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ORIGINAL ARTICLE

Phylogenetic Diversity and Antibacterial Activity of Culturable Fungi Derived from the Zoanthid *Palythoa haddoni* in the South China Sea

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Abstract Investigation on diversity of culturable fungi mainly focused on sponges and corals, yet little attention had been paid to the fungal communities associated with zoanthid corals. In this study, a total of 193 culturable fungal strains were isolated from the zoanthid *Palythoa haddoni* collected in the South China Sea, of which 49 independent isolates were identified using both morphological characteristics and internal transcribed spacer (ITS) sequence analyses. Thirty-five strains were selected for phylogenetic analysis based on fungal ITS sequences. The results indicated that 18 genera within eight taxonomic orders of two phyla (seven orders of the phylum Ascomycota and one order of the phylum Basidiomycota) together with one unidentified fungal strain have been achieved, and *Cladosporium* sp. represented the dominant culturable genus. Particularly, 14 genera were isolated from a zoanthid for the first time. The antibacterial activities of organic extracts of mycelia and fermentation broth of 49 identified fungi were evaluated, and 29 (59.2 %) of the isolates displayed broad-spectrum or selective antibacterial activity. More interestingly, more than 60 % of the active fungal strains showed strong activity against two aquatic pathogenic bacteria *Nocardia brasiliensis* and *Vibrio parahaemolyticus*, compared with other pathogenic bacteria, indicating that zoanthid-derived fungi may protect its host against pathogens. This is the first report of systematically phylogenetic diversity and extensively antibacterial activity of zoanthid-derived fungi.

Keywords Cnidaria · Zoanthid · *Palythoa haddoni* · Marine fungi · Phylogenetic diversity · Antibacterial activity

Introduction

The biodiversity of the marine environment and the associated chemical diversity constitute a practically unlimited resource of new active substances in the field of the development of marine bioactive products (Carté 1996; Kobayashi and Tsuda 2004; Simmons et al. 2005; Strobel 2003). Fungi, as an important composition of marine microorganisms, have attracted more attention in recent years. Secondary metabolites from marine-derived fungi have proved to be rich sources of structurally novel and biologically active compounds that have become significant chemical entities for drug discovery (Blunt et al. 2014; Newman and Cragg 2012).

Marine fungi grow on a wide variety of substrata ranging from wood, sediments, soils, algae, corals, and calcareous tubes of mollusks to decaying leaves of mangroves, intertidal grasses, and living animals (Kohlmeyer and Kohlmeyer 1979; Hyde 1996). About 800–1,000 taxa of marine fungi representing a wide range of pathogens and symbionts had been identified from the complex inhabitants in coral reefs (Herndl and Weinbauer 2003). It has been reported that many marine invertebrates are marine filter feeders that can filter large volumes of surrounding water through a unique aquiferous system (Rohwer et al. 2001; Rohwer et al. 2002; Nithyanand et al. 2011). As a result, they become a rich

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reservoir of diverse, highly concentrated marine microorganisms, although some of which have not been cultured yet (Paul et al. 2009; Priess et al. 2000; Wang et al. 2008). Many host-derived metabolites had a striking similarity to known microbial metabolites, which suggested that many natural products from marine organisms could be of microbial origin (Faulkner 2002). An increasing number of bioactive metabolites are isolated from marine microorganisms derived from sponges, soft corals, and ascidians; accumulating evidence has been obtained for microorganisms as the real producers of bioactive metabolites originally isolated from their hosts (Schupp et al. 2002). Moreover, fungal diversity has been widely investigated into many marine organisms, such as sponges, corals and ascidians (Claudia et al. 2010; Ding et al. 2011; Paul et al. 2009; Priess et al. 2000; Xu et al. 2011; Wang et al. 2008; Zhang et al. 2012b), but fungi derived from zoanthids are very limited.

Zoanthids are found in a wide range of environments from shallow tropical coral reefs (Burnett et al. 1997) to cold seeps in the deep sea (Reimer et al. 2007), which includes three families. The family Sphenopidae includes the genus *Palythoa*. Neozoanthidae is monogeneric and monospecific and known only from Madagascar. The third family, the Zoanthidae, is the only zoanthid taxa that does not utilize encrustation and includes three genera, *Acrozoanthus*, *Isaurus*, and *Zoanthus. Palythoa* and *Zoanthus* in particular are quite popular on coral reefs and known as sources of palytoxin (*Palythoa*) (Moore and Scheuer 1971) and fluorescent proteins (*Zoanthus*) (Labas et al. 2002).

Investigation on the composition of zoanthid-fungal communities will lay a basis for the revelation of ecological function of zoanthid-fungi association. Therefore, it is very meaningful to investigate the fungi associated with zoanthids. It was reported that Brazilian marine-derived fungi have been isolated from the zoanthids *Palythoa caribaeorum*, *Palythoa variabilis*, and *Zoanthus solanderi* (Da Silva et al. 2008). Several representatives of *Aspergillus*, *Fusarium*, *Peacilomyces*, *Penicillium,* and *Trichoderma* were identified. Furthermore, two new indole alkaloids, 2-(3,3-dimethylprop-1-ene)-costaclavine and 2-(3,3-dimethylprop-1-ene)-epicostaclavine, together with three known compounds, costaclavine and fumgaclavines A and C, were obtained from the fungus *Aspergillus fumigatus* isolated from a zoanthid *Zoanthus* sp. collected from Amami Island, Japan (Zhang et al. 2012a). However, a systematic research on the diversity of fungi derived from zoanthids in the South China Sea has been rarely done. Recently, a pyrosequencing research on the diversity of microbes derived from the zoanthid *Palythoa australiae* in the South China Sea was reported (Sun et al. 2014). As part of our ongoing investigation on the culturable microorganisms derived from marine invertebrates, the marine fungi associated with the zoanthid *Palythoa haddoni* attracted our attention for their relatively high diversity and strong antibacterial activities.

This work aims to evaluate cultivable fungal diversity derived from the zoanthid *P. haddoni* in the South China Sea and antibacterial activities of fungal extracts. The diversity of fungi was evaluated based on morphologic observations and phylogenetic relationships of internal transcribed spacer (ITS)-ribosomal DNA (rDNA) sequences. Antibacterial activity of fungal extracts was evaluated against seven pathogenic bacteria and two aquatic pathogenic bacteria, and chemical investigation of three bioactive fungal strains was also studied.

