Isolation and Characterization of *Bacillus* sp. Producing Broad-Spectrum Antibiotics Against Human and Plant Pathogenic Fungi

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A strain of bacterium producing antifungal antibiotic was isolated and identification of the strain was attempted. We could identify the bacterium as being a *Bacillus* sp., based on morphological observation, physiological characteristics, and 16S rDNA sequence analysis, thus leading us to designate the strain as *Bacillus* sp. AH-E-1. The strain showed potent antibiotic activity against phytopathogenic and human pathogenic fungi by inducing mycelial distortion and swelling and inhibiting spore germination. The antibiotic metabolite produced by the strain demonstrated excellent thermal and pH (2–11) stability, but was labile to autoclaving. From these results, we could find a broader antifungal activity of *Bacillus* genus. Isolation and characterization of the active agent produced by the strain are under progress.

Keywords: Antifungal ability, antifungal spectrum, *Bacillus* sp., identification

The number of available drugs for treatment of fungal infections remains limited and they show different degrees of undesirable side effects. Besides this, owing to the increase in opportunistic infections in immunocompromised hosts and about 4.5 billion people exposed to the deleterious effects of *Fusarium*, *Aspergillus* sp., *etc*. in developing countries [20, 24, 28], exploring and researching new antifungal agents from new resources have attracted major attention of research scientists and clinicians.

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Bacillus sp., including *B. cereus*, *B. amyloliquefaciens*, *B. subtilis*, *B. licheniformis*, *B. polymyxa*, *B. thuringiensis* and *B. pumilus* [2–4, 6, 7, 13, 15], which are known as producers of a number of antifungal compounds, have been explored for biocontrol agents for several years [18, 19, 21, 25]. It has been fully demonstrated that all of them could inhibit some phytopathogenic fungi successfully. However, it has been barely reported that *Bacillus* sp. could inhibit clinical pathogenic fungi strongly. Although *B. subtilis* and *B. pumilus* were both inhibitory to clinical filamentous fungi pathogens, there were only one or two fungi invstestigated in previous reports [4, 14]. In particular, to date, no *Bacillus* sp. have been reported to inhibit the clinical yeast pathogens *C. parapsilosis*, *C. glabrata*, and *C. krusei*.

Here, we firstly report an isolated *Bacillus* sp. AH-E-1, which exhibited a broad antifungal spectrum. Not only can it inhibit 13 strains of phytopathogenic fungi, but it also showed excellent antibiotics ability against 20 strains of important clinical filamentous fungi and yeasts. Moreover, the thermal and pH stabilities of antifungal compounds generated by this bacterium were investigated and the effects on spore production, germination, and hyphal morphology were also observed.

MATERIALS AND METHODS

Strains and Reagents

A bacterium with antifungal property, AH-E-1 strain, which was isolated from rotten trees, was used as an antifungal microorganism in this work. All the other strains used in this study are listed in Table S1. The human pathogenic fungi were isolated from specimens at the First Hospital and Research Center for Medical Mycology of Peking University (FHRCMM; Beijing, China) or purchased from

the National Center for Medical Culture Collections (CMCC). The plant pathogenic fungi were purchased from the China Forestry Culture Collection Center (CFCC). All of them were maintained on potato-dextrose agar (PDA). The known *Bacillus* sp. in this study was purchased from the China General Microbiological Culture Collection Center (CGMCC). *Staphylococcus aureus* and *Escherichia coli* were obtained from the American Type Culture Collection (ATCC). All the bacteria described above were cultured on trypticase soy agar (TSA). Molecular biological reagents, Genomic DNA extraction kit, Easy spin column DNA gel extraction kit, and Single-step competent cell preps kit were purchased from Katara Biotechnology Co. Ltd (Dalian, China). Amphotericin B was from Sigma (USA). All the culture media including PDA and TSA were from Becton, Dickinson and Company (BD, France).

Identification of Microorganism
Morphological and physiological characteristics. From the morphological observation and physiological characteristics, the microorganism was identified according to the description in *Bergey's Manual of Determinative Bacteriology* [5]. Morphological characteristics were examined under light microscopy (Olympus, Japan).

