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Screening for antibacterial and cytotoxic potential of marine fungi isolated from samples collected in Ly Son Island, Quang Ngai Province

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ABSTRACT

The ocean covers almost three-quarters of our planet's surface. Marine fungi are fungi that live in marine habitats. Most are microscopic, either microfungi or yeasts, but many marine species inhabiting the world's oceans are unknown. They are well recognized as a source of many novel compounds with valuable pharmacological properties. In the present study, we isolated and assessed marine fungi's antimicrobial and cytotoxic activities from samples collected in Ly Son Island, Quang Ngai Province, Vietnam. From 20 samples, a total of 44 marine fungi were isolated. As a result, 33/44 strains were resistant to at least one tested microorganism. Among them were two isolates, VM10 and VM25, which showed a broad spectrum of antibacterial activity against five to six tested pathogenic ATCC strains with low MIC values. Moreover, 4/44 extracts (VM10, VM15, VM25, and VM39) showed remarkable cytotoxic effects against HepG2 human hepatoma, MCF-7 breast cancer, and A549 lung cancer cell lines, with the recorded viability of tested cancer cells ranging more than 50%. The two candidate strains were subsequently identified as *Aspergillus versicolor* VM10 and *Metarhizium* sp. VM25 by using 18S rRNA sequence analysis. The promising candidate isolates were analyzed in a phylogenetic tree based on MegaX software.

Keywords: *Aspergillus versicolor,* antimicrobial activity, Cytotoxic, *Metarhizium,* MIC, Marine fungi.

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INTRODUCTION

Although there are not many studies about marine fungi, less than other marine microbial, marine fungi, much is left to be discovered. However, they have been found widespread throughout most ocean habitats; they are present on the surface water and the deep sea. Theory predicts that under "desert" type, extreme environments, the species can successfully survive in such high temperatures, pressures, and pH variations from acidic to alkaline with low nutrients are predicted to produce several promising secondary compounds with unique structural features and essential bioactivities. Exploration of fungi from these ecosystems will undoubtedly yield good active metabolites and hold a promising way ahead [1].

According to the latest report on www.marinefungi.org, until 05/03/2024, 2,041 marine fungi species are distributed in 814 genera, 278 families, 107 orders, 34 classes, and 10 phyla. The above include fungi documented from sporulating material in the intertidal-submerged material in the sea (drift and attached wood, leaves, fruits, pneumatophores), those isolated from seawater, deep sea, and mangrove sediments, endophytes isolated from algae, corals and submerged parts of mangrove plants and those parasitic on plankton on seagrasses, etc. Whether marine-derived fungi are obligate or facultative, these organisms are fast researched as promising sources of important natural compounds. The diversity of potent secondary metabolites produced by marine fungi is related to antimicrobial, anticancer, antiviral, antioxidant, and anti-inflammatory activities. Since the vital role in the production of valuable compounds of marine microbes was unveiled, besides studying marine macroorganisms, along with modern technology and techniques, scientists are also interested in exploring marine microbes, which have been recognized as renewable sources (under controlled culture conditions in the laboratory or on an industrial scale) [2, 3].

This is an ongoing project investigating bioactive natural compounds from marine-derived fungi. This research aims to probe and evaluate the marine fungi extracts with unique physiological features in order to find and discover biopharmaceutical products.

MATERIAL AND METHODS

Sampling

Samples were collected in Ly Son Island, Quang Ngai Province, Vietnam (Fig. 1) at different coordinates by SCUBA diving and pickaxe. The samples were placed in 50 mL falcon tubes or plastic boxes filled with 30% glycerol in an icebox and transported to the the Institute of Marine Biochemistry laboratory for further analysis. In the Lab., the samples were stored at 4 ^{o}C until used. All sample boxes must be labeled with the site name, the date and time of the sample collection, and other information [4].

Figure 1. Sampling location

The research team commits to using samples in this study in accordance with international guidelines and considering the conservation of marine resources.

Isolation of marine fungal strains

An amount of 0.5 g of sample was ground, suspended in 4.5 mL of sterile distilled water, and homogenized by vortexing for 1 min. Next, 0.5 mL of the homogenized solution was used for serial dilution in sterile distilled marine water to 10^{-3}.