Materials and Methods

Zoanthid Material

Colonies of the zoanthid *P. haddoni* was collected from coral reefs on Weizhou Island (109° 10′ E, 20° 54′ N) in the South China Sea in April, 2010 (Fig. 1). They were identified as *P. haddoni* (Anthozoa, Zoanthidea, Epizoanthidae, Palythoa) by Dr. Weizhou Chen at the Marine Biology Institute, Shantou University. The samples were placed in ziplock bags containing seawater, transported to the laboratory, and processed immediately for isolation and cultivation of fungi. Alternatively, the zoanthid was frozen and stored at −20 °C for future use.

Isolation of Zoanthid-Derived Fungi

To isolate fungi, the specimen was rinsed three times with sterile artificial seawater (ASW; Li and Liu 2006) to remove sediments and loosely attached microorganisms. The surface of specimen was sterilized with 75 % ethanol for 30 s and washed three times with sterile water. The washed sample was cut into 1 cm^3 pieces with a sterile scalpel and thoroughly homogenized using a blender containing 2 mL ASW under aseptic conditions. The resulting homogenate was diluted with ASW at three dilutions (1:10, 1:100, and 1:1,000). For fungal cultivation, 100 μL of each dilution was plated in quadruplicate onto corresponding medium (Table 1). The inoculated plates were cultured at 25 °C for 1–3 weeks and re-plated several times until the morphology of the fungi could be distinguished. Fungal isolates were chosen and transferred onto new corresponding agar plates on the basis of their morphological differences, especially the visible examination of growth characteristics, mycelia, and diffusible pigments. Benzylpenicillin (25 mg/mL), streptomycin (25 mg/mL), and rose bengal (33 mg/mL) were added to prohibit the growth of bacteria. The strains were deposited at the Key Laboratory of Marine Drugs, the Ministry of Education of China, School of Medicine and Pharmacy, Ocean University of China, Qingdao, People's Republic of China.

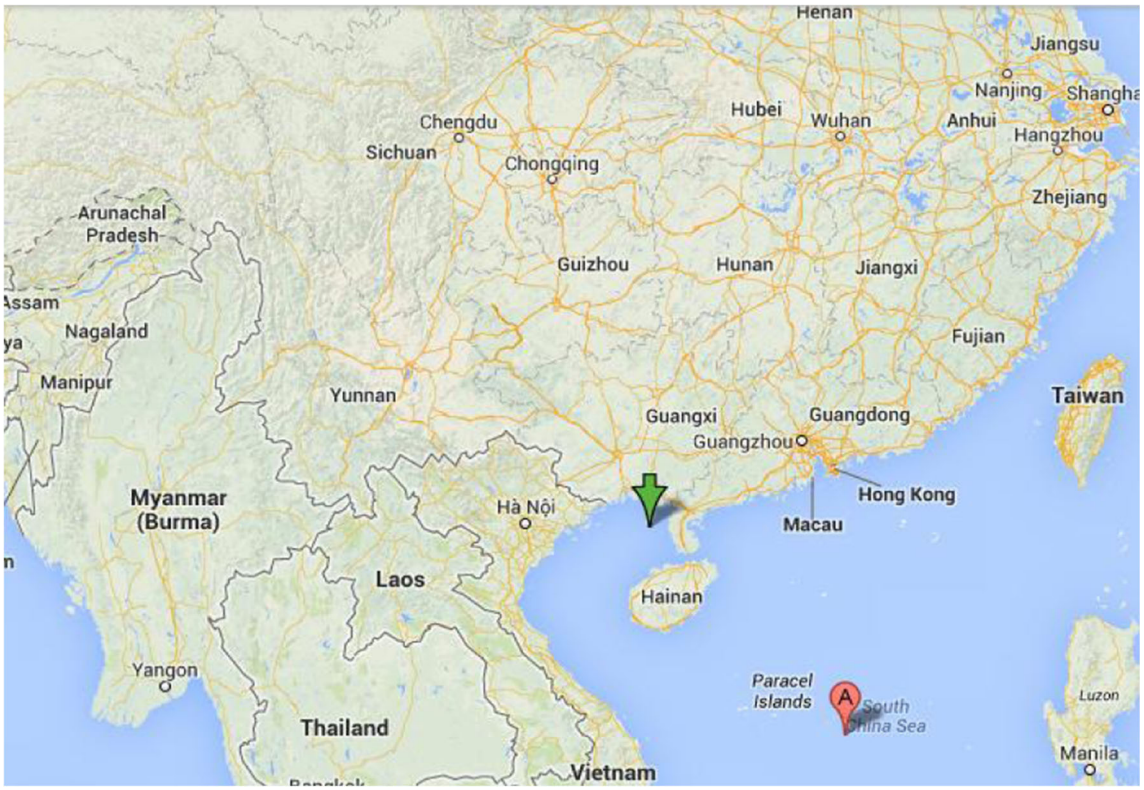


Fig. 1 Map of the South China Sea (*red arrow*) and location of the sampling site (*green arrow*)

DNA Extraction, PCR Amplification, and Sequencing

About 100 mg of fresh fungal mycelium was collected in an Eppendorf tube (1.5 mL) to extract genomic DNA from the fungus using the Fungal DNA kits (50) (E.Z.N.A., Omega) according to the manufacturer's protocol. The resulting genomic DNA was used as template to amplify the fungal ITS-rDNA gene fragment, and nearly full-length ITS sequences were amplified by polymerase chain reaction using primers ITS1F (5′-CTTGGTCATTTAGAGGAAGTAA-3′) (Gardes and Bruns 1993) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) (White et al. 1990). The PCR reactions were performed in a final volume of 50 μL, which was composed of template DNA (2 μL), 5 μL 10X PCR buffer, 1 μL dNTP, 0.5 μL ITS1F, 0.5 μL ITS4 (20 μmol/mL each), 0.25 μL Taq polymerase, and appropriate ultrapure water. Amplification reaction was carried out with the following thermal cycles profiles: 1 cycle for 5 min at 94 °C; then 30 cycles of 40 s at 94 °C, 40 s at 52 °C, and 60 s at 72 °C; and followed by a final extension at 72 °C for 10 min. Then, PCR products (5 μL) were loaded onto an agarose gel (1.2 % agarose in 0.5×TAE, 5 μL of ethidium bromide 1 %*m*/*v* solution per 100 mL of gel); then, they were isolated from a gel extraction kit (E.Z.N.A., Omega) according to the manufacturer's protocol after electrophoresis at 100 V for 35 min. PCR products were submitted for sequencing (Invitrogen, Shanghai) with the primer ITS4 or ITS1F. The sequence results were compared in GenBank using the Basic Local Alignment Search Tool.