16S rDNA Sequence Analysis
DNA preparation and PCR amplification. Bacterial cells were obtained from late-exponential cultures shaken in trypticase soy broth (TSB) at 37°C. Total DNA of strain AH-E-1 was prepared and purified using a Genomic DNA Extraction kit. The 16S rRNA gene was amplified for sequencing using forward primer p1: 5'-cc**ggatcc**agagtttgatcctggtcagaacgaacgct-3' and reverse primer p6: 5'-cg**gggatcc**tacggctaccttgttacgacttcacccc-3' (*Bam*HI sites are in bold). The 50 μl PCR reaction mixture contained 100 μmol/l of each dNTP, 50 pmol of each primers, 10× buffer (with 25 mmol/l MgCl$_2$), 2 U *Taq* polymerase, and 1 mol/l of template DNA. The polymerase chain reaction (PCR) temperature program was 94°C for 3 min, followed by 94°C for 1 min, 52°C for 1 min, and 72°C for 3 min for 35 cycles. Following amplification, the PCR products were analyzed by means of electrophoresis with 0.8% agarose gels stained with ethidium bromide. Amplicons were visualized by an ImageQuant 350 (GE, USA) [9].

Transformation of 16S rRNA Gene and Sequencing
The 16S rRNA gene purified from gels with the Easy spin column DNA gel extraction kit and PUC18 were digested with *Bam*HI, respectively, and ligated at 16°C for 16 h with T4 DNA ligase. Then, the combined plasmid was used to transform competent *E. coli* DH5α. Blue colonies are selected for the presence of the combined plasmid on plates containing X-Gal and ampicillin. The 16S rRNA recombined in *E. coli* DH5α was sequenced by Katara Biotechnology Co., Ltd (Dalian, China).

Analysis of 16S rDNA
Computational analysis of DNA sequence data, sequence editing, and multisequence alignment were performed by using DNAstar software. The contiguous sequences were subsequently used to submit and query the nonredundant sequence database at the National Center for Biotechnology Information (NCBI), using the BLAST algorithm. These analyses provided the sequences that were most closely related to the query sequence, based on the percent of nucleotide similarity.

Sequences closely similar to that of AH-E-1 were downloaded and manually aligned and analyzed by using BioEdit (version 7.0.9). Phylogenetic trees were created by using the neighbor-joining algorithm based on the model Kimura-2 parameter through the Mega4.0 program. The reliability of the phylogenetic tree topology was tested by bootstrap resampling with 1,000 replicates.

Preparation of Culture Filtrate of *Bacillus* sp. AH-E-1
Bacillus sp. AH-E-1 was cultured on potato-dextrose broth (PDB) at 30°C for 24 h. After centrifugation (4,000 rpm) at 4°C for 20 min, the cell-free supernatant was collected and filtered aseptically through a sterile 0.22-μm-pore-size membrane filter (Millex-FG; Millipore, USA).

Assay for Antimicrobial Activity of Culture Filtrate
The antimicrobial effects were tested by the disc diffusion method [22]. *S. aureus*, *E. coli*, and *C. albicans* were indicative microorganisms of Gram-positive bacteria, Gram-negative bacteria, and fungi, respectively. Molten PDA precooled to 45°C media was seeded with the indicative strains, respectively, to give a final concentration of 10^7 CFU/ml. The seeded agar was then poured immediately into sterile Petri dishes. After the plates were cooled, wells of 5 mm diameter were made in the medium using a sterile 5-mm-diameter cork borer. Ten μl of the sample solution or standards of Amphotericin B (1 mg/ml) was added into the wells with a Gilson Pipetman, randomly and in triplicate. The plates were incubated at 30°C (fungi) or 37°C (bacteria). After incubation for 24 h, the diameters of the zones of inhibition produced by different samples were compared to assess antimicrobial activity.

