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Mangrove soil-derived *Bacillus* sp. strain IPS8 exhibits anticandidal potential

Gerwin Louis T. Dela Torre[1], Melody S. Garin[1], Angelo D. Dela Tonga[2], Erna C. Arollado[1,3], Bryan Paul I. Bulatao[1,4], and Richelle Ann M. Manalo-Cabalinan*[1,3]

[1]Institute of Pharmaceutical Sciences, National Institutes of Health, University of the Philippines Manila, Ermita, Manila, 1000, Philippines
[2]Institute of Molecular Biology and Biotechnology, National Institutes of Health, University of the Philippines Manila, Ermita, Manila, 1000, Philippines
[3]Department of Clinical, Social and Administrative Pharmacy, College of Pharmacy, University of the Philippines Manila, Ermita, Manila, 1000, Philippines
[4]Department of Industrial Pharmacy, College of Pharmacy, University of the Philippines Manila, Ermita, Manila, 1000, Philippines

ABSTRACT

The unmet research need and the increasing public health threat of *Candida* infections warrant the search for sources of novel anticandidal agents. The present study isolated and screened mangrove soil-derived bacteria for anticandidal activity against *C. albicans*, *C. glabrata,* and *C. tropicalis*. Six bacteria were isolated from the mangrove forest subsoils of Alabat Island, Quezon Province, Philippines. Two of these bacteria, namely strains IPS7 and IPS8, showed antagonistic activities via the cross-streak and agar plug methods. However, only the cell-free culture supernatant (CFCS) containing the bacterial metabolites of IPS8 showed inhibitory capacity against *C. albicans* and *C. tropicalis* in the agar well diffusion assay. Interestingly, the activity of the IPS8 CFCS was not statistically different from the activity of amphotericin B against *C. albicans* ($p = 0.05267$). Based on the combined morphological, biochemical, and genomic data, the strain IPS8 was putatively identified as *Bacillus velezensis*. The results of this study demonstrate that mangrove soil-derived bacteria, such as strain IPS8, are potential sources of anticandidal agents.

*Corresponding author
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INTRODUCTION

Fungal infections represent a significant but often overlooked contributor to mortality worldwide, with over 150 million severe cases and approximately 1.7 million deaths occurring annually (Kainz et al. 2020; van Rhijn and Bromley 2021). Acknowledging the rising threat posed by fungal infections, the World Health Organization (WHO) released a fungal pathogen priority list that requires public health attention and immediate research and development (WHO 2022). Among the 19 pathogens listed are several *Candida* species. Of these, *C. albicans* was identified as one of the critical-priority pathogens due to its association with high mortality rates, signifying one of the greatest public health threats globally. Meanwhile, *C. glabrata* and *C. tropicalis* were considered high-priority pathogens due to their increasing prominence in causing fungal

KEYWORDS

anticandidal activity, *Bacillus velezensis*, *Candida* spp., cell-free culture supernatant, mangrove soil

infections in the past years. The prioritization focused on the capacity of these pathogens to cause invasive fungal infections, for which challenges of antifungal drug resistance exist. Reports of resistant *Candida* species against several antifungal classes, such as azoles and echinocandins, have increased, highlighting the urgent need to discover novel antifungal agents (Pristov and Ghannoum 2019)—a field that has seen limited progress in the past years (Vahedi-Shahandashti and Lass-Flörl 2020). These *Candida* species are normally part of a healthy human microbiome but can become opportunistic pathogens and cause candidiasis if an imbalance of competing microorganisms develops or the host's immune competence is reduced (Ciurea et al. 2020). The pathogenicity of these *Candida* species can be attributed to virulence factors, including adherence, phenotypic switching, production of hydrolytic enzymes, and biofilm formation, which are implicated in antifungal resistance (Pristov and Ghannoum 2019).

Addressing the urgency of combating *Candida* infections, the WHO recommends focusing research on novel chemical classes of antifungal agents (WHO 2022). Tracing the history of drug discovery, soil microorganisms, particularly those under the genus *Bacillus*, have been documented as a significant source of antimicrobials (Sumi et al. 2015). Although the majority of compounds discovered from soil microorganisms were antibacterials, there were also antifungals, such as the cyclic lipopeptides, iturins, and fengycin (Yousfi et al. 2024). Extending these findings, two promising antifungal lipopeptide leads, AF_4 and AF_5 of the class bacillomycin and derived from *Bacillus subtilis* isolated from soil have entered the antifungal research pipeline (Ramachandran et al. 2014). They are currently being investigated due to their potent activities against *Candida*, *Cryptococcus*, and *Aspergillus* pathogens in both in vitro and in vivo studies (Ramesh et al. 2023; Paul et al. 2025).

With the notion that soil microorganisms hold the potential to produce antifungals, different soil samples have been put into research, such as mangrove soils (Parboteeah et al. 2023). The unique environmental conditions of the mangrove ecosystem characterized by high salinity, fluctuating temperatures, and nutrient limitation, provide an interesting backdrop for the search for soil microorganisms capable of producing novel bioactive metabolites (Alhassan and Aljahdali 2021). In the Philippines, mangrove soils have already been explored for their microbial diversity, leading to the isolation of *Bacillus* species with antibacterial activities (Tabao and Monsalud 2010; Creencia et al. 2022). However, due to the historical underrecognition of fungal infections and a predominant focus on antibacterial discovery, it is likely that these mangrove soil microorganisms have not been systematically screened for antifungal activity (Vanreppelen et al. 2023). To address the gap in the underexplored antifungal potential of mangrove soil microorganisms, the present study was conducted to isolate and characterize bacteria from mangrove soils capable of inhibiting the growth of select *Candida* species.

MATERIALS AND METHODS

The conduct of the study was approved by the Institutional Biosafety and Biosecurity Committee of the University of the Philippines Manila with approval number 2023-047.