Then, 50 µL aliquots were spread over seven different selective agar media used for the isolation of fungi, including A1 (10 g soluble starch, 4 g yeast extract, 2 g peptone, 30 g instant ocean, 15 g agar); MEA (5 g malt extract, 1 g peptone, 30 g instant ocean, 15 g agar); PDA (30 g potato extract, 20 g dextrose, 5 g soluble starch, 30 g instant ocean, 15 g agar); NZSG (20 g soluble starch, 5g yeast extract, 10 g glucose, 5g NZ amine A, 30 g instant ocean, 15 g agar); SWA (30 g instant ocean, 15 g agar); PMDA (30 g potato extract, 20 g dextrose 10 g malt extract, 30 g instant ocean, 15 g agar), CZ (30 g Saccharose, 2 g $NaNO_3$, 1 g $K_2HPO_4.3H_2O$, 0.5 g KCl, 0.5 g $MgSO_4.7H_2O$, 0.01 g $FeSO_4.7H_2O$, 30 g instant ocean,15 g agar); All add distilled water until the volume is 1 L; pH 7.0. Independent fungi colonies were picked up and streaked onto the PDA agar medium. Pure isolates were recovered and stored in 30% glycerol at -20°C [4, 5].

Preparation of crude extracts of culture broth

Fungal strains were cultivated at 28°C in sterile 1,000 mL flasks containing 500 mL PDA broth medium, pH 7.0, shaken at 200 rpm and 28°C. After 7 days of cultivation, the culture broths were filtered by filter paper (thickness 0.35–0.5 mm, particle retention 3 µm) and then extracted with 300 mL ethyl acetate (5 times × 15 minutes). The extracts were then evaporated under reduced pressure (250 mbar, heating bath at 45°C) to yield crude extracts [4, 6].

Screening for antimicrobial activity of extracts from fungi

Crude extracts were diluted in DMSO at 1% concentration (10 mg/1 mL) and used in screening experiments for antagonistic properties against the test microorganisms. Seven pathogenic microorganisms including three Gram-negative bacteria (*Escherichia coli* ATCC25922, *Pseudomonas aeruginosa* ATCC27853, *Salmonella enterica* ATCC13076), and three Gram-positive bacteria (*Enterococcus faecalis* ATCC29212, *Staphylococcus aureus* ATCC25923, *Bacillus cereus* ATCC 14579) and one yeast *Candida albicans* ATCC10231. The test microbes were grown in 96 well plates containing LB broth - supplemented with the crude extracts at different concentrations. The crude extract was diluted in DMSO at a decreasing concentration range: 256, 128, 64, 32, 16, 8, 4, and 2 µg/mL for the number of experiments. The final test microbial concentration of 2×10^5 CFU/mL per well. Streptomycin was used as a positive control for bacteria and cycloheximide for the yeast *C. albicans*. All plates were incubated at 37°C with shaking at 120 rpm. After 24 h, read the MIC value as the value at the well with the lowest concentration of antimicrobial agent that completely inhibits the visible growth of a test microbial, accomplished by spreading cultures out on an agar plate. All experiments were done three times [7, 8].

Cytotoxicity assay

Three human cancer cell lines: human liver cancer HepG2, human breast cancer MCF-7 and human lung cancer A549 were used for determining the cytotoxic activity of extracts from all fungi strains. The antiproliferative MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium) assay was employed and conducted following the previously described protocols [9]. Experiments were carried out in triplicate for the accuracy of data. The cells were then cultured for an additional 48 h. At the end of the treatment period, MTT (final concentration 1 mg/mL) was added to each well, which was then incubated at 37°C in 5% CO_2, 98% humidity. The coloured crystals of the produced formazan were dissolved in DMSO (dimethyl sulfoxide, Sigma, USA). The absorbance was measured at 540 nm using an ELx800 Microplate Reader (Bio-Tek Instruments). Ellipcine 10 µg/mL was used as a positive control and DMSO served as a negative control [4, 9]. A decrease in cell viability to less than 50 % was noted as a positive outcome. The inhibitory rate of cell growth (*IR*) was calculated according to the following equation:

$$IR = 100\% - \left[\left(OD_{sample} - OD_{blank}\right)\right] / \left[\left(OD_{DMSO} - OD_{blank}\right)\right] \times 100$$

OD_{sample} is the average *OD* value of the treated sample; OD_{DMSO} is the average *OD* value of the negative control wells; OD_{blank} is the average *OD* value of the blank wells with culture medium only. The TableCurve 2Dv4 software was used for data analysis and IC_{50} calculation.