Thermal and pH Stabilities of Antifungal Substances in Culture Filtrate
To investigate the thermal stability, the culture filtrate was treated at 100°C for 5 min, autoclaved at 121°C for 20 min, and placed at room temperature for 4 days. The residual inhibitory activity to fungi was then disc-diffusing assayed as described above. To research pH stability, the culture filtrate was diluted with various pH buffers. The residual inhibitory activity to fungi was then disc-diffusing assayed as described above.

Antifungal Activity of the Whole Culture of *Bacillus* sp. AH-E-1
The assays against the filamentous fungi were performed according to Choi *et al.* [7]. Briefly, a mycelial column (5 mm in diameter) was cut off from the white perimeter portion of the colony, which had been cultured on PDA plates at 28°C for 5 days, and placed at a distance 2 cm away from the rim of a fresh PDA plate. Then, *Bacillus* sp. AH-E-1 growing in TSB (a concentration of 10^8 CFU/ml) was streaked in a straight line on the PDA plate (2 cm away from the other rim). All of the filamentous fungi were treated with *Bacillus* sp. AH-E-1 as described above. Simultaneously, control plates inoculated in different mycelial columns were prepared without *Bacillus* sp. AH-E-1. These plates were cultured at 28°C for 5–7 days, and then the zones of inhibition were measured. The degree of inhibition was calculated using the following equation: The degree of inhibition = (area of the fungal colony on the control– area of the fungal colony on the treatment with bacteria)/area of the fungal colony on the control×100%. The scale was the following: - = no visible inhibition, (+) = weak inhibition in relation to the area of the fungal colony on the control, + = no fungal growth on 0.1– 3% in relation to the area of the fungal colony on the control, ++ =

no fungal growth on 3−8% in relation to the area of the fungal colony on the control, and +++ = no fungal growth on >8% in relation to the area of the fungal colony on the control [8, 26].

A dual plate assay was used to determine the inhibitory ability of the bacterial strain against the yeasts [8, 26]. *Bacillus* sp. AH-E-1 was inoculated in two 2 cm lines on PDA plates and incubated at 30°C for 24 h. Then, 10 ml of 45°C soft PDA (0.5% agar) containing *C. albicans* (10^8 CFU/ml) was poured onto one of these agar plates and incubated at 30°C for 48 h. The other yeasts were treated as done for *C. albicans*. The degree of inhibition was calculated as the area of inhibited growth in relation to the total area of the Petri dish, and the scale was defined as previously described.

Effect of *Bacillus* sp. AH-E-1 on Fungal Hyphae

The effect on hyphal growth was tested by the coverslip insertion method. Firstly, PDA plates with *A. fumigates*, *A. nidulans*, *T. rubrum*, *F. compactum*, *M. canis*, and *Bacillus* sp. AH-E-1 were prepared respectively as described above. After incubation for 2−3 days, several clean coverslips were inserted obliquely (from 30° to 45°) at the rim of the normal mycelial colonies and near the rim of the inhibited zones caused by *Bacillus* sp. AH-E-1. All assays were performed in triplicates. When the hyphae on the coverslips were 0.5−1.0 cm, they were observed under an electron microscope (×400). Each experiment was performed in duplicates.

Activity of Culture Filtrate on Spore Germination and Germ Tube Growth

The spores of *A. nidulans*, *P. capsici*, and *P. verrucosa*, which had been maintained on PDA at 25°C for 7−10 days, were harvested by sterile distilled water and filtered through eight layers of sterile cheese-cloth [2]. To evaluate the effect of the culture filtrate on the spore germination and germ tube growth, 2 ml of the culture filtrate was mixed with 1 ml of spore suspension ($1×10^7$ spores/ml in sterile distilled water) completely in an Eppendorf tube. Controls consisted of 2 ml of PDB and 1 ml of the same spore suspension. A 100 μl aliquot of the separate mixtures described above was respectively added to different hollow-ground slides with covers, and then incubated in humidified Petri dishes at 28°C for 72 h [17]. All assays were performed in triplicates. At regular intervals, the number of germinated spores and the length of germ tubes were examined by counting and measuring a total of 200 spores in some random fields under an electron microscope (×400) [29]. The results were expressed as the percentage of spore germination (PSG) and germ tube morphology [17].

