2013).

Isolates belonging to the *R. marisflavi* group did not show clear antagonistic activity in vitro. Likewise, *R. marisflavi* RHF2.1-7 was unable to control rice blast by induced systemic resistance (Lam et al. 2021). *Priestia* strains were variable in their effectiveness, with *P. megaterium* RHF1.1-1 and RHF1.1-16 and *P. koreensis* 5.5-4 being the most performant. Comparison of their genomes with that of the other *Priestia* isolates did not reveal the presence of known BGCs in the three strains. Therefore, the antifungal activity may be due to (an) unknown metabolite(s) produced by these strains. The *Priestia* strains and three isolates of the *R. marisflavi* group possess a BGC that encodes a rhizobactin 1021/schizokinen-

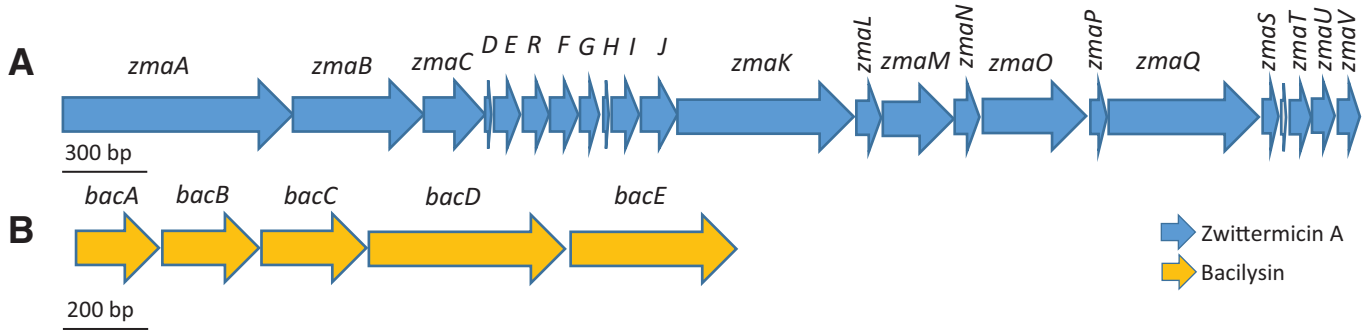


Fig. 8. A, The zwittermicin gene cluster encoded by *zma* genes present in the genomes of *Bacillus thuringiensis* strains RHF5.1-15 and RHF5.1-28. **B,** The bacilysin gene cluster encoded by *bac* genes present in the genomes of the *B. altitudinis* strains.

type siderophore. Rhizobactin 1021 is produced by the bacterium *Sinorhizobium meliloti* (Lynch et al. 2001). *P. megaterium* isolates produce a siderophore named schizokinen (Mullis et al. 1971; Santos et al. 2014); however, the BGC responsible for its production has not been investigated. Both siderophores are structurally closely related, and schizokinen is an intermediary product in the biosynthesis of rhizobactin 1021 (Årstøl and Hohmann-Marriott 2019; Lynch et al. 2001). In *S. meliloti*, the gene products of *rhbABCDEF* are needed for synthesizing the rhizobactin 1021 precursor schizokinen (Lynch et al. 2001), and a very similar gene cluster was found in our *Priestia* and *Rossellomorea* strains. Chemical analysis revealed that our strains indeed produced schizokinen. To the best of our knowledge, the role of either rhizobactin 1021 or schizokinen in antagonistic activity against phytopathogens has not been investigated.

We previously demonstrated the usefulness of *B. siamensis* (*velezensis*) and *B. altitudinis* strains in controlling rice blast on rice grown in ASS or potting soil. Their potential to control other rice diseases remains to be investigated. Likewise, it would be worthwhile to assess the biocontrol potential of the *Priestia* and *B. thuringiensis* strains in plant experiments. It is likely that these *Bacillaceae* contribute to plant health in ASSs, because we predominantly isolated them from healthy rice plants in Vietnam. Additionally, it remains to be determined which specialized metabolites are imperative in the antagonistic activity against the pathogens tested. In the case of *B. siamensis*, we have already shown that the CLiPs surfactin, fengycin, and iturin play a role in direct antagonism against *P. oryzae* on rice, whereas fengycin and iturin work synergistically in the induction of systemic resistance to rice blast (Lam et al. 2021). The antifungal potential of these *Bacillus* species provides an opportunity to further evaluate their usage for disease control on rice grown under ASS conditions in Vietnam.

Data availability. The DNA-seq raw read data and assembled genomes reported in this publication are available in NCBI under BioProject ID PRJNA993114. The sequencing read accessions are SRR25318621 to SRR25318647. The genome accessions are *Rossellomorea marisflavi* RHF1.1-4: SAMN36379369; *R. marisflavi* RHF2.2-1: SAMN36379370; *R. marisflavi* RHF2.1-7: SAMN36379371; *R. marisflavi* RHF2.5-7: SAMN36379372; *R. marisflavi* RHF4.1-12: SAMN36379373; *Priestia megaterium* RHF1.1-1: SAMN36379374; *P. megaterium* RHF3.5-3: SAMN36379375; *P. megaterium* RHF1.1-16: SAMN36379376; *P. megaterium* RHF3.1-8: SAMN36379377; *Priestia koreensis* RHF5.5-4: SAMN36379378; *P. megaterium* RHF5.1-14: SAMN36379379; *P. megaterium* RHF5.1-27: SAMN36379380; *Bacillus thuringiensis* RHF1.1-17: SAMN36379381; *B. thuringiensis* RHF1.1-2: SAMN36379382; *B. thuringiensis* RHF2.1-6: SAMN36379383; *B. thuringiensis* RHF3.3-1: SAMN36379384; *B. thuringiensis* RHF3.1-21: SAMN36379385; *B. thuringiensis* RHF4.1-24: SAMN36379386; *B. thuringiensis* RHF5.1-15: SAMN36379387; *B. thuringiensis* RHF5.1-28: SAMN36379388; *Bacillus altitudinis* RHF1.4-2: SAMN36379389; *B. altitudinis* RHF3.1-9: SAMN36379390; *B. altitudinis* RHF3.1-20: SAMN36379391; *B. altitudinis* RHF4.5-6: SAMN36379392; *B. altitudinis* RHF4.1-26: SAMN36379393; *Bacillus siamensis* RHF4.1-25: SAMN36379394; and *B. siamensis* RHF1.1-3: SAMN36379395.

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