Taxonomic characterization of promising strains

Taxonomic characterization of strain is based on a combination of molecular information to classify species, and morphological taxonomy uses morphology and phenotypic.

Morphological characterization of fungi strains was observed according to spore-producing, diffusible pigments, melanin pigment formation, and hyphae and conidia in culture media. The isolated fungi were photographed and measured using a compound microscope (Nikon ECLIPSE 80i, Japan).

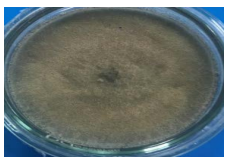
For molecular identification, sequences of 18S rRNA were used for taxonomical identification of the fungal strains. The PCR mixture (50 µL) contained 2.0 µL fungi DNA, 25 µL Master Mix 2X, 19 µL of H_2O, 2.0 µL of 10 pmol/µL for each primer NS3F (5'-GCAAGTCTGG TGCCAGCAGCC-3') and NS8R (5'-TCCGCAGGTTCA CCTACGGA-3') [10]. The mixtures were amplified using the following cycling profile: 94°C for 3 mins, 30 cycles at 94°C for 1 min, primer annealing at 58°C for 1 min, and 72°C for 1 min 20 secs, extension at 72°C for 8 mins. The PCR products were analyzed using capillary electrophoresis on a DNA analyzer (ABI PRISM 3100, Applied Bioscience). Gene sequences were analyzed by BioEdit version 7.2 and aligned with known 18S rRNA gene sequences in the GenBank database by the CLUSTAL W program. Evolutionary analysis was performed using the Maximum Likelihood method, whereas the evolutionary history was inferred using the Maximum Likelihood method and JTT matrix-based model [6, 11].

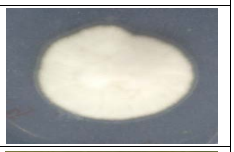
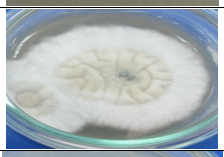
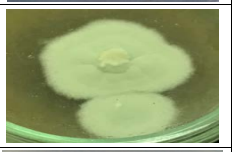
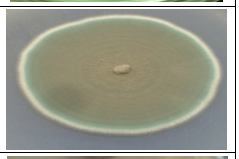
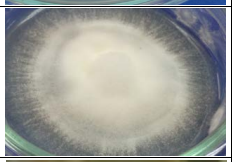
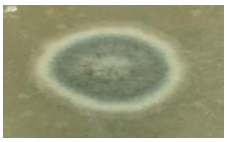
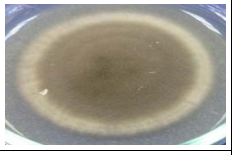
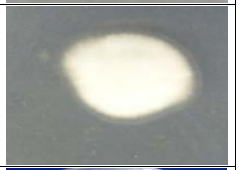
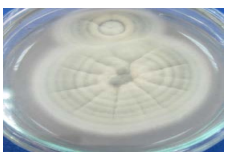
RESULTS

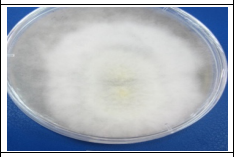
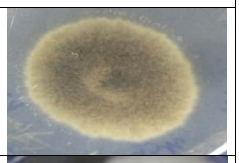
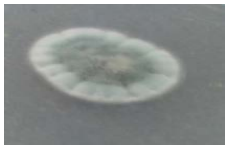
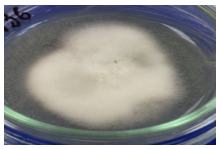
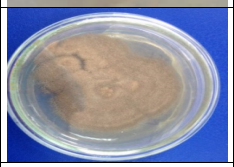
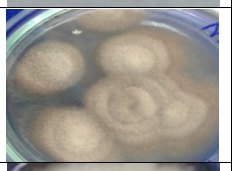
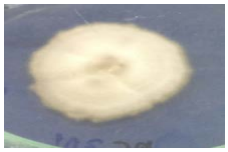
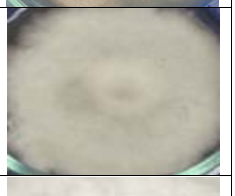
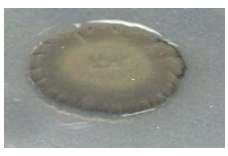
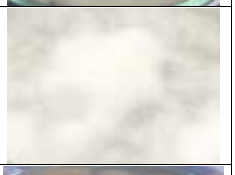
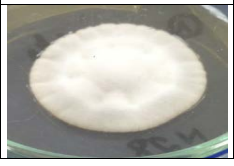
Sampling and isolation of marine fungal strains