Statistical Analysis

The data obtained were subjected to analysis of variance (ANOVA) using SPSS 17.0 software for Windows (SPSS Inc., Chicago, IL, USA). The mean values were compared using the least significant difference (LSD) test, at P < 0.05.

RESULTS

Identification of Antifungal Bacteria

Colonies of AH-E-1 were opaque, flat, dry, and circular, with unsmooth margin. AH-E-1, Gram-positive, is a spore-forming, rod-shaped bacterium. Its cylindrical spores are
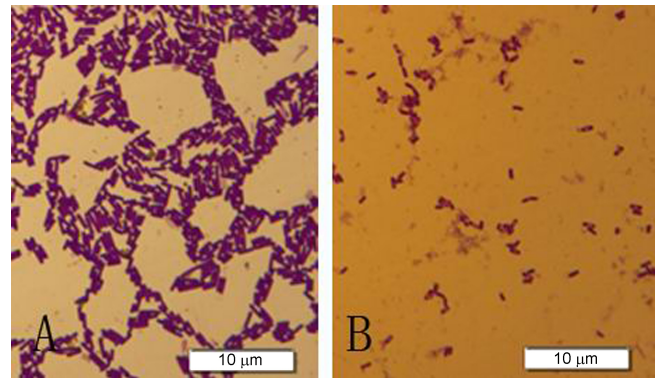

Fig. 1. Light micrographs showing the shapes and sizes of strain AH-E-1 after 18 h and 24 h incubation on TSA at 37°C.
Cells were stained by crystal violet and observed under an electron microscope (×1,000 magnification). (**A**) Cell morphology of strain AH-E-1 cultured for 18 h; (**B**) cells with spore morphology of strain AH-E-1 cultured for 24 h.

developed centrally or paracentrally in non-swollen sporangia after 24 h incubation, which is the specific characteristic of genus *Bacillus*.

Some biochemical characteristics of the strain AH-E-1 were investigated (Table S2). It can be concluded that the reported *Bacillus* sp. with antifungal property, which included *B. thuringiensis*, *B. subtilis*, *B. licheniformis*, *B. cereus*, *B. amyloliquefaciens*, and *B. pumilus*, were not totally identical to *Bacillus* sp. AH-E-1 in biochemical properties. Furthermore, morphological observation, as given in Fig. 1, showed strain AH-E-1 was greatly different from *B. thuringiensis*, *B. subtilis*, *B. licheniformis*, *B. cereus*, *B. amyloliquefaciens*, and *B. pumilus* in shape and size after 18 h and 24 h incubation on TSA (Supplemental data Fig. S1). Moreover, the 16S rDNA sequence of AH-E-1 was determined and deposited into GenBank (Accession No. AY485275). The phylogenetic tree based on 16S rDNA sequences, as shown in Fig. 2, displayed that it was different from its nearest neighbor *B. subtilis* and *B. amyloliquefaciens*. Although there were differences between it and the reported *Bacillus* sp., according to the morphological observation, physiological characteristics, and 16S rDNA sequence analysis, we could still name it *Bacillus* sp. AH-E-1.