Soil sample site collection

The collection of mangrove soils, in cooperation with the Municipal Environment and Natural Resources Office (MENRO) of Alabat, Quezon, was carefully planned, ensuring no harm was inflicted on the mangrove ecosystem. Samples of mangrove soil were collected from the seaward zone of two mangrove forests in the town of Alabat, situated on an island on the east coast of Southern Luzon, in the province of Quezon, Philippines. The mangrove forests in the town of Alabat were chosen as the study site due to the dense vegetation present, which supports a diverse soil bacterial community (Malabrigo et al. 2018; Wang et al. 2014). This microbial diversity increases the likelihood of isolating a wide array of bacterial species for analysis. The first mangrove forest lies in Barangay Gordon (14.063702°N, 122.062102°E), while the second is located in Barangay 5 (14.096982°N, 122.011434°E). The subsoil layers with a depth of 15–30 cm were collected using a multi-stage sediment sludge sampler (290.1, AMS Inc., Idaho, USA). The pH of the soil was measured by mixing approximately 20 g of mangrove soil with 100 mL of distilled water, vigorously shaking the mixture, and allowing it to stand for 10 min. A pH meter (PH220S, Lutron, Taipei, Taiwan) was then used to record the pH of the resulting wet slurry, with measurements taken in triplicate. About 500 g of mangrove soil samples were air-dried, packed in properly labelled resealable plastic bags, and transported to the laboratory of the Institute of Pharmaceutical Sciences, National Institutes of Health, University of the Philippines Manila for processing. Soil samples were stored at 2–8 °C before the soil pretreatment.

Isolation and purification of soil bacteria

Mangrove soil samples were pretreated by heating 1 g for 60 min at 120 °C. Subsequently, a soil suspension was prepared by placing the pretreated sample in 10 mL sterile distilled water and vortexing (Vortex-Genie 2, Scientific Industries, Inc., New York, USA) for 1 min. A 1:10 dilution was prepared by adding 1 mL of the soil suspension to 9 mL sterile peptone water (1403, Condalab, Madrid, Spain), and vortexing the mixture afterward. Tyrosine agar (M362, HiMedia Laboratories, Mumbai, India) plates were prepared and supplemented with 50 mg/L potassium dichromate (32606, Techno PharmaChem, Bahadurgarh, India) and 25 mg/L fluconazole (PHR1160, Sigma Aldrich, Missouri, USA) to inhibit the growth of Gram-negative bacteria and fungi, respectively. An aliquot (0.1 mL) of the mixture was spread onto tyrosine agar plates, followed by incubation for 7 days at 28 °C (Heratherm, IGS400, Thermo Fisher Scientific, Massachusetts, USA). After incubation, morphologically distinct colonies were picked and further purified in tyrosine agar plates using the same conditions. Purified cultures were preserved using the Microbank™ preservation system (Pro-Lab Diagnostics, Texas, USA). Briefly, colonies were transferred into cryovials containing cryobeads submerged in cryopreservative solution until a 3.0 McFarland standard was achieved. Cryovials were closed, inverted five times to emulsify the bacteria, and were left to stand for 2 min. Afterward, the cryopreservative solution was removed and the cryovials were placed in a cryobox before storing in an ultralow temperature freezer (TDE, Thermo Fisher Scientific, Massachusetts, USA) at −80 °C. Retrieval of bacteria in cryobeads followed the manufacturer's guidelines prior to the anticandidal activity assays and bacterial characterization.

Candida species

The anticandidal properties of the mangrove soil-derived bacteria were tested against *C. albicans* ATCC 10231, *C. glabrata* ATCC 15126, and *C. tropicalis* ATCC 750 (KWIK-STIK™ Microbiologics, Virginia, USA). These strains were previously identified to be susceptible to Amphotericin B (Chang et al. 2000; Kubera et al. 2025). All microorganisms were first cultured for 48 h at room temperature in Sabouraud Dextrose Agar (SDA) (GM063, HiMedia Laboratories, Mumbai, India) plates and transferred to the Microbank™ preservation system as previously described.

Anticandidal activity tests

Mangrove soil-derived bacteria were tested using successive anticandidal activity tests. Preliminary screening was performed to check the inhibitory activity against the *Candida* species via the cross-streak method (Claverías et al. 2015). Each of the six bacteria was streaked on one side, approximately 1 cm from the edge of the SDA plate, and incubated for 7 days at 28 °C. All three *Candida* species were then streaked perpendicularly towards the edge of the growth of the mangrove soil-derived bacteria ensuring contact. Proper distance between the candidal pathogens was made to avoid any overlap. The plates were then incubated for 48 h at room temperature. The *Candida* streaks were observed for any growth inhibitions. A semi-quantitative evaluation of the inhibitory activities was conducted as follows: (-) no growth inhibition; (+) < 10% growth inhibition; (++) 10-50% growth inhibition; and (+++) > 50% growth inhibition. Two mangrove soil-derived bacteria that inhibited more than half of the *Candida* streak were selected, and their anticandidal activity was further confirmed using the agar plug assay (Balouiri et al. 2016). Briefly, a 7-day-old mangrove soil-derived bacterial lawn was prepared in SDA. Plugs of 6 mm diameter were punched aseptically using a sterile pipette tip. These plugs were inverted and transferred to the surface of SDA plates previously inoculated with one of the *Candida* species under study. Inoculation was done by dipping a sterile cotton swab into the fungal suspension and swabbing the SDA three times, rotating the plates by roughly 60° between streaking to ensure even distribution of *Candida*. Plates were then incubated at room temperature for 48 h. Uninoculated SDA plugs were used as a negative control. Tests per *Candida* species were performed in quadruplicate. Inhibition zones were measured using the inhibition zone reader of an automatic colony counter (SCAN500, Interscience, Saint-Nom-la-Bretèche, France). Mangrove soil-derived bacteria whose plugs showed inhibition zones were considered active and were further subjected to agar well diffusion assay to verify the production of anticandidal metabolites.