Marine samples were collected with SCUBA diving at a depth of 10–13 m under sea level, and the water temperature ranged from 26–30°C in different geographic coordinates on Ly Son island. Observations of colony features such as colour, size, shape, hyphae, conidia, conidiophores, and arrangement of spores visible by the naked eye were used classically to identify fungi. Colonies that showed powder consistency or stuck firmly to the agar surface from the isolation plates were purified through several rounds of transfer to suitable culture media. A total of 44 strains were isolated from the marine samples. The numbers of samples and isolates in each sample are presented in Table 1. All potential colonies were purified and stored in glycerol stock at -80°C.

Table 1. Information on coordinates, sampling depth, sample name, sample symbol and colony morphology of fungal strains isolated in Ly Son Island

No.	Strain name	Coordinates; Sampling depth; Sample name	Colony	No.	Strain name	Coordinates; Sampling depth; Sample name	Colony
1	VM1	15°21'52" - 109°9'4"; 12 m; Sediment.		23	VM24	15°23'19" - 109°9'11"; 12 m; Sponge.	
2	VM2	15°21'55" - 109°9'27"; 10 m; Echinoderm.		24	VM25	15°21'55" - 109°9'27"; 10m; Sediment.	

No.	Strain name	Coordinates; Sampling depth; Sample name	Colony	No.	Strain name	Coordinates; Sampling depth; Sample name	Colony
3	VM3	15°21’55” - 109°9’27”; 10 m; Seaweed.		25	VM26	15°21’52” - 109°9’4”; 12 m; Echinoderm.	
4	VM4	15°21’55” - 109°9’27”; 10 m; Mollusk.		26	VM27	15°21’55” - 109°9’27”; 10 m; Sponge.	
5	VM5	15°21’55” - 109°9’27”; 10 m; Sediment.		27	VM28	15°23’19” - 109°9’11”; 13 m; Echinoderm.	
6	VM7	15°21’52” - 109°9’4”; 12 m; Echinoderm		28	VM29	15°21’52” - 109°9’4”; 12 m; Seaweed	
7	VM8	15°21’55” - 109°9’27”; 10 m; Seaweed		29	VM30	15°21’52” - 109°9’4”; 12 m; Seaweed	
8	VM9	15°21’52” - 109°9’4”; 12 m; Sponge		30	VM31	15°21’52” - 109°9’4”; 12 m; Echinoderm	
9	VM10	15°21’55” - 109°9’27”; 10 m; Sponge		31	VM32	15°23’19” - 109°9’11”; 13 m; Echinoderm	
10	VM11	15°21’55” - 109°9’27”; 10 m; Seaweed		32	VM33	15°21’52” - 109°9’4”; 12 m; Sponge	
11	VM12	15°23’19” - 109°9’11”; 13 m; Echinoderm		33	VM34	15°21’52” - 109°9’4”; 12 m; Echinoderm	
12	VM13	15°21’52” - 109°9’4”; 12 m; Sponge		34	VM35	15°21’55” - 109°9’27”; 10 m; Seaweed	

No.	Strain name	Coordinates; Sampling depth; Sample name	Colony	No.	Strain name	Coordinates; Sampling depth; Sample name	Colony
13	VM14	15°23’19” - 109°9’11”; 12 m; Soft coral		35	VM36	15°21’52” - 109°9’4”; 12 m; Mollusk	
14	VM15	15°23’19” - 109°9’11”; 13 m; Echinoderm		36	VM37	15°21’52” - 109°9’4”; 12 m; Echinoderm	
15	VM16	15°23’19” - 109°9’11”; 13 m; Sediment		37	VM38	15°21’52” - 109°9’4”; 12 m; Echinoderm	
16	VM17	15°23’19” - 109°9’11”; 13 m; Soft coral		38	VM39	15°21’52” - 109°9’4”; 12 m; Mollusk	
17	VM18	15°21’55” - 109°9’27”; 12 m; Seaweed		39	VM40	15°23’19” - 109°9’11”; 13 m; Seaweed	
18	VM19	15°23’19” - 109°9’11”; 13 m; Echinoderm.		40	VM41	15°21’55” - 109°9’27”; 10 m; Echinoderm	
19	VM20	15°21’52” - 109°9’4”; 12 m; Sponge		41	VM42	15°21’52” - 109°9’4”; 12 m; Mollusk	
20	VM21	15°21’55” - 109°9’27”; 12 m; Sediment.		42	VM43	15°21’52” - 109°9’4”; 12 m; Echinoderm	
21	VM22	15°21’55” - 109°9’27”; 10 m; Mollusk		43	VM44	15°21’52” - 109°9’4”; 12 m; Soft coral	
22	VM23	15°23’19” - 109°9’1”; 12 m; Mollusk		44	VM45	15°23’19” - 109°9’11”; 13 m; Echinoderm	