Assay for Antimicrobial Activity of Culture Filtrate

The antibiotic spectrum was estimated with Gram-positive *S. aureus*, Gram-negative *E. coli*, and fungus *C. albicans* as indicator strains, respectively. The results showed that the filtered culture fluid of *Bacillus* sp. AH-E-1 inhibited *C. albicans*, but had no effect on *S. aureus* and *E. coli*. Furthermore, the antifungal activity of the filtered culture fluid of *Bacillus* sp. AH-E-1 was much higher than Amphotericin B (1 mg/ml) in equal volume (Fig. 3).
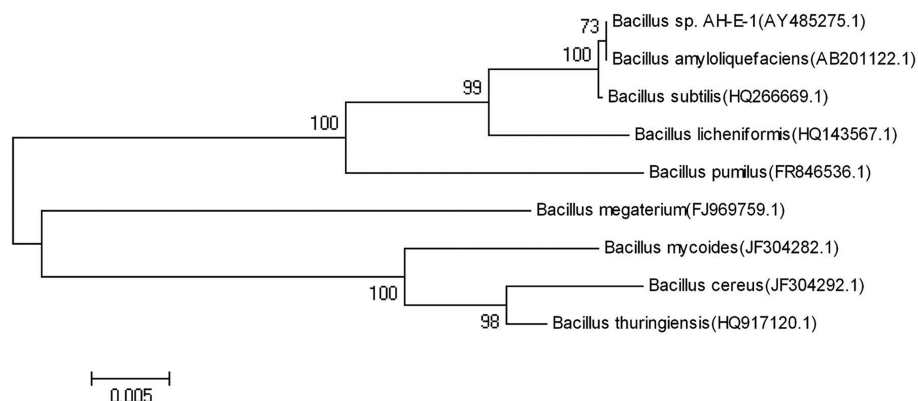
Fig. 2. Phylogenetic relationships between *Bacillus* sp. with antifungal property, based on 16S rDNA sequences.
The tree was constructed based on 1,400 bp. Bootstrap support values (1,000 replicates) above 50% are shown at nodes.

Thermal and pH Stabilities of Antifungal Substances in Culture Filtrate

After different thermal treatments, the residual inhibitory activity of antifungal culture filtrate against *C. albicans* was measured. It was found that the fungicides were remarkably thermostable. The inhibitory activity of the sample heated at 100°C for 5 min was even higher than that of untreated samples, possibly because of a concentration effect. However, the samples denatured at 121°C for 20 min were not resistant to *C. albicans* (Fig. 3). In addition, antifungal activity was hardly affected when the metabolites were placed at room temperature for 3 days, but antifungal activity decreased evidently after 4 days.

The effect of pH on the antifungal activity of the metabolites against *C. albicans* was studied at various pH values. It was found that the inhibitory activity was not significantly influenced by variation of pH from 2 to 11. The inhibitory activity was highly retained even at extreme pH.

Antifungal Activity of Whole Culture of *Bacillus* sp. AH-E-1

The antifungal activities exhibited by the *Bacillus* sp. AH-E-1 are presented in Table 1. It was shown there were different levels of activities [(+) to +++] against 33 strains of facultative indicator fungi (Supplementary Data Fig. S2, S3, and S4).

For the clinical pathogenic fungi, the strongest inhibitory activities (+++) were observed against the growth of *A. fumigates*, *A. flavus*, *A. niger*, *A. versicolor*, *T. mentagrophytes*, *F. compactum*, *P. verrucosa*, *M. racemosus*, *M. canis*, *C. albicans*, and *C. glabrata*. Secondly, the inhibitory activities (++) against *A. nidulans*, *C. krusei*, and *C. parapsilosis* were also demonstrated. Then, there were evident inhibitory activities of + against *F. pedrosoi*, *C. carrionii*, *T. rubrum*, *C. tropicalis*, and *C. neoformans*. Therefore, the inhibitory effects of *Bacillus* sp. AH-E-1 on the clinical pathogenic fungi as indicators were evident, except for *S. schenckii*. Furthermore, it was exciting that there were the strongest inhibitory activities (+++) against all plant pathogenic indicator fungi including *G. bidwellii*, *P. arachidicola*, *P. malkoffii*, *M. grisea*, *R. stolonifer*, *V. mali*, *C. lunata*, *C. gossypii*, *F. graminearum*, *B. dothidea*, *P. capsici*, *A. solani*, and *R. cerealis*.