In the agar well diffusion assay, the cell-free culture supernatant (CFCS) of the two active mangrove soil-derived bacteria weretested. One cryobead of the target bacteria was inoculated in 50 mL sterile Sabouraud Dextrose Broth (SDB) contained in a 500 mL Erlenmeyer flask. These were incubated at 28 °C in an orbital temperature shaker (NB205-LF, N-Biotek, Bucheon-Si, South Korea) set at 150 rpm. After 7 days of incubation, the bacterial cultures were then centrifuged (Digicen 21R, Orto Arlesa, Madrid, Spain) for 15 min, set at 5000 rpm and 4 °C, to separate the bacterial cells. The supernatants passed through a 0.45 µm syringe-driven disk membrane filter, and the resulting CFCS were collected. To ensure that the CFCS were indeed free of any viable bacteria, 50 µL were spread in tyrosine agar and incubated at 28 °C for 7 days. The CFCS of the mangrove soil-derived bacteria were tested against the three *Candida* species via agar well diffusion method (Balouiri et al. 2016). The SDA plates inoculated with the three *Candida* species were prepared as previously described. Wells (6 mm) were created by punching the inoculated SDA with a sterile pipette tip and removing the resulting plug. The wells were filled with 100 µL of CFCS, a solution of amphotericin B in DMSO (15 µg) as the positive control, and DMSO and uninoculated SDB as the negative controls. Afterwards, the diameter of the inhibitory zones that appeared around the wells was measured using the inhibition zone reader of an automatic colony counter. Tests were performed in quadruplicate.

Phenotypic characterization

The active mangrove soil-derived bacteria were characterized phenotypically. The growth characteristics in terms of colony size, form, elevation, margin, surface, opacity, and color were observed in SDA after 7-day incubation. The SDA was selected for characterization to ensure consistency with the media used for the anticandidal assay, where they demonstrated anticandidal activities. Gram-staining (R40080 Remel, Thermo Fisher Scientific, Massachusetts, USA) was likewise performed and examined using an optical microscope (Axioscope 5, Zeiss, Oberkochen, Germany).

Biochemical characterization

The biochemical profile of the active mangrove soil-derived bacteria was determined using a carbohydrate test kit (KB009 HiCarbo™ Kit, HiMedia Laboratories, Mumbai, India). It is a comprehensive test system containing 35 different sugars used for the analysis of microbial carbohydrate utilization. A homogenous suspension of the mangrove soil-derived bacteria was prepared in sterile saline. About 50 µL of the suspension was transferred to the surface of each well containing the carbohydrate. The kits were then incubated at 37 °C for 48 h. Color reactions in the wells were observed and compared to the color chart included in the kit.

Molecular characterization

The genomic characterization and capillary sequencing were conducted at the Philippine Genome Center, DNA Sequencing Core Facility. The active mangrove soil-derived bacteria were extracted using Quick-DNA Fungal/Bacterial Miniprep Kit (Zymo Research, California, USA) and were assessed by running the samples through a gel electrophoresis system to ensure purity, integrity, and size distribution. Gene amplification from the extracted DNA was performed using 27F and 1492R primers. The PCR reaction mixture was made with the genomic DNA, PCR buffer, $MgCl_2$, dNTP mixture, forward and reverse primers, and Taq DNA polymerase. Analysis was performed in a thermocycler (T100 Thermal Cycler, Bio-Rad, California, USA) and programmed as follows: initial denaturation at 95 °C for 5 min, followed by 29 cycles of denaturation step at 94 °C for 1 min, annealing at 55 °C for 45 s, extension at 72 °C for 1 min, final extension at 72 °C for 10 min, and the reaction mixture was held at 4°C. The PCR amplicons were run on 1% agarose gel, and the base pair (bp) length was checked. These were bi-directionally sequenced with the BigDye Terminator v3.1 Cycle Sequencing Kit on the ABI 3730xl Genetic Analyzer using a 50 cm 96-capillary array and POP-7™ polymer (Applied Biosystems, Massachusetts, USA) following the protocols supplied by the manufacturer. Base calling was conducted using Sequencing Analysis Software v5.4.

The generated sequences were manually curated and trimmed with Molecular Evolutionary Genetics Analysis (MEGA) version 11.0.13. The resulting sequences were searched in the National Centre for Biotechnology Information (NCBI) data bank using the nucleotide Basic Local Alignment Search Tool (BLAST) and compared with closely related sequences deposited. Phylogenetic analysis was performed in MEGA through the construction of a phylogenetic tree via the neighbor-joining statistical method and the Kimura 2-parameter substitution model with gamma-distributed rates among sites. Confidence values of individual branches were determined using bootstrap analysis based on 1000 replicates.

Statistical analysis

GraphPad Prism version 8.0.2 was used in the statistical analyses. The data on pH and zones of inhibition were reported as mean ± standard deviation. Statistical differences between anticandidal activities were evaluated using either independent t-test or one-way ANOVA. A p-value < 0.05 was considered statistically significant.

RESULTS

Isolation and purification of mangrove soil-derived bacteria

The subsoil collected in the mangrove forests of Barangay Gordon was found to have a pH of 6.88 ± 0.23, while the subsoil from the mangrove forest of Barangay 5 has a pH of 7.05 ± 0.19. Four morphologically distinct bacterial colonies were identified in the samples from Barangay Gordon, while two were observed from Barangay 5. These bacterial colonies were subcultured in tyrosine agar to ensure purity. The four isolates from Barangay Gordon were designated as IPS4, IPS5, IPS6, and IPS7, while the remaining two from Barangay 5 were IPS8 and IPS9.

Anticandidal activity of mangrove soil-derived bacteria

The anticandidal activities of the isolated mangrove soil bacteria were first checked via the cross-streak method. Results showed that only two isolates, IPS7 and IPS8, had antagonistic activities against the three *Candida* species (Table 1).

Table 1: Anticandidal activity of mangrove soil-derived bacteria against three *Candida* species via the cross-streak method.