Table 1 Composition of media used for fungal isolation

Medium	Composition (L^{-1})
Potato dextrose agar (PDA) medium	200 g potato extract[a], 20 g dextrose, 1 g peptone, 3 g K_2HPO_4, 15 g agar
Rose bengal medium (RBM)	5 g peptone, 10 g glucose, 0.033 g rose bengal, 1 g K_2HPO_4, 0.5 g $MgSO_4$, 15 g agar
Czapek-Dox medium	30 g sucrose, 15 g agar, 3 g $NaNO_3$, 0.5 g KCl, 1 g K_2HPO_4, 1.5 g $MgSO_4 \cdot 7H_2O$, 0.01 g $FeSO_4$, 0.5 g KCl
Luria Bertani (LB) agar medium[b]	10 g peptone, 5 g yeast extract, 10 g NaCl, 15 g agar

All media were prepared with artificial sterile seawater (ASW; Li and Liu 2006), and adjusted to pH 7.2 prior to autoclaving at 121 °C for 20 min

[a] The unpeeled potato (200 g) was washed, cut into pieces, and boiled in seawater for 30 min

[b] The LB medium was used in antibacterial activity test

Phylogenetic Analysis

Fungal ITS-rDNA sequences were edited with Lasergene Software SeqMan (DNAStar Inc.). For preliminary identification, sequences of fungal ITS-rDNA regions obtained from the zoanthid were compared with related sequences in the National Center for Biotechnology Information (NCBI). Each of these sequences was then aligned to sequences available in the NCBI database to determine the identity of the sequence. All fungal ITS sequences were aligned using the Clustal X (1.83) software, applying the default parameters (Thompson et al. 1997). The phylogenetic tree was generated using neighbor-joining (NJ) algorithms in the MEGA 4 software combined with bootstrap analysis using 1,000 replicates incorporating fungal sequences showing the highest homology to sequences amplified in this study.

Antibacterial Activity of Separated Fungi

The fungi were inoculated into 1-L Erlenmeyer flasks containing 400 mL of liquid medium (200 g of washed but unpeeled potato slices were boiled in 1 L seawater for 30 min, glucose 20 g in 1 L seawater) and cultured at 27 °C for 28 days without shaking. The fermented broth was separated through cheese cloth to obtain the filtrate and the mycelia. The filtrate was extracted three times with an equal volume ethyl acetate at room temperature and further concentrated in vacuo to give dried residues (l) prior to antimicrobial assays. The mycelia were extracted with methanol by aid of ultrasound to generate the mycelia extracts(s). The extracts were dissolved in dimethyl sulfoxide (DMSO) to give a stock solution (1 mg/mL). Three repeats were tested in every inhibition experiment. The antibacterial activities against nine bacteria, including seven pathogenic bacteria *Staphylococcus aureus* (ATCC 27154), *Staphylococcus epidermidis* (ATCC 12228), *Bacillus subtilis* (ATCC 6633), *Bacillus cereus* (ATCC 11077), *Kocuria rhizophila* (ATCC 9341), *Tetragenococcus halophilus* (ATCC 13623), and *Escherichia coli* (ATCC 25922) and two aquatic pathogenic bacteria *Nocardia brasiliensis* (ATCC 19019) and *Vibrio parahaemolyticus* (ATCC 17802) were determined by a serial dilution technique using 96-well microtiter plates. Bacteria were cultured overnight at 37 °C in Luria Bertani (LB) broth and diluted to 10^6 cfu/mL when being used. LB broth, DMSO, and ciprofloxacin were used as a blank control, a negative control, and a positive control, respectively. The plates were incubated at 37 °C for 24 h. The results were observed with a Labsystems MK3 at 630 nm.

Nucleotide Sequence Accession Number

Fungal ITS-rDNA sequence was deposited in GenBank under the accession number HM 565952 and numbers ranging from JF819129 to JF819176 (Tables 2 and 3).

Table 2 The classification of cultivable fungi associated with the zoanthid *P. haddoni*

	Phylum	Class	Order	Genus	Number
	Ascomycota	Dothideomycetes	*Capnodiales* (11)	*Cladosporium*	10
				Teratosphaeria	1
			Pleosporrales (18)	*Exserohilum*	7
				Cochliobolus	4
				Alternaria	3
				Leptosphaerulina	1
				Massarina	1
				Microsphaeropsis	1
				Stagonospora	1
		Sordariomycetes	*Trichosphaeriales* (7)	*Nigrospora*	7
			Hypocreales (8)	*Fusarium*	2
				Stachybotrys	2
				Trichoderma	2
				Myrothecium	1
				Unidentified	1
			Diaporthales (1)	*Phomopsis*	1
			Xylariales (1)	*Pestalotiopsis*	1
		Eurotiomycetes	*Eurotiales* (2)	*Penicillium*	2
	Basidiomycota	Agaricomycete	*Agaricales* (1)	*Coprinellus*	1
Total	2	4	8	18+ (unidentified)	49

Table 3 Phylogenetic affiliations of cultivable fungi associated with the zoanthid *P. haddoni*