Effect of *Bacillus* sp. AH-E-1 on Fungal Hyphae

The mycelia from the periphery of the zones of inhibition produced by *Bacillus* sp. AH-E-1 were examined. Microscopic observations showed that the hyphae distorted, and large vesicles and swelling appeared in them (Fig. 4B), which demonstrated that *Bacillus* sp. AH-E-1 induced morphological abnormalities in hyphae of *A. nidulans*, *M. canis*, *A. fumigatus*, *F. compactum*, and *T. rubrum*. Otherwise, the mycelia from the controls were intact, elongated, and smooth without any distortion (Fig. 4A).

Fig. 3. Thermal stability of the antifungal metabolites (10 μl per well).
(A) Autoclaved PDB; (B) culture filtrate of *Bacillus* sp. AH-E-1 autoclaved at 121°C for 20 min; (C) culture filtrate of *Bacillus* sp. AH-E-1 heated at 100°C for 5 min; (D) culture filtrate of *Bacillus* sp. AH-E-1; E: Amphotericin B (1 mg/ml).

Table 1. Inhibitory effects of *Bacillus* sp. AH-E-1 against indicator fungi (twenty-seven filamentous fungi and six yeasts).

Indicator fungus		Indicator fungus		Indicator fungus	
Aspergillus		*Microsporum*		Phytopathogenic	
A. fumigatus	+++	*M. canis*	+++	*P. arachidicola*	+++
A. flavus	+++			*P. malkoffii*	+++
A. niger	+++	*Mucor*		*P. capsici*	+++
A. versicolor	+++	*Mucor racemosus*	+++	*M. grisea*	+++
A. nidulans	++			*B. dothidea*	+++
		Sporothrix		*F. graminearum*	+++
Trichophyton		*S. schenckii*	(+)	*C. gossypii*	+++
T. rubrum	+			*V. mali*	+++
T. mentagrophytes	+++	Yeasts		*A. solani*	+++
		C. albicans	+++	*R. cerealis*	+++
Dematiaceous fungi		*C. tropicalis*	+	*C. lunata*	+++
F. pedrosoi	+	*C. krusei*	++	*G. bidwellii*	+++
F. compactum	+++	*C. glabrata*	+++	*R. stolonifer*	+++
P. verrucosa	+++	*C. parapsilosis*	++		
C. carrionii	+	*C. neoformans*	+		

Filamentous fungi: The degree of inhibition = (area of the fungal colony on control−area of the colony with bacteria)/area of the fungal colony on the control×100%.

Yeasts: The degree of inhibition was calculated as the area of inhibited growth in relation to the total area of the Petri dish

The scale was the following: - = no visible inhibition, (+) = weak inhibition in relation to the area of the fungal colony on the control, + = no fungal growth on 0.1−3% in relation to the area of the fungal colony on the control, ++ = no fungal growth on 3−8% in relation to the area of the fungal colony on the control, and +++ = no fungal growth on >8% in relation to the area of the fungal colony on the control.

Effects of Culture Filtrate on Spore Germination and Germ Tube Growth

Effects of the culture filtrate on the spore germination and germ tube growth of *A. nidulans*, *P. capsici*, and *P. verrucosa* are given in Table 2 and Table S3. The results demonstrated that the culture filtrates were strongly inhibitory to spore germination and could give suppression to germ tube growth. As observed in Table 2, spore germination percentages (SGP) of the controls increased greatly from 0% at 8 h to almost 50% at 16 h. Nevertheless, no spore
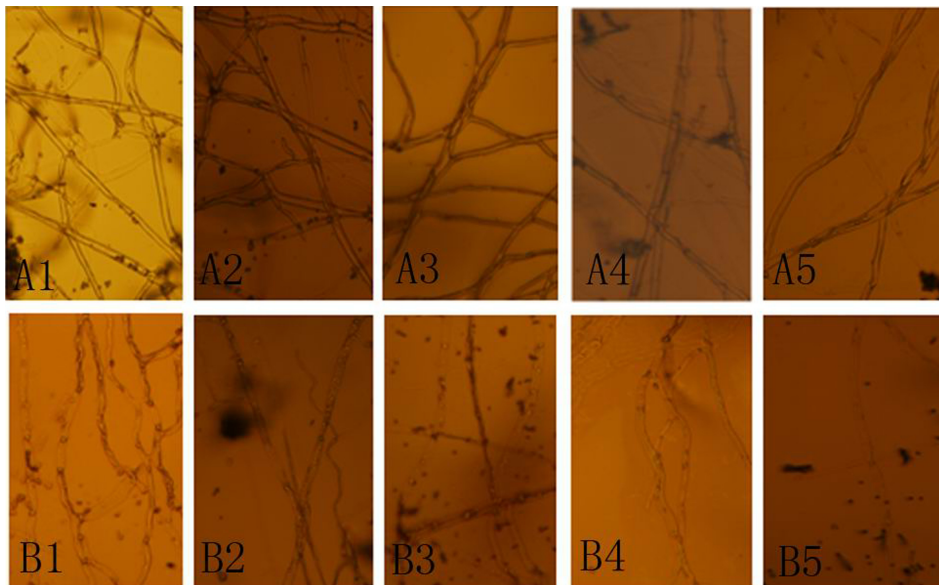