Screening for antimicrobial activity of extracts from fungi

Results of screening the antibacterial activity of ethyl acetate crude extract of 44 fungal strains expressed through minimum inhibitory concentration (MIC) values showed that 33/44 strains had antibacterial activity from 1 to 6 strains of test microorganisms. 19/44 isolates showed anti-*C. albicans* activities. 20/44 strains against at least 2 strains of test microorganisms. The two isolates, VM10 and VM25, were highly active, especially in the ability to resist the group of microorganisms that cause intestinal diseases with MIC values equal to or lower than the positive control. The detailed results are presented in Table 2.

A total of 44 fungi isolates were tested for activity against 7 pathogenic microorganisms. Among all the marine fungi crude extracts tested, MIC was determined for 30 fungi isolates against *E. faecalis*. In particular, strains VM10 and VM25 exhibited antagonistic activity against 5/7 microbial pathogens strains with MIC values from 16-256 µg/mL. Subsequently, these 44 crude extracts were tested for their ability to inhibit the viability of 3 cancer cell lines HepG2, A549, and MCF-7.

Table 2. Antibacterial activity of the extracts from 33 fungal strains against microbial pathogens

No.	Fungal strains	ATCC microbial pathogens						
		Gram +			Gram -			Yeast
		E. faecalis	*S. aureus*	*B. cereus*	*E. coli*	*P. aeruginosa*	*S. enterica*	*C. albicans*
1	VM1	64	64	128	-	-	-	128
2	VM2	32	-	-	-	-	-	32
3	VM3	128	-	-	-	-	-	-
4	VM4	32	32	16	-	-	-	64
5	VM5	64	-	128	-	-	-	-
6	VM7	128	-	-	-	-	-	128
7	VM8	32	-	32	-	-	-	16
8	VM9	64	-	-	-	-	-	-
9	VM10	64	128	32	-	-	64	64
10	VM11	256	-	-	-	-	-	256
11	VM12	256	-	-	-	-	-	128
12	VM13	256	-	-	-	-	-	256
13	VM14	128	-	-	-	-	-	-
14	VM18	64	-	-	-	-	-	128
15	VM19	64	-	-	-	-	-	256
16	VM20	-	-	-	-	-	-	256
17	VM21	128	-	-	-	-	-	128
18	VM22	256	-	-	-	-	-	-
19	VM23	-	128	32	-	-	-	-
20	VM25	32	32	32			16	16
21	VM26	128	-	-	-	-	-	64
22	VM27	64	-	-	-	-	-	-
23	VM28	-	64	-	-	-	-	256
24	VM30	32	32	32	-	-	-	8
25	VM31	256	-	-	-	-	-	-
26	VM32	128	256	-	-	-	-	-
27	VM33	128	-	256	-	-	-	-
28	VM34	256	-	-	-	-	-	-

No.	Fungal strains	ATCC microbial pathogens						
		Gram +			Gram -			Yeast
		E. faecalis	*S. aureus*	*B. cereus*	*E. coli*	*P. aeruginosa*	*S. enterica*	*C. albicans*
29	VM36	256	-	-	-	-	-	-
30	VM37	256	-	-	-	-	-	-
31	VM39	256	-	-	-	-	-	128
32	VM44	128	256	-	-	-	-	-
33	VM45	128	-	-	-	-	-	64
Streptomycin		256	256	128	32	256	128	-
Cyclohexamide								32

Notes: Positive control: Streptomycin, Cycloheximide; (-): Inactive.

Cytotoxicity assay

Inhibitory effect of crude extracts (at 100 μg/mL) on the growth of 3 cancer cell lines. Cell viability was assessed by MTT test as reported in the methods. To the cytotoxicity assay results of the 44 extracts, only 4/44 strains showed cytotoxic effects on 1 to 2 test cell lines with significant differences. Values are the mean ±SD of three separate experiments in triplicate. The in vitro results are summarized in Table 3.