Isolate ID	Order	Genus	Accession number	Closest identified relative	Identity (%)	Overlap (bp)
TA26-2	*Capnodiales*	*Cladosporium*	JF819130	*Cladosporium oxysporum* (EU759979)	100	500
TA26-5			JF819131	*Cladosporium oxysporum* (EU759979)	100	502
TA26-6			JF819132	*Cladosporium oxysporum* (JX156364)	99	528
TA26-7			JF819133	*Cladosporium cladosporioides* (AY361968)	99	490
TA26-10			JF819134	*Cladosporium sphaerospermum* (GU017501)	99	509
TA26-11			JF819135	*Cladosporium* sp. (JN546221)	100	502
TA26-12			JF819136	*Cladosporium colocasiae* (EU076964)	99	501
TA26-13			JF819137	*Cladosporium cladosporioides* (EF405864)	99	510
TA26-60			JF819174	*Cladosporium sphaerospermum* (GU017501)	99	503
TA26-61			JF819175	*Cladosporium oxysporum* (EU759979)	99	499
TA26-63		*Teratosphaeria*	JF819176	*Teratosphaeria* sp. (JN709043)	99	527
TA26-9	*Trichosphaeriale*	*Nigrospora*	HM565952	*Nigrospora* sp. (JN207298)	99	526
TA26-27			JF819148	*Nigrospora oryzae* (JQ863242)	99	505
TA26-32			JF819152	*Nigrospora oryzae* (HQ262527)	100	499
TA26-34			JF819154	*Nigrospora sphaerica* (HQ608063)	100	507
TA26-38			JF819158	*Nigrospora oryzae* (FJ487918)	99	504
TA26-42			JF819161	*Nigrospora oryzae* (FJ487918)	99	533
TA26-58			JF819172	*Nigrospora sphaerica* (HQ608063)	99	503
TA26-20	*Pleosporrales*	*Exserohilum*	JF819144	*Exserohilum rostratum* (FJ949084)	98	540
TA26-31			JF819151	*Exserohilum rostratum* (FJ949084)	99	554
TA26-41			JF819160	*Exserohilum* sp. (JN711431)	99	553
TA26-49			JF819166	*Exserohilum rostratum* (FJ949084)	99	540
TA26-52			JF819167	*Exserohilum rostratum* (FJ949084)	97	545
TA26-53			JF819168	*Exserohilum rostratum* (FJ949084)	99	552
TA26-54			JF819169	*Exserohilum* sp. (JQ388288)	99	399
TA26-15		*Cochliobolus*	JF819139	*Cochliobolus hawaiiensis* (JN601029)	100	520
TA26-17			JF819141	*Cochliobolus hawaiiensis* (JN601029)	100	547
TA26-43			JF819162	*Cochliobolus dactyloctenii* (AF158106)	99	515
TA26-46			JF819163	*Cochliobolus lunatus* (JN943462)	100	545
TA26-16		*Alternaria*	JF819140	*Alternaria tenuissima* (FJ949080)	99	540
TA26-37			JF819157	*Alternaria alternata* (AB693900)	99	522
TA26-47			JF819164	*Alternaria alternate* (AB693900)	99	516
TA26-14		*Leptosphaerulina*	JF819138	*Leptosphaerulina* sp. (HM771013)	99	540
TA26-18		*Massarina*	JF819142	*Massarina* sp. (JQ889692)	98	522
TA26-19		*Microsphaeropsis*	JF819143	*Microsphaeropsis arundinis* (JQ647902)	99	540
TA26-24		*Stagonospora*	JF819146	*Stagonospora* sp. (EU009968)	97	543
TA26-23	*Hypocreales*	Unidentified	JF819145	Unidentified fungus clone (GU721260)	91	522
TA26-55		*Stachybotrys*	JF819170	*Stachybotrys* sp. (JX077027)	98	525
TA26-59			JF819173	*Stachybotrys* sp. (JX077027)	99	527
TA26-30		*Fusarium*	JF819150	*Fusarium chlamydosporum* (GU134902)	99	520
TA26-56			JF819171	*Fusarium chlamydosporum* (GU134902)	100	490
TA26-28		*Trichoderma*	JF819149	*Trichoderma aureoviride* (EU816400)	99	540
TA26-36			JF819156	*Trichoderma aureoviride* (EU816400)	99	540
TA26-35		*Myrothecium*	JF819155	*Myrothecium masonii* (AY254153)	99	530
TA26-1	*Eurotiales*	*Penicillium*	JF819129	*Penicillium sclerotiorum* (HM595498)	99	550
TA26-48			JF819165	*Penicillium oxalicum* (JQ946374)	99	537
TA26-26	*Agaricales*	*Coprinellus*	JF819147	*Coprinellus radians* (HM595561)	99	540
TA26-39	*Diaporthales*	*Phomopsis*	JF819159	*Phomopsis liquidambari* (HQ328002)	99	526
TA26-33	*Xylariales*	*Pestalotiopsis*	JF819153	*Pestalotiopsis uvicola* (FJ790875)	99	550

Results

Phylogenetic Diversity of Culturable Fungi Associated with Zoanthid

A total of 193 isolates were isolated from the zoanthid *P. haddoni*, and morphological traits were examined to exclude reduplicate strains by the published taxonomic keys (Kohlmeyer and Kohlmeyer 1979; Kohlmeyer and Volkmann-Kohlmeyer 1991; Hyde et al. 2000). Consequently, 49 independent strains were selected for sequencing and identification based on ITS sequences. According to the sequences deposited into NCBI, among the 49 identified fungi, 48 belonged to the phylum Ascomycota including seven taxonomic orders: *Capnodiales*, *Diaporthales*, *Eurotiales*, *Hypocreales*, *Pleosporrales*, *Trichosphaeriales*, and *Xylariales* (Table 2). Particularly, one representative strain TA26-26 was grouped in the genus *Coprinellus* of the order *Agaricales*, the phylum Basidiomycota. These identified fungi and their best matches in the NCBI database are summarized in Table 3. Most of the isolates matched their closest relatives with 98 to 100 % similarity except for TA26-23 (91 %), TA26-24 (97 %), and TA26-52 (97 %). It should be noted that the strain TA26-23 was unsuccessfully identified based on fungal ITS sequences.

Further phylogenetic analysis was carried out on 35 strains because we selected the representative strains which may belong to different fungal species after we aligned the sequences with the Clustal X software (Fig. 2). In total, all identified fungi belonged to 18 genera in eight orders and two phyla: *Alternaria*, *Cladosporium*, *Cochliobolus*, *Coprinellus*, *Exserohilum*, *Fusarium*, *Leptosphaerulina*, *Massarina*, *Microsphaeropsis*, *Myrothecium*, *Nigrospora*, *Penicillium*, *Pestalotiopsis*, *Phomopsis*, *Stachybotrys*, *Stagonospora*, *Teratosphaeria*, and *Trichoderma* as well as one unidentified strain (TA26-23) (Table 3). *Pleosporrales* was the dominant group of the identified fungi accounting for 36.7 % in this study. The fungal community was dominated by *Cladosporium*, comprising 10 isolates, accounting for 20.4 %, followed by *Exserohilum* and *Nigrospora* with seven isolates, respectively (Fig. 3). Most of the remaining genera occurred as singletons or doubletons.

Screening of Antibacterial Activities of the Extracts from Fungal Broth and Mycelia