Fig. 4. Effect of *Bacillus* sp. AH-E-1 on hyphae morphology after incubation at 28°C for 5−7 days.
(**A**) Normal hyphae of *A. nidulans* (A₁), *M. canis* (A₂), *A. fumigatus* (A₃), *F. compactum* (A₄), and *T. rubrum* (A₅) (control). (**B**) Hyphal deformations of *A. nidulans* (B₁), *M. canis* (B₂), *A. fumigatus* (B₃), *F. compactum* (B₄), and *T. rubrum* (B₅) produced by strain AH-E-1 (×400 magnification).

Table 2. Inhibitory effects of the culture filtrate on spore germination (n=3).

Incubation time (h)	PSG					
	A. nidulans		*P. capsici*		*P. verrucosa*	
	Treatment	Control	Treatment	Control	Treatment	Control
8	0.0^a±0.0	0.0^a±0.0	0.0^a±0.0	0.0^a±0.0	0.0^a±0.0	0.0^a±0.0
12	0.0^a±0.0	50.0^b±2.0	0.0^a±0.0	43.8^b±1.2	0.0^a±0.0	29.7^b±2.1
16	0.0^a±0.0	58.5^b±2.0	0.0^a±0.0	55.3^b±2.1	0.0^a±0.0	46.0^c±1.0
20	17.0^b±1.0	97.3^c±0.6	0.0^a±0.0	92.2^c±0.6	0.0^a±0.0	79.3^d±1.2
24	19.3^b±0.6	100.0^d±0.0	9.2^b±2.3	100.0^d±0.0	0.0^a±0.0	99.3^e±0.6
36	23.5^c±1.7	100.0^d±0.0	27.2^c±0.6	100.0^d±0.0	0.0^a±0.0	100.0^e±0.0
48	39.5^d±1.2	100.0^d±0.0	33.8^d±1.2	100.0^d±0.0	0.0^a±0.0	100.0^e±0.0
72	40.3^d±2.3	100.0^d±0.0	34.7^d±0.6	100.0^d±0.0	15.0^b±0.6	100.0^e±0.0

PSG: the percentage of spore germination. Means followed by the same letter within the same columns for each fungus are not significantly different by the LSD test (P≤0.05).

germination was observed for the treatments. Although the incubation time was up to 24 h and spore germination percentages of the controls almost reached to 100%, no spore germination could be observed for the treatment groups of *P. verrucosa* and low spore germination percentages of 19.3% and 9.2% for the treatments of *A. nidulans* and *P. capsici* were found. When the incubation time was extended to 72 h, SGP of *A. nidulans* and *P. capsici* showed no remarkable increase compared with those of 48 h, which were separately near to a point (40% and 34%, respectively). In addition, the germ tubes of the treatments showed limited extension and were difficult to grow into compact mycelia (Table S3). Furthermore, it was found to be most effective on conidial germination and germ tube elongation of *P. verrucosa*, whose spore germination was observed until 3 days.