Mangrove soil-derived bacteria	Anticandidal activity		
	Candida albicans	***Candida glabrata***	***Candida tropicalis***
IPS4	-	-	-
IPS5	-	-	-
IPS6	-	-	-
IPS7	+++	+++	+++
IPS8	+++	+++	+++
IPS9	-	-	-

Interpretation: (-) no growth inhibition; (+++) > 50% growth inhibition

The agar plug method was used as another screening technique for the anticandidal activity of the mangrove soil-derived bacteria (Figure 1A). Inhibition zones of the agar plugs of IPS7 and IPS8 against *C. albicans* (Figure 1B), *C. glabrata* (Figure 1C), and *C. tropicalis* (Figure 1D) were noted, and data were subsequently analyzed (Figure 1E). The findings on the agar plug method showed that the activities of IPS7 across the three *Candida* species were statistically different ($p < 0.0001$), wherein the highest activity was observed against *C. albicans*, followed by *C. glabrata,* then *C. tropicalis*. These observations were also evident in the activities of IPS8 against all three *Candida* species ($p < 0.0001$). Between IPS7 and IPS8, the anticandidal activities were not significantly different against *C. albicans* ($p = 0.08676$) and *C. glabrata* ($p > 0.99999$), except for *C. tropicalis*, wherein the inhibition of IPS8 was statistically higher ($p = 0.00005$) compared to IPS7.

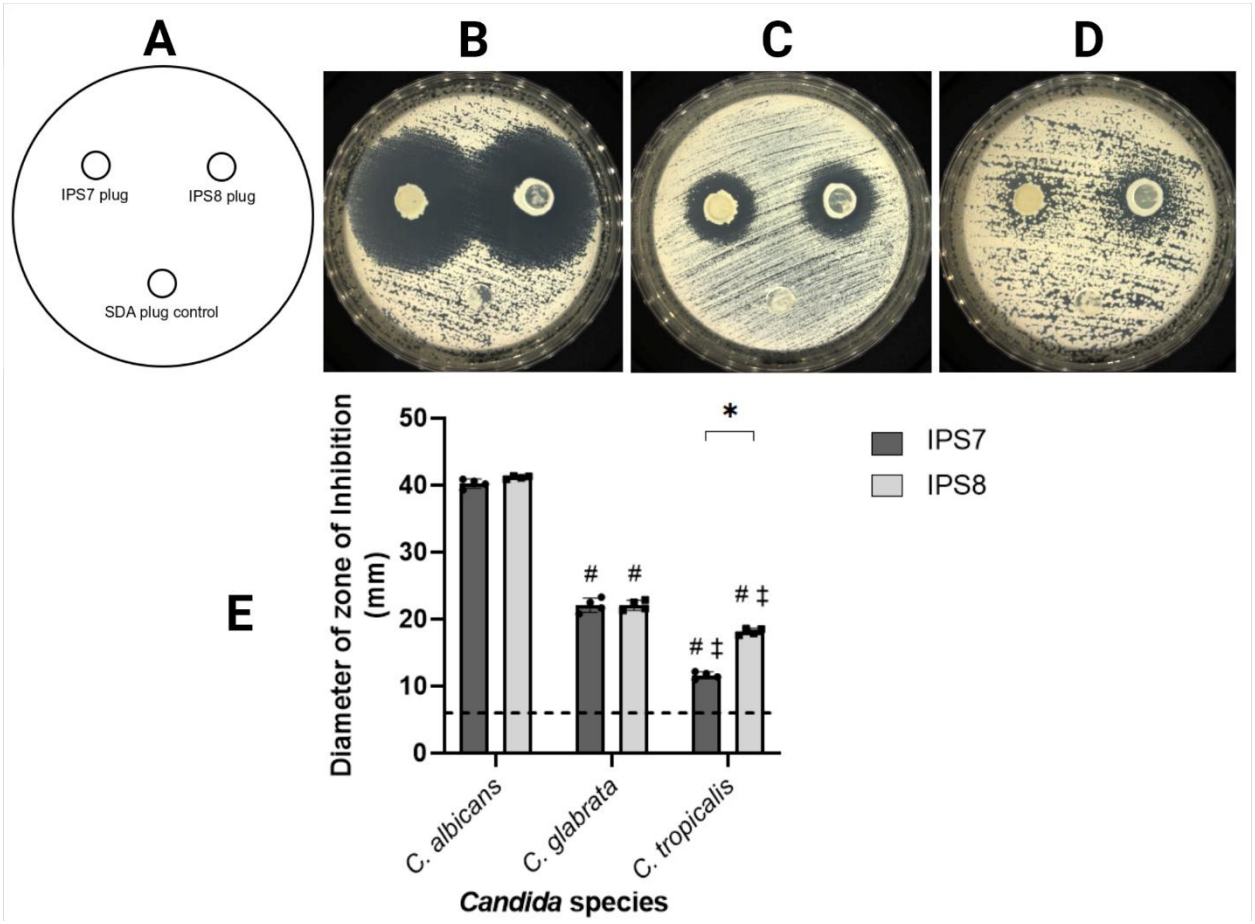

Figure 1: (A) Agar plug set-up used for the anticandidal screening of IPS7 and IPS8 against (B) *C. albicans*, (C) *C. glabrata*, and (D) *C. tropicalis*. (E) Data on the anticandidal activities of IPS7 and IPS8 (n = 4). The SDA agar plug controls have no activities and were not included in the graph. Dashed line corresponds to the 6 mm size of the agar plug. * indicates statistically significant difference between the activities of IPS7 and IPS8; # indicates statistically significant differences in the activities of IPS7 and IPS8 against *C. albicans*; and ‡ indicates statistically significant differences in the activities of IPS7 and IPS8 against *C. glabrata* ($p < 0.05$).