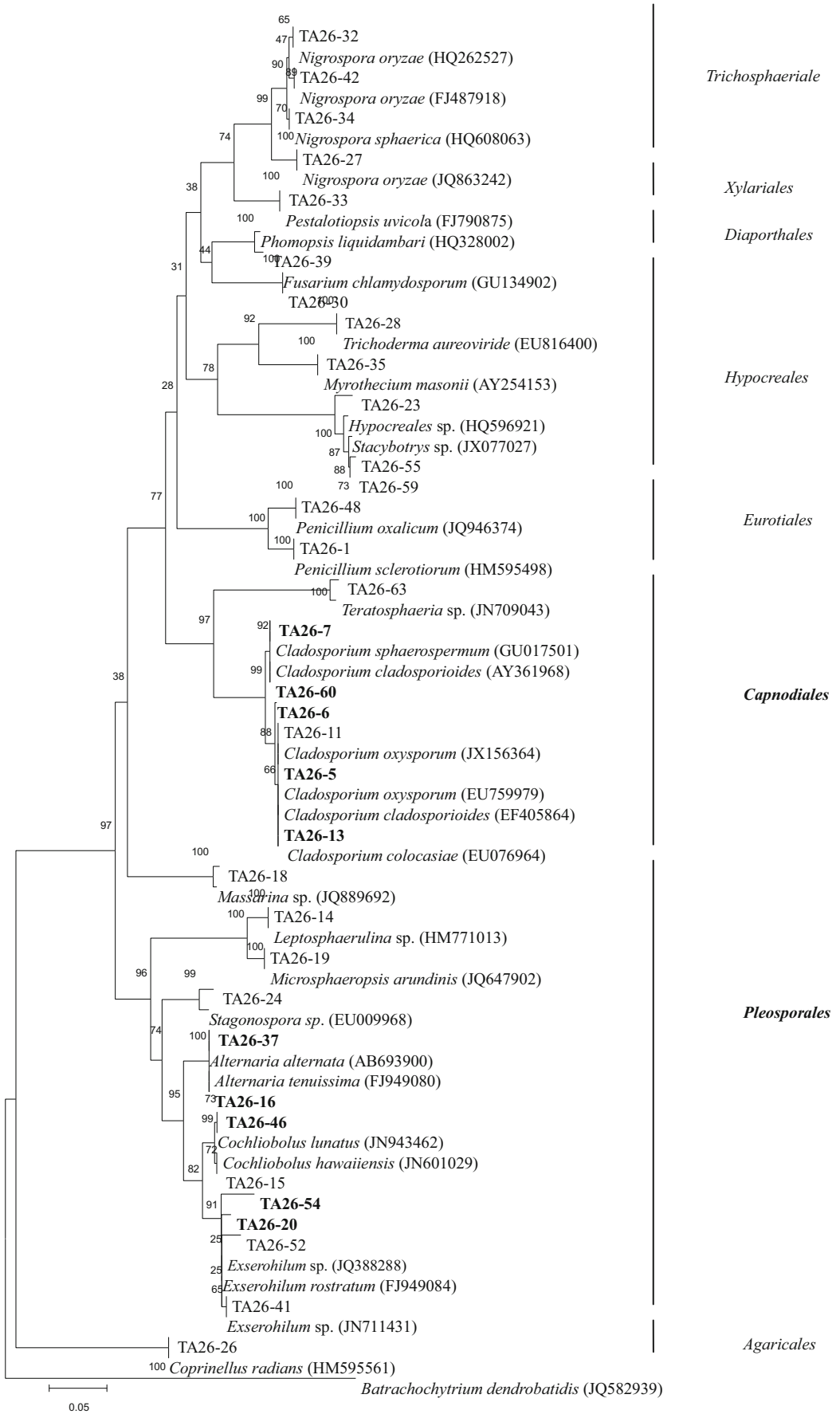
Organic extracts of fermentation broth and mycelia of the 49 identified fungi were screened for antibacterial activities against nine indicator bacteria with the organic extracts from the fermentation broth and mycelia. As showed in Table 4, 29 strains (59.2 %) of the identified fungi displayed different levels of antibacterial activities against the tested pathogenic bacteria. Additionally, mycelia extracts exhibited higher inhibitory activities than their fermentation broth in most cases. Interestingly, among all the active fungal strains, more than 60 % isolates showed equal or stronger activities against all three Gram-negative bacteria *E. coli*, *N. brasiliensis*, and *V. parahaemolyticus* than the positive control ciprofloxacin.

As shown in Table 4, the difference in the antibacterial activity of fungi was found between different genera and strains. Fungi classified within the orders of *Capnodiales* and *Pleosporrales* showed the main contribution to the antibacterial activity against two aquatic pathogenic bacteria *N. brasiliensis* and *V. parahaemolyticus*. Additionally, five fungal isolates, *Cladosporium* (TA26-2 and TA26-6), *Nigrospora* (TA26-9 and TA26-58), and *Fusarium* (TA26-56) displayed strong (+++) antibacterial activity toward all the tested bacteria. It suggested that these strains could produce interesting and useful antibacterial compounds. Two isolates (TA26-16 and TA26-20) strongly inhibited all tested bacteria except for *S. epidermidis*, which were grouped into *Alternaria* and *Exserohilum*, respectively. Furthermore, *Cochliobolus* (TA26-43 and TA26-46) and *Exserohilum* (TA26-20, TA26-53, and TA26-54) had strong antibacterial activity against *S. aureus*, *T. halophilus*, *E. coli*, *N. brasiliensis*, and *V. parahaemolyticus*. It seemed that all the identified fungi belonging to the genera *Alternaria*, *Cochliobolus*, and *Exserohilum* in the order *Pleosporrales* were not active against *S. epidermidis*. In contrast, all fungi (TA26-9, TA26-27, TA26-38, TA26-42, and TA26-58) in the genus *Nigrospora* showed selective and strong inhibitory activity against *S. epidermidis*. Most of the remaining active singletons or doubletons showed a weak activity against several bacteria or only strong activity against one or two tested bacteria.

Fig. 2 Neighbor-joining phylogenetic tree based on ITS sequences of cultured fungi associated with the cnidarian zoanthid *P. haddoni*. The values at each node represent the bootstrap values from 1,000 replicates, and the *scale bar*=0.05 substitutions per nucleotide. *Batrachochytrium dendrobatidis* JQ582939 was used as outgroup. Strains with strong activity against two aquatic pathogenic bacteria are in *bold*

Discussion

The chemistry and biology of palytoxin and its analogs from the genus *Zoanthus* have been concentrated in the past three decades (Moore and Bartolini 1981; Moore and Scheuer 1971; Ciminiello et al. 2009; Ciminiello et al. 2014), while investigation on phylogenetic diversity of zoanthid-associated fungi is relatively rare (Da Silva et al. 2008; Zhang et al. 2012a; Sun et al. 2014). In this study, 49 of the 193 isolates were selected for sequence excluding some reduplicate strains by the direct observations of sporulating structure. Forty-eight