Table 3. The cytotoxic activity of 5 bioactive strains against human cancer cell lines

Crude extracts	Percent growth inhibition of cells (% Inhibition ± SD)		
	HepG2	A549	MCF-7
VM10	38.66 ± 1.30	63.14 ± 1.42	43.76 ± 0.77
VM15	15.67 ± 1.81	46.41 ± 1.46	51.34 ± 1.56
VM26	23.93 ± 1.32	55.21 ± 1.32	31.23 ± 1.42
VM39	29.66 ± 2.12	36.21 ± 2.21	57.36 ± 1.45
Camptothecin * (5 μg/mL)	85.23 ± 1.37	79.31 ± 1.08	88.26 ± 1.39

Notes: * Camptothecin: anticancer compound used as the standard.

From the results of preliminary screening for antimicrobial and cytotoxic activities, 2 strains (VM10 and VM25) with the best biological activity were selected and identified. Strain VM10 inhibited 5/7 test microbial strains and inhibited 63.14 ± 1.42% of A549 cancer cells. Although strain VM25 did not inhibit the 3 test cancer cell lines, it had very good inhibitory activity against 5/7 test microbial strains, with MICs from 16–32 μg/mL. While VM15, VM26 and VM39 strains did not show good antimicrobial activity, they were capable of causing cytotoxicity to human breast cancer MCF-7 and human lung cancer A549 cell lines.

Taxonomic characterization of promising strains

The fungal strains were cultured for 3 days to 10 days on CZ agar medium (Czapek) at 28^{o}C, to observe colony morphology, the color of aerial mycelium, and substrate mycelium. The shape and size of fungal hyphae, ascospores, spore-forming organs, and conidia were observed using a Japanese Nikon ECLIPSE 80i optical microscope.

Description of VM10 (Fig. 2): Colonies on Czapek medium grow slowly, with a 2–3 cm diameter after 2 weeks at 28^{o}C. The colony is grooved, partitioned, and has many soft, silky aerial fibres. The colony's surface has many spore-forming structures, initially white-green, grey-green, then dark green, colourless to reddish brown secretions; The left side is dark orange to reddish brown. Spore-forming stalks are produced from the basal mycelium, 80–700 μm × 3.5–9 μm, colorless to light yellow-brown, thick and smooth walls. One hypha has a pear-shaped to nearly nearly spherical shape

sporangium, about 9–20 μm in diameter, at the tip. Conidia are produced in long dry chains which aggregated in 2 columns. Spores already have been 4.5 μm in size, with sharp spines, form chains.

Description of VM25 (Fig. 3): Colonies on Czapek medium at 28°C after 7 days d reaching 1.8–2.2 cm diameter, initially pink-white, then turning purple-pink with age, with a velvety to slightly spongy surface, thin edges. Fungal hyphae are colorless, light brown, smooth, and 1.5–2 μm in diameter. Sporangiophores are unbranched or have 1–2 branches, septate, straight or curved, slightly tapering towards the apex, 1–2 μm × 10–20 μm in size, the tip has a regular zigzag shape, 1 μm wide, 10–30 μm long. Conidia are spherical or nearly spherical, no-walled, smooth, colorless, 2–2.5 μm in diameter.

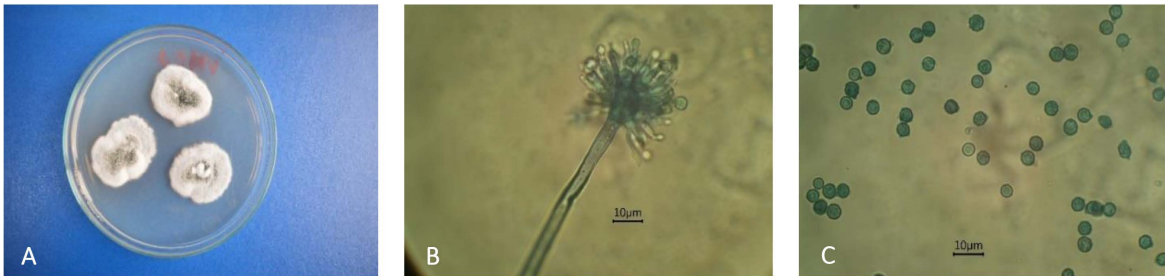


Figure 2. (A) VM10 Colonies on Czapek at 28°C/7 days; (B) Sporangium; and (C) Conidia × 1,000