Agar well diffusion assay was performed to determine the antifungal activity of the metabolites released in the culture media by the mangrove soil-derived bacteria (Figure 2A). In this assay, only the CFCS of IPS8 showed clear zones of inhibition against *C. albicans* (Figure 2B) and *C. tropicalis* (Figure 2C), while faint inhibitions were found against *C. glabrata* (Figure 2D), and thus were not considered in the analyses. However, the activity of amphotericin B against *C. glabrata* was still evident at 31.45 ± 0.69 mm. As shown in Figure 2E, the activity of IPS8 CFCS was statistically higher in *C. albicans* compared to *C. tropicalis* ($p < 0.0001$), a result similar to the observed activities of the positive control amphotericin B ($p < 0.0001$). Interestingly, the inhibitory activity of IPS8 CFCS against *C. albicans* was not significantly different from amphotericin B ($p = 0.05267$), however, its activity against *C. tropicalis* was significantly lower ($p < 0.000001$).

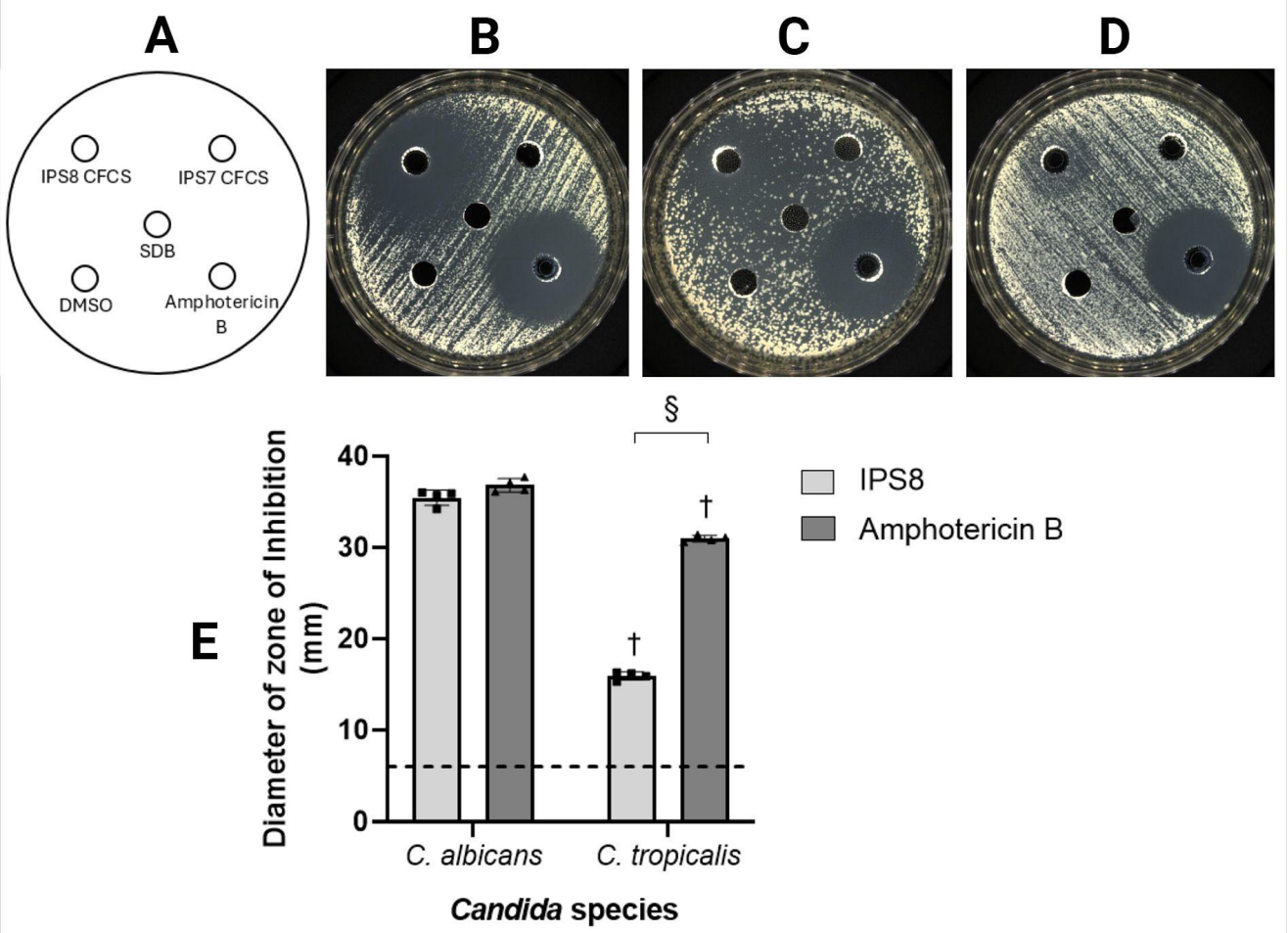

Figure 2: (A) Agar well diffusion set-up used for the anticandidal activity analysis of the CFCS of IPS7 and IPS8 against (B) *C. albicans*, (C) *C. tropicalis*, and (D) *C. glabrata*. (E) Data on the anticandidal activities of the CFCS of IPS8 and amphotericin B (n = 4). The CFCS of IPS7, SDB, and DMSO have no activities and were not included in the graph. Dashed line corresponds to the 6 mm size of the agar well. § indicates statistically significant difference between the activities of IPS8 and amphotericin B; and † indicates statistically significant differences in the activities of IPS8 and amphotericin B against *C. albicans* ($p < 0.05$).

Characterization of mangrove soil-derived bacteria with anticandidal activities

The colony morphologies on SDA were recorded and summarized in Table 2. Representative images of streak plate morphology, single colony morphology, and Gram stain were also obtained for IPS7 (Figure 3A) and IPS8 (Figure 3B). Both were rod-shaped Gram-positive bacteria. Biochemical characterization showed that IPS7 was able to utilize fructose, dextrose, trehalose, sucrose, L-arabinose, D-arabinose, mannose, salicin, ortho-Nitrophenyl-β-galactoside (ONPG), esculin, citrate, and malonate, while IPS8 can utilize fructose, dextrose, sucrose, mannose, mannitol, and cellobiose.