DISCUSSION

Exploring and researching effective antifungal therapy and broad-spectrum but safe antifungal agents from new resources have been highly developed in recent years. It has been reported that a great number of bacteria including *Schinus terebinthifolius* [11], lactic acid bacteria [26], *Bacillus*, *Burkholdria*, and *Pseudomonas* [1] can produce antifungal compounds. However, *Bacillus* sp. is considered as an excellent potential antifungal microorganism owing to producing a variety of antifungal agents such as volatiles, antibiotics, and enzymes [2, 10, 16].

Here, we reported an isolated bacterium with broad-spectrum property, *Bacillus* sp. AH-E-1. Compared with the reported *Bacillus* sp. with antifungal activity, which included *B. cereus*, *B. amyloliquefaciens*, *B. subtilis*, *B. licheniformis*, *B. polymyxa*, *B. thuringiensis*, and *B. pumilus*, *Bacillus* sp. AH-E-1 showed a broader antifungal spectrum and the stronger antifungal activity, which was stable during

5–7 days. It could significantly inhibit phytopathogenic fungi including rice pathogen *M. grisea*, wheat pathogens *F. graminearum* and *R. cerealis*, peanut pathogen *P. arachidicola*, corn pathogen *C. lunata*, cotton pathogens *C. gossypii* and *P. malkoffii*, and vegetable and fruit pathogens *P. capsici*, *A. solani*, *B. dothidea*, *G. bidwellii*, *V. mali*, and *R. stolonifer*. More importantly, it could strongly inhibit the growth of some common clinical fungi, which included superficial infection pathogens *Microsporum* and *Trichophyton* sp. and deep infection pathogens *Aspergillus*, Dematiaceae, and *Mucor* sp. Furthermore, the inhibitory activities against some clinical organ and opportunistic yeast pathogens (*C. albicans*, *C. glabrata*, and *C. krusei*) were also strong.

The *Bacillus* sp. AH-E-1 was isolated from rotten trees. It was identified primarily by the traditional methods and phylogenetic analyses. By the morphological observation and 16S rDNA sequences analyses, it could be certain that strain AH-E-1 belonged to the genus *Bacillus*. Species demarcation of some *Bacillus* isolates is quite complex and bacterial taxonomy has been a tedious, esoteric, and uncertain discipline. However, a simple and rapid approach to define the variability between the species of a specific genus and the specificity of the genus, by using the parameters of signatures (generated by MEME), restriction enzyme (RE) sites, nucleotide stretches "generated" by RE, and the phylogenetic framework, has been developed [12, 21]. To further classify *Bacillus* sp. AH-E-1, the method of Signature Analysis was performed. Nevertheless, there was no unique signature in 16S rDNA sequences of *Bacillus* sp. AH-E-1. Therefore, it could not be specifically identified and we named it *Bacillus* sp. AH-E-1.

The thermal stability of the antifungal compound from *Bacillus* sp. AH-E-1 was remarkable, and the inhibitory activity was not significantly affected by variation of pH, even at extreme pH. However, no antifungal activity was found when it was autoclaved. It was similar with the

reported antifungal peptides or proteins of *B. subtilis* and *B. cereus* [23, 27, 30]. Although antifungal metabolites of AH-E-1 were not further characterized in this study, according to previous reports about antifungal metabolites of *Bacillus* sp., we could suppose that the antifungal metabolites belong to proteins or peptides. Moreover, for the antifungal stability of 5−7 days and the broad spectrum shown in Table 1, this strain should be a potential application in production of antifungal compounds, especially in the pharmaceutical industry. Thus, the purification of the antifungal metabolites is necessary to further characterize this fungicide.

In summary, we described here a bacterium with broad-spectrum antifungal property, *Bacillus* sp. AH-E-1. It exhibited excellent inhibitory activities against 13 strains of phytopathogenic fungi, and 11 strains of important clinical pathogenic fungi. Therefore, it may be utilized as a potential antagonist against fungi in medicine and agriculture, and thus isolation and characterization of the active agent produced by the strain are necessary.

Acknowledgment

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