TA26-32
Nigrospora oryzae (HQ262527)
TA26-42
Nigrospora oryzae (FJ487918)
TA26-34
Nigrospora sphaerica (HQ608063)
TA26-27
Nigrospora oryzae (JQ863242)
TA26-33
Pestalotiopsis uvicola (FJ790875)
Phomopsis liquidambari (HQ328002)
TA26-39
Fusarium chlamydosporum (GU134902)
TA26-30
TA26-28
Trichoderma aureoviride (EU816400)
TA26-35
Myrothecium masonii (AY254153)
TA26-23
Hypocreales sp. (HQ596921)
Stacybotrys sp. (JX077027)
TA26-55
TA26-59
TA26-48
Penicillium oxalicum (JQ946374)
TA26-1
Penicillium sclerotiorum (HM595498)
TA26-63
Teratosphaeria sp. (JN709043)
TA26-7
Cladosporium sphaerospermum (GU017501)
Cladosporium cladosporioides (AY361968)
TA26-60
TA26-6
TA26-11
Cladosporium oxysporum (JX156364)
TA26-5
Cladosporium oxysporum (EU759979)
Cladosporium cladosporioides (EF405864)
TA26-13
Cladosporium colocasiae (EU076964)
TA26-18
Massarina sp. (JQ889692)
TA26-14
Leptosphaerulina sp. (HM771013)
TA26-19
Microsphaeropsis arundinis (JQ647902)
TA26-24
Stagonospora sp. (EU009968)
TA26-37
Alternaria alternata (AB693900)
Alternaria tenuissima (FJ949080)
TA26-16
TA26-46
Cochliobolus lunatus (JN943462)
Cochliobolus hawaiiensis (JN601029)
TA26-15
TA26-54
TA26-20
TA26-52
Exserohilum sp. (JQ388288)
Exserohilum rostratum (FJ949084)
TA26-41
Exserohilum sp. (JN711431)
TA26-26
Coprinellus radians (HM595561)
Batrachochytrium dendrobatidis (JQ582939)
0.05
Trichosphaeriale
Xylariales
Diaporthales
Hypocreales
Eurotiales
Capnodiales
Pleosporales
Agaricales

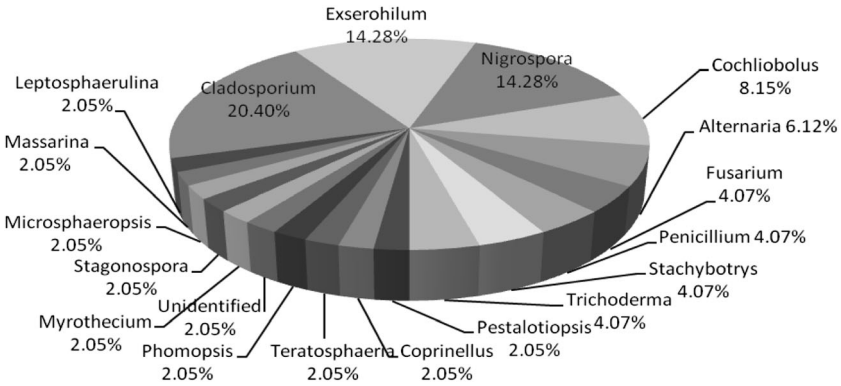


Fig. 3 Percentage of genus of cultivable fungi associated with cnidarian zoanthid *P. haddoni*

identified fungi were successfully classified at the genus level based on ITS region sequences with relatives in the NCBI database except for one isolate (TA26-23) (Table 3). A previous study reported that only eight genera (*Aspergillus*, *Fusarium*, *Peacilomyces*, *Penicillium*, *Trichoderma*, *Cladosporium*, *Eutypella*, and *Khuskia*) were isolated from three Brazilian zoanthids *P. caribaeorum*, *P. variabilis*, and *Z. solanderi* (Da Silva et al. 2008). Another study demonstrated that the fungus *A. fumigatus* was isolated from a zoanthid *Zoanthus* sp. collected from Amami Island, Japan (Zhang et al. 2012a). Recently, the microbial community associated with the zoanthid *P. australiae* in the South China Sea was investigated using 454 pyrosequencing. Using this technology, the bacterial diversity was revealed fully, and 2,353 bacterial, 583 archaeal, and 36 eukaryotic microbial ribotypes were detected, respectively (Sun et al. 2014). Eighteen genera were isolated from *P. haddoni* and 14 genera of them have not been previously reported from zoanthids (Table 3, Fig. 2). The above results indicated that a high diversity of fungi can be recovered from zoanthids in the South China Sea.

So far, few fungi of the phylum Basidiomycota have been isolated from marine invertebrates, such as gorgonians and sponges (Ding et al. 2011; Baker et al. 2009). In this study, only one isolate TA26-26 in the phylum Basidiomycota was culturable, the remaining 47 strains belonged to the phylum Ascomycota. Basidiomycota fungi could be very difficult to culture than *Ascomycota* fungi. Identification of strains TA26-23 (JF819145) was unsuccessful, but it was definitely different from other strains. However, it showed 91 % similarity with an uncultured fungal clone (GU721260) when compared with sequences in NCBI. The low sequence similarity and phylogenetic analysis suggested that it might belong to a novel taxon. Some genera, to which the novel isolates belong, are well-known and prolific metabolite producers (Iwatsuki et al. 2006; Wicke et al. 2000). The novel strain may produce a number of commercially interesting and potentially useful products.

The fungal community associated with the zoanthid *P. haddoni* was dominated by *Cladosporium*, followed by *Exserohilum* and *Nigrospora*. A comprehensive study of fungal diversity of gorgonians, soft corals, sponges, and other invertebrates in coral reef ecosystems indicated that *Aspergillus* and *Penicillium* are the very common genera (Ding et al. 2011; Wang et al. 2011; Zuluaga-Montero et al. 2010). In this research, no *Aspergillus* fungi were identified from the zoanthid *P. haddoni*, while *Penicillium* was not the dominant genus. It should be noted that *Cladosporium* is the dominant genus in *P. haddoni*. Furthermore, it was reported that fungi of the genus *Cladosporium* displayed antibacterial, antitumoral, and antioxidant activities (Wang et al. 2011; Zhang et al. 2012b; Henríquez et al. 2014), indicating a potential source of bioactive natural products, which may have important chemical and ecological significance in the chemical defensive system of marine invertebrates. The existence of a relatively large number of *Cladosporium* fungi might play an important role during the long-term survival options for the zoanthid *P. haddoni*.