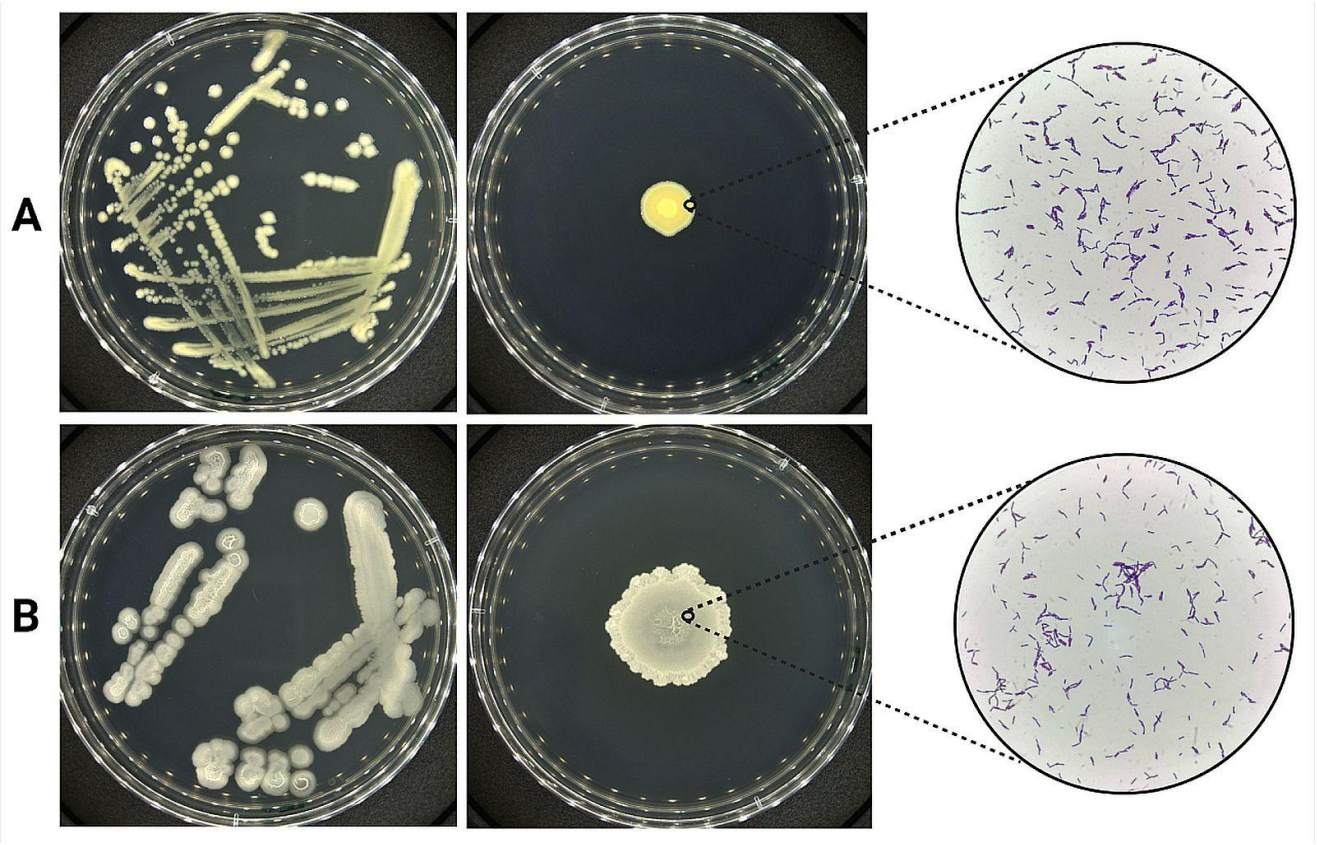

Figure 3: Representative streak plate in SDA, single colony morphology in SDA, and Gram-stain of (A) IPS7 and (B) IPS8.

Table 2: Colony morphology of the mangrove soil-derived bacteria with anticandidal activity.

Colony characteristics	Mangrove soil-derived bacteria	
	IPS7	**IPS8**
Size	> 1 mm	> 1 mm
Form	Circular	Circular
Elevation	Crateriform	Flat
Margin	Entire	Undulate
Surface	Butyrous	Wrinkled
Opacity	Opaque	Opaque
Color	Yellowish	Off-white

The genome assembly and annotation produced consensus sequences with a length of 1381 bp and 1398 bp for IPS7 and IPS8, respectively. To check their putative identity, the 16S rRNA sequences were analyzed through NCBI BLAST. Strain IPS7 was closely related to *B. aerius* 24K, having a 100% identity similarity, whereas IPS8 was closely related to *B. velezensis* FZB42 with a sequence similarity of 99.93%. Their close relationship was further supported by the generated phylogenetic tree (Figure 4) with confidence values of individual branches determined using bootstrap analysis. Given the high similarity of 16S rRNA gene sequences among closely related *Bacillus* species, both isolates were conservatively classified at the genus level as *Bacillus* sp. The 16S rRNA sequences of IPS7 and IPS8 were submitted to NCBI GenBank, with accession numbers PQ409435 and PQ409436, respectively.