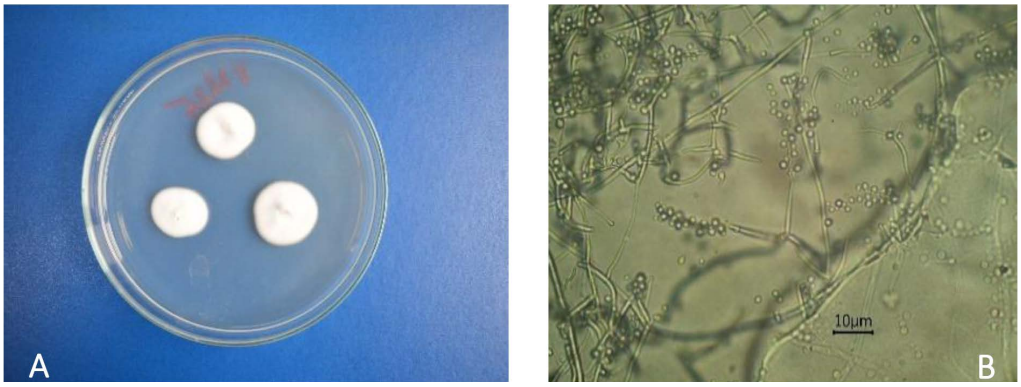


Figure 3. (A) VM25 Colonies on Czapek at 28°C/7 days; (B) Sporangium and conidia x 1000

Taxonomic characterization of promising strains

By analyzing the 18S rRNA gene sequence, two isolated strains possessing potent antimicrobial activity, the 18S rRNA sequence of VM10 had a close phylogenetic relationship with NG_067623.1 *Aspergillus versicolor* NRRL 238 and VM25 showed more than 99% similarity to strains of NG_062608.1 *Metarhizium viride* CBS 348.65. The tree shows the affiliations of the 11 fungal strains based on 18S rRNA gene sequencing and constructed by MEGA-X software. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. Evolutionary distances were computed using the Neighbor-Joining method. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1,000 replicates) are shown next to the branches. The bar length represents 0.01 substitutions per nucleotide site. The phylogenetic tree showed three clusters in which VM10 and VM25 were grouped in the clade of *Penicillium*, *Metarhizium*, and *Aspergillus* genus, and their 18S rRNA gene sequence were deposited in GenBank under accession numbers PP425895 (VM10) and PP425869 (VM25) (Fig. 4).

In recent decades, researchers have focused on screening the possibility of synthesizing

bioactive metabolites from marine microbes (actinomyces, fungi, bacteria). Marine-derived fungi produce unique structures and bioactivity secondary metabolites, such as polyketides, alkaloids, peptides, lactones, terpenoids, steroids, etc. They exhibit anticancer, antibacterial, antifungal, antiviral, anti-inflammatory, antioxidant, antibiotic, and cytotoxic activities [12, 13]. The marine-derived fungus *Aspergillus unguis* and *Penicillium citrinum* isolated from *Callyspongia* sp. showed good cytotoxic and antibacterial activity [3]. Marine fungus *Aspergillus ochraceus* isolated from *Acanthostrongylophora ingens* sponge had antibacterial activity against *Vibrio cholerae*, *E. faecalis*, MDRPA, *P. aeruginosa*, MRSA, *S. aureus*, and *M. tuberculosis* H37Rv [14]. Study about the *Metarhizium* genus, the *Metarhizium anisopliae* fungus toxin acts as an effective chemical substance for pests: *Aedes aegypti, Anopheles stephensi, Culex quinquefasciatus* at 24 h post-treatment [15].