In an investigation on the antibacterial activity of organic extracts of the culturable fungi from *P. haddoni*, more than half of the identified fungi displayed different levels of antibacterial activity against pathogenic bacteria in their fermentation broth and/or mycelia (Table 4), suggesting that the culturable fungi could fend off or develop resistance to certain microbial diseases of zoanthids. A few previous reports stated that 20–70 % of culturable microorganisms in marine invertebrates exhibited antimicrobial activity (Nithyanand and Pandian 2009; Shnit-Orland and Kushmaro 2009). It was reported that fungi associated with the gorgonian coral *Echinogorgia rebekka* (Wang et al. 2011), and culturable microorganisms associated with the South China Sea black coral *Antipathes dichotoma* (Zhang et al. 2012b), exhibited moderate to potent antibacterial activities to some pathogenic bacteria. In addition, the fungi associated with Antarctic sponges, particularly *Geomyces*, would be valuable sources of antimicrobial and antitumor compounds (Henríquez et al. 2014). In this report, 59.2 % fungi showed antibacterial activities which agreed with a few previous reports showing that 20–70 % of culturable microorganisms in marine invertebrates exhibited antimicrobial activity (Nithyanand and Pandian 2009; Shnit-Orland and Kushmaro 2009). More than

Table 4 Antibacterial activity of the EtOAc extracts from the fermentation broth and mycelia of the active fungi

Fungal strain			*B. subtilis*		*B. cereus*		*K. rhizophila*		*S. epidermidis*		*S. aureus*		*T. halophilus*		*E. coli*		*N. brasiliensis*		*V. parahaemolyticus*	
Order	Genus	Strain	s	l	s	l	s	l	s	l	s	l	s	l	s	l	s	l	s	l
Capnodiales	*Cladosporium*	TA26-2	+++	−	+	−	+++	−	+	−	+++	−	+++	+	+++	−	+++	+	+++	+++
		TA26-5	+	−	−	−	−	−	−	−	−	−	+++	+++	+++	−	+++	+++	+++	+++
		TA26-6	+++	−	+++	−	+++	−	+++	−	+++	−	+++	+++	+++	−	+++	+++	+++	+++
		TA26-7	+	−	−	−	+++	−	+++	−	−	−	−	+++	−	−	+++	+++	+	+++
		TA26-13	+++	−	+	−	−	−	−	−	+++	−	+++	−	+++	−	+++	−	+++	−
		TA26-60	+	−	+	−	−	−	−	−	+++	+++	+++	−	−	−	+++	−	+++	−
		TA26-61	+++	+	+++	+	+++	−	−	−	−	−	+++	+++	+++	+++	+++	+	+++	+
	Teratosphaeria	TA26-63	−	−	−	−	−	−	−	+++	−	−	+	−	−	−	+	−	+++	−
Pleosporrales	*Cochliobolus*	TA26-43	+	+	+	−	−	+	−	−	+++	−	+++	+++	+++	−	+++	−	+++	+
		TA26-46	+++	+	−	−	+	−	−	−	+	−	+++	−	+	−	+++	−	+++	−
	Alternaria	TA26-16	+++	+++	+++	+++	+++	+++	−	−	+++	+++	+++	+++	+++	+++	+	+++	+	+++
		TA26-37	+	−	−	−	−	−	−	−	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
	Exserohilum	TA26-20	+++	−	+++	+	+++	+++	−	−	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
		TA26-52	+++	−	+++	+	−	−	−	−	−	−	+++	−	+++	−	−	−	+++	+
		TA26-53	−	−	+	−	−	−	−	−	+++	+++	+++	−	+++	+++	+++	+++	+	+++
		TA26-54	+++	+	−	−	−	−	−	−	+++	−	+++	+++	+++	−	+++	+++	+++	+
	Stagonospora	TA26-24	+	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	+
Hypocreales	*Hypocreales*	TA26-55	−	+	+	+	−	+++	+++	−	−	+	−	−	+	+	−	+	−	−
		TA26-59	−	−	+	−	−	+++	−	−	−	+++	+	−	−	+	−	−	+	−
	Trichoderma	TA26-28	+++	−	+++	−	+	−	−	−	−	−	+	+	+++	−	+	+++	+	−
	Myrothecium	TA26-35	−	−	−	−	−	+	+	−	−	+	−	−	−	+	+	+	−	−
	Fusarium	TA26-56	+++	−	+++	−	+++	+++	+++	−	+++	+++	−	+++	+++	+++	+++	−	+++	−
Trichosphaeriales	*Nigrospora*	TA26-9	+++	+++	+++	+++	−	+	+	+++	+	+++	+	+++	+++	+++	+	−	+++	+++
		TA26-27	−	+++	−	+	−	+	+++	+	−	−	−	−	+++	+++	+++	+++	+++	+++
		TA26-38	−	−	−	−	−	−	+++	+++	−	−	−	−	−	−	+	−	−	−
		TA26-42	−	−	−	−	−	−	+++	+++	−	−	−	−	−	−	−	−	+	+
		TA26-58	−	+++	−	+++	−	+	+++	+	−	+++	−	+	−	+++	+	+++	+++	+++
Eurotiales	*Penicillium*	TA26-1	+	−	−	−	+++	−	−	+	−	−	+	−	−	−	−	+	+++	−
		TA26-48	−	−	−	−	+	−	+++	−	+	+	−	−	−	−	+++	−	−	+
Diaporthales	*Phomopsis*	TA26-39	−	−	−	+	−	−		+++	−	−	−	−	−	−	+	−	−	−

Positive control: ciprofloxacin (20 μM); negative control: dimethyl sulfoxide (DMSO)

s the exacts from the fungal mycelia (1 mg/mL); *l* the exacts from the fermentation broth (1 mg/mL); – no antibacterial activity; + weak inhibitory activity, the activity between the positive contrast and negative contrast; +++ strong inhibitory activity (equivalent or stronger compared with those of the positive control), no bacteria can grow in the 96-well microtiter plates

60 % of the active fungal strains isolated from *P. haddoni* which were classified within the orders *Capnodiales* and *Pleosporrales* showed strong inhibition against the two pathogenic bacteria *N. brasiliensis* and *V. parahaemolyticus* (Table 4; Fig. 2). Fungi classified within the order *Pleosporrales* have been shown to produce novel antimicrobial compounds (Bhadury et al. 2006; Bugni and Ireland 2004; Paul et al. 2009). Although no report showed that fungi of the order *Capnodiales* possess antimicrobial activity, the genus *Cladosporium* displayed relatively high antibacterial activity in some studies (Wang et al. 2011; Zhang et al. 2012b; Henríquez et al. 2014). Fungi associated with the zoanthid *P. haddoni* showed significant activity against several bacteria, especially two aquatic pathogenic bacteria *N. brasiliensis* and *V. parahaemolyticus*, suggesting that fungi could participate in avoiding their hosts subjected to marine erosion and infection of pathogens.