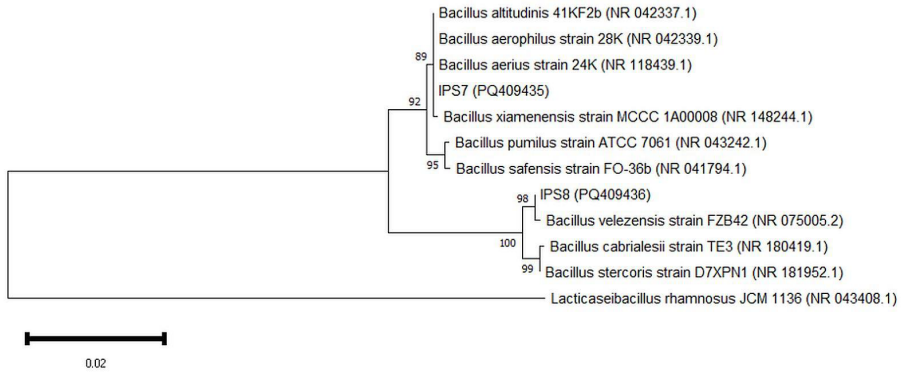

Figure 4: Phylogenetic relationships between IPS7, IPS8 and other *Bacillus* species. The tree was constructed using the neighbor-joining algorithm with confidence values based on a bootstrap analysis of 1000 replicates. The scale bar corresponds to an evolutionary distance of 0.020 nucleotides per position in the sequence. *Lacticaseibacillus rhamnosus* was used as the outgroup.

DISCUSSION

The seaward mangrove forests in Alabat, Quezon, have an estimated stand density of 1050 stems per hectare (Malabrigo et al. 2018). The dense vegetation makes these mangrove forests rich sources of diverse soil microorganisms. High vegetation has been shown to promote a more diverse soil microbial community (Wang et al. 2014). Moreover, the unique blend of terrestrial, marine, and freshwater microorganisms in mangrove ecosystems collectively forms the mangrove microbiome, making the area a hotspot of microbial diversity (Baskaran et al. 2023). These microorganisms play a key role in several biogeochemical cycles in the maintenance of mangrove forests. Through these cycles, microorganisms generate diverse microbial metabolites, making them a treasure house of natural products that can be used in the search for novel anticandidal agents (Li et al. 2022). One naturally abundant resident of mangrove forest soils that grows in a wide range of pH, with peak growth at pH 7, is the genus *Bacillus* (Liu et al. 2017; Iqbal et al. 2024). In recent years, *Bacillus* species derived from mangrove soils have gained attention as promising sources of antibacterial agents (Liu et al. 2017). However, their potential as sources of antifungal metabolites, specifically against human candidal pathogens, remains underexplored and warrants further investigation.

In the present study, IPS7 and IPS8, which were isolated in mangrove forest subsoils with near-neutral pH, were first microscopically characterized as Gram-positive bacilli. Further analysis of the 16S rRNA sequences revealed that IPS7 was closely related to *B. aerius* 24K. To support the molecular findings, carbohydrate utilization profiles were compared with previously published biochemical data for related strains. The majority of carbohydrates utilized by IPS7 were also reported to be used by various strains of *B. aerius* in earlier studies (Shivaji et al. 2006; Shafi et al. 2017). The strain-specific differences in carbohydrate utilization may be ascribed to the genomic differences between strains (Goyal et al. 2022). Lastly, the colony morphology of IPS7 resembled that of *B. aerius* reported in other studies, despite the use of non-SDA media, including the yellowish color (Adeniyi et al. 2024), and the crater-like appearance at the center of the colony (Galaviz-Silva et al. 2018). These collective observations led to the putative identification of strain IPS7 as *B. aerius*. As for IPS8, our present study found that it was phylogenetically similar to *B. velezensis* FZB42. The morphological characteristics of *B. velezensis* IPS8 in SDA were comparable to the data of Zhou et al. (2022) in nutrient agar, and Li et al. (2023) in Luria-Bertani agar. The carbohydrate utilization profiles were compared to published data for related strains and were found to closely match that of *B. velezensis* BM21 (Wang et al. 2020), except for the non-utilization of arabinose and glucose by IPS8. Similar to IPS7, the genomic differences between strains may be responsible for the differences in carbohydrate utilization, as evidenced by the study of Thanh Tam et al. (2023), wherein differences in genes and gene clusters involved in carbohydrate transport and metabolism from different strains of *B. velezensis* were identified. Based on the morphological, biochemical, and the 99.93% similarity of the partial 16S rRNA sequence to known sequences in the database, IPS8 was putatively identified as *B. velezensis*. It is important to note that the identification of IPS7 and IPS8 at the species level was putative and based solely on the findings obtained in this study. The first steps in bacterial identifications (namely: phenotypic, biochemical, and molecular characterizations) have their limitations and may not reliably distinguish closely related species (Plummer et al. 2015; Franco-Duarte et al. 2019). It is therefore recommended to pursue whole genome sequencing and genomic comparative analyses to provide a better taxonomic identity with a comprehensive genomic profile for the isolated bacteria (Tian et al. 2024).

The broad-spectrum antifungal activity of *B. velezensis* against phytopathogenic fungi, belonging to the genus *Colletotrichum*, *Cylindrocarpon*, *Rhizoctonia*, and *Sclerotinia*, among others, has been previously documented (Song et al. 2022). Our study provided insights that the mangrove soil-derived bacterium IPS8, putatively identified as *B. velezensis*, could be a potential agent against *Candida* species of public health importance, aligning with the work of Sahal et al. (2023). Various strains of *B. velezensis* possess a great number of biosynthetic genes, such as the non-ribosomal peptide synthase and polyketide synthase gene clusters (Mullins et al. 2020; Rabbee et al. 2023). These genes are considered to be an integral part of a defense system, responsible for the specialized antimicrobial metabolite production (Chun et al. 2019). It was identified that *B. velezensis* harbors biosynthetic genes related to the production of macrolactin, bacillaene, surfactin, as well as iturin, and fengycin which are known to have anticandidal activities (Yousfi et al. 2024). The presence of these biosynthetic genes that produce anticandidal metabolites may be related to the potent and comparable activity noted in the CFCS of IPS8 when compared with the positive control, amphotericin B, against *C. albicans*.

It is interesting that the *Candida* species used in our study showed varying susceptibility to the mangrove soil-derived bacteria. This difference may be attributed to variations in their cell morphology (Kauser et al. 2024). The inner cell wall, which consists of chitin and glucan, is thinner in *C. albicans* compared to *C. glabrata* and *C. tropicalis* (Walker and Munro 2020), making *C. albicans* highly susceptible to anticandidal compounds. In our study, IPS8 showed the highest inhibition against *C. albicans*.