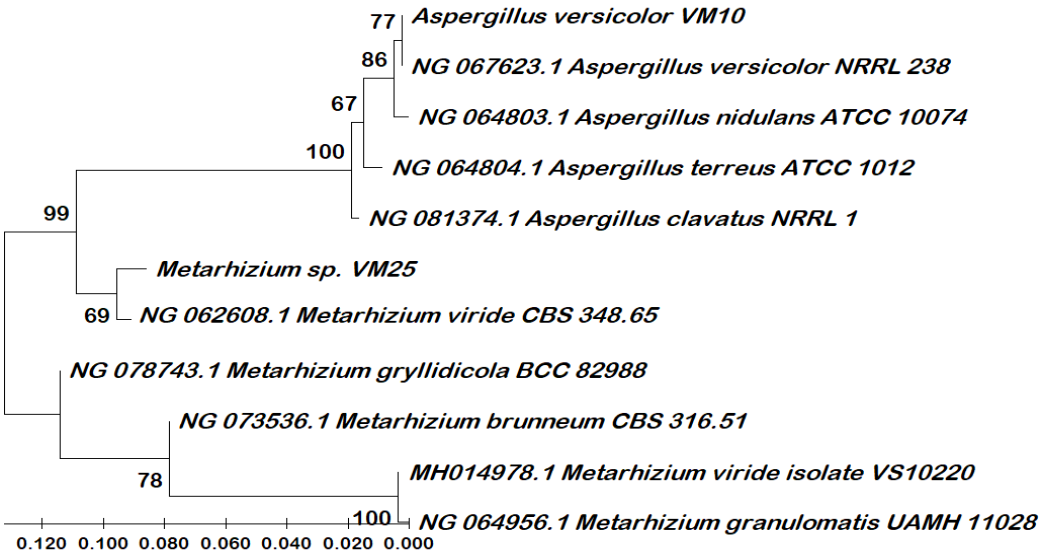


Figure 4. Phylogenetic tree based on 18S rRNA gene sequences showing relationships between 2 studied strains with representative members of genera *Aspergillus* and *Metarhizium* genus

A very valuable study by scientists at Swansea University, UK, showed that *Metarhizium brunneum* strains produced a wide array of volatile organic compounds, which were screened against a range of rhizosphere and non-rhizosphere microbes, including three Gram-negative bacteria (*Escherichia coli, Pantoea agglomerans, Pseudomonas aeruginosa*), five Gram-positive bacteria (*Micrococcus luteus, Staphylococcus aureus, Bacillus subtilis, B. megaterium, B. thuringiensis*), two yeasts (*Candida albicans, Candida glabrata*), and three plant pathogenic fungi (*Pythium ultimum, Botrytis cinerea, Fusarium graminearum*). This study provided new insights into how *Metarhizium brunneum* may protect plants from pathogenic microbes and correspondingly promote healthy growth [16].

In Viet Nam, a study by Minh et al., the investigation of ethyl acetate extract from solid fermentation of the marine-derived fungus *Penicillium citrinum* VM6 from Ly Son Island led to the isolation of eight metabolites, including one citrinin dimer dicitrinone F. Of these, compound 3β-hydroxy-5,9-epoxy-(22E,24R)-ergosta-7,22-dien-6-one was isolated for the first time from the *Penicillium* genus and compound dicitrinone F with carbon-bridged C-7/C-7’ linkage is rarely reported. All isolated compounds exhibited selective antimicrobial activity against the tested Gram-positive bacteria and *C. albicans* with MICs of 32–256 µg/mL. Compounds dicitrinone F and melithasterol B exhibited cytotoxicity against all tested cell lines A549, MCF7, MDA-MB-231, Hela, and AGS with IC_{50} values of 6.7 ± 0.2 to

29.6 ± 2.2 µg/mL, whereas compound phenol A acid had selective cytotoxicity against the MCF7 cell lines with IC50 of 98.1 ± 7.8 µg/mL [18].

CONCLUSION

The vast diversity of marine habitats is why researchers to find novel metabolite producers. Herein, we report the isolation and assessment of antimicrobial and cytotoxic activities of marine fungi isolated from samples collected in Ly Son Island. Of these, 2 isolates, VM10 and VM25, displayed a broad spectrum of antibacterial activity against five to six tested pathogenic ATCC strains with low MIC values. Cytotoxic assays revealed that 4/44 extracts (VM10, VM15, VM25, and VM39) exhibited cytotoxicity against 3 cancer cell lines. These results suggest that VM10 and VM25 are potential natural antibacterial and cytotoxic secondary compound sources. The strains VM10 and VM25 taxonomy were identified using 18S rRNA gene sequence analysis and compared with fungal 18S rRNA sequences in the GenBank database by the NBCI Blast program. Their 18S rRNA gene sequences were registered on the GenBank database with the accession numbers PP425895 (Aspergillus versicolor VM10) and PP425869 (Metarhizium sp. VM25).

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