Candida species are usually cultured in SDA or SDB. Accordingly, these media were also utilized in culturing mangrove soil-derived bacteria to ensure assay compatibility. El-Sayed et al. (2021, 2023) similarly utilized Sabouraud-based media in evaluating the antifungal activities of soil bacterial isolates, reinforcing its suitability for such assays. Additionally, this approach explores the idea of one strain, many compounds (OSMAC) strategy, which emphasizes the influence of nutrients present in the culture medium on metabolite expression (de Jesus et al. 2024). The results suggest that IPS7 and IPS8, which exhibited anticandidal activity in both cross-streak and agar plug assays, produced bioactive compounds in response to the nutrients available in SDA. Interestingly, in the agar well diffusion assay, the CFCS of IPS7 lost its activity against the three *Candida* species, while the CFCS of IPS8 showed weak activity against *C. glabrata*, as observed with faint zones of inhibition around the well. The three methods performed to determine the anticandidal activity exhibited varying levels of sensitivity. Our study demonstrated that the agar well diffusion assay proved to be less sensitive compared to the other two methods. This finding corroborates with Chau et al. (2020), noting that while antimicrobial activities are often detected via both cross-streak and agar well diffusion assay, the latter shows weaker to no activity at times. The observed weak or no activity may be ascribed to the relative variable expression of anticandidal compounds in liquid versus solid media. Different bacterial metabolites are produced depending on whether a bacterium is cultured in solid or liquid media, and it is highly possible for a microorganism to produce antimicrobial metabolites in solid media but not in liquid media (Kibret et al. 2018; Uddin et al. 2013). For instance, in the study of Kibret et al. (2018), soil-derived *Streptomyces* sp. Go-475 elicited inhibitory activities against the yeasts *C. albicans* and *Cryptococcus neoformans* when subjected to solid media fermentation, but no activity was observed when liquid media fermentation was utilized. It is also possible that an enhanced production of bioactive bacterial metabolites occurred in solid

media as it mimics the soil (Lajtai-Szabó et al. 2022), an environment where both IPS7 and IPS8 inhabited naturally. In addition, the weak or no anticandidal activity of bacterial compounds in CFCS could result from the inactivation, modification, or binding of the active anticandidal compound to the various liquid media components (Sapkota et al. 2020). While the physical forms of culture media partly dictate the profile of secondary metabolites produced by a bacterium, other factors, such as culture media pH, incubation time, and incubation temperature, likewise impact the bacterial metabolite production significantly (Elazzazy et al. 2024). This indicates that the release of secondary metabolites of a bacterium depends on the culture environment, and this multifactorial condition needs to be optimized to better utilize these bioactive compounds.

Additionally, there is a distinct difference in the setup of the cross-streak and agar plug methods versus the agar well diffusion assay that can be observed. In the cross-streak and agar plug methods, a mangrove soil-derived bacterium and a *Candida* species were in contact and grown together on SDA, somewhat simulating a co-culture system (Hossain 2024). This co-culture technique, wherein two or more microorganisms are grown with some degree of contact between them, mimics a natural microbial community and is used to activate the biosynthetic gene clusters of microorganisms, inducing the production of new bioactive metabolites (Goers et al. 2014; Sun et al. 2022). During the 7-day incubation of mangrove soil-derived bacteria, bacterial metabolites are produced and diffused in SDA. It is likely that the production of more or new anticandidal metabolites by the mangrove soil-derived bacteria occurred upon its contact with the *Candida* species and diffused as well in the agar to eliminate its competitor for the nutrients available on the plate. In contrast, the agar well diffusion method utilized the CFCS from mangrove soil-derived bacteria incubated in the absence of *Candida* species. In this setup, the potential activation of silent gene expression by competing microorganisms and the subsequent generation of new or potent anticandidal metabolites might be absent. This translated into the lack of inhibitory activity when the CFCSs were tested against the *Candida* species in the agar well diffusion assay. However, further tests are necessary to validate these possibilities. Given that IPS8 showed anticandidal activities across all three tests, it can be inferred that it is a better candidate than IPS7 for sourcing anticandidal compounds, possibly producing these compounds intrinsically. For a more robust interpretation of results, it is recommended that subsequent similar studies include a reference bacterial strain with an established anticandidal activity. Including such a control would allow for a more accurate benchmarking of the bioactivities of the mangrove soil-derived bacteria.

CONCLUSION

The present study reinforces the scientific evidence that mangrove forest soils harbor bacteria with remarkable anticandidal activities. In particular, IPS8, putatively identified as *B. velezensis*, was found to be capable of inhibiting the growth of three *Candida* species and, therefore, can be a viable source of anticandidal compounds. It is therefore recommended that future research on IPS8 should include whole genome sequencing to confirm its true identity, with a focus on identifying biosynthetic gene clusters, followed by the isolation, purification, and characterization of bioactive anticandidal compounds. This could stimulate the research pipeline in drug discovery, addressing the current stagnation in the search for novel anticandidal compounds.

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CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any potential conflict of interest.

CONTRIBUTIONS OF INDIVIDUAL AUTHORS

Gerwin Louis T. Dela Torre: Conceptualization and design of work, data gathering, acquisition, analysis, and interpretation of data, writing – original draft. Melody S. Garin: Data gathering, acquisition, analysis, and interpretation of data, writing – review and editing. Angelo D. Dela Tonga: Data analysis and interpretation of data, supervision, writing – review and editing. Erna C. Arollado: Conceptualization and design of work, data analysis and interpretation of data, supervision, writing – review and editing. Bryan Paul I. Bulatao: Conceptualization and design of work, data analysis and interpretation of data, supervision, writing – review and editing. Richelle Ann M. Manalo-Cabalinan: Conceptualization and design of work, data analysis and interpretation of data, supervision, writing – review and editing.

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