It should be mentioned that we have studied the secondary metabolites of three highly antibacterial strains *Nigrospora* sp. (TA26-9), *Cochliobolus* sp. (TA26-46), and *Trichoderma* sp. (TA26-28). Ten antibacterial hydroanthraquinone analogs, including two new compounds, 4α-*epi*-9α-methoxydihydrodeoxybostrycin and 10-deoxybostrycin, were obtained from the strain *Nigrospora* sp. (TA26-9) and their structure-activity relationships were also discussed. More importantly, 3-acetoxy-4-deoxybostrycin exhibited a promising activity against *B. cereus* with an MIC value of 48.8 nM, which was stronger than that of the positive control ciprofloxacin (MIC 1,250 nM) (Yang et al. 2012). Moreover, a series of 14-membered resorcylic acid lactones (RALs), including three new metabolites, cochliomycins isolated from the gorgonian-derived fungus *Cochliobolus lunatus*, was also recently isolated from a zoanthid-derived *Cochliobolus* sp. (TA26-46). Most RALs showed potent antifouling activity against the barnacle *Balanus amphitrite* larvae (Shao et al. 2011). Consequently, these zoanthid-derived fungi may produce antibacterial compounds that could be used against emerging medical pathogens.

In summary, a total of 193 culturable fungal strains were isolated from a zoanthid *P. haddoni* in the South China Sea, of which 49 independent isolates were identified. Eighteen genera within eight taxonomic orders of two phyla (seven orders of the phylum Ascomycota and one order of the phylum Basidiomycota) together with one unidentified fungal strain have been achieved, and *Cladosporium* sp. represented the dominant culturable fungi. Particularly, 14 genera were isolated from zoanthids for the first time. Twenty nine of the isolates (59.2 %) displayed a broad-spectrum or selective antibacterial activity. More interestingly, more than 60 % of the active fungal strains showed a strong activity against two aquatic pathogenic bacteria *N. brasiliensis* and *V. parahaemolyticus*. This is the first report of systematically phylogenetic diversity and extensively antibacterial activity of zoanthid-derived fungi. Our study may contribute to our knowledge of zoanthid-derived fungi, and continued investigation of these unexplored fungi represents a promising strategy for a significant resource of potential structurally interesting molecules.

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References

Baker PW, Kennedy J, Dobson A, Marchesi JR (2009) Phylogenetic diversity and antimicrobial activities of fungi associated with *Haliclona simulans* isolated from Irish coastal waters. Mar Biotechnol 11:540–547

Bhadury P, Mohammad BT, Wright C (2006) The current status of natural products from marine fungi and their potential as anti-infective agents. J Ind Microbiol Biotechnol 33:325–337

Blunt JW, Copp BR, Keyzers RA, Munroa MHG, Prinsep MR (2014) Marine natural products. Nat Prod Rep 31:160–258

Bugni TS, Ireland CM (2004) Marine-derived fungi: a chemically and biologically diverse group of microorganisms. Nat Prod Rep 21: 143–163

Burnett WJ, Benzie JAH, Beardmore JA, Ryland JS (1997) Zoanthids (Anthozoa, Hexacorallia) from the Great Barrier Reef and Torres Strait, Australia: systematics, evolution and a key to species. Coral Reefs 16:55–68

Carté BK (1996) Biomedical potential of marine natural products. BioScience 46:271–286

Ciminiello P, Dell'Aversano C, Dello Iacovo E, Fattorusso E, Forino M, Grauso L, Tartaglione L, Florio C, Lorenzon P, De Bortoli M, Tubaro A, Poli M, Bignami G (2009) Stereostructure and biological activity of 42-hydroxy-palytoxin: a new palytoxin analogue from Hawaiian *Palythoa* subspecies. Chem Res Toxicol 22:1851–1859

Ciminiello P, Dell'Aversano C, Dello Iacovo E, Forino M, Tartaglione L, Pelin M, Sosa S, Tubaro A, Chaloin O, Poli M, Bignami G (2014) Stereoisomers of 42-hydroxy palytoxin from Hawaiian *Palythoa toxica* and *P. tuberculosa*: stereostructure elucidation, detection, and biological activities. J Nat Prod 77:351–357

Claudia M, Rafaella CB, Paula B, Michel P, Carlos S, Mariana RJ, Rebeca RL, Fabiana FG, Valeria MO, Roberto GB, Lara DS (2010) Microbial diversity associated with algae, ascidians and sponges from the north coast of Sao Paulo state, Brazil. Microbiol Res 165:465–482

Da Silva M, Passarini MRZ, Bonugli RC, Sette LD (2008) Cnidarian-drived filamentous fungi from Brazil: isolation, characterisation and RBBR decolourisation screening. Environ Technol 29:1331–1339

Ding B, Yin Y, Zhang FL, Li ZY (2011) Recovery and phylogenetic diversity of culturable fungi associated with marine sponges *Clathrina luteoculcitella* and *Holoxea* sp. in the South China Sea. Mar Biotechnol 13:713–721

Faulkner DJ (2002) Marine natural products. Nat Prod Rep 19:1–48

Gardes M, Bruns TD (1993) ITS primer with enhaneeds specificity for basidiomycetes application to the identification of mycorrizae-andrusts. Mol Ecol 2:113–118

Henríquez M, Vergara K, Norambuena J, Beiza A, Maza F, Ubilla P, Araya I, Chávez R, San-Martín A, Darias J, Darias MJ, Vaca I

(2014) Diversity of cultivable fungi associated with Antarctic marine sponges and screening for their antimicrobial, antitumoral and antioxidant potential. World J Microbiol Biotechnol 30:65–76

Herndl GL, Weinbauer MG (2003) Marine microbial food web structure and function. In: Wefer G, Lamy F, Mantoura F (eds) Marine science frontiers for Europe 265–277

Hyde KD (1996) Marine fungi. In: Grgurinovic C, Mallett K (eds) Fungi of Australia, Vol 1B 39–64

Hyde KD, Sarma VV, Jones EBG (2000) Morphology and taxonomy of higher marine fungi. In: Hyde KD, Pointing SB (eds) Marine mycology: a practical approach. Fungal Diversity Press, Hong Kong, pp 172–204

Iwatsuki M, Tomoda H, Uchida R, Gouda H, Hirono S, Lariatins ŌS (2006) Antimycobacterial peptides produced by *Rhodococcus* sp. K01−B0171, have a lasso structure. J Am Chem Soc 128:7486–7491

Kobayashi J, Tsuda M (2004) Bioactive products from Okinawan marine micro- and macroorganisms. Phytochem Rev 3:267–274

Kohlmeyer J, Kohlmeyer E (1979) Marine mycology—the higher fungi. Academic, New York, pp 1–690

Kohlmeyer J, Volkmann-Kohlmeyer B (1991) Illustrated key to the filamentous higher marine fungi. Bot Mar 34:1–61

Labas YA, Gurskaya NG, Yanushevich YG, Fradkov AF, Lukyanov KA, Lukyanov SA, Matz MV (2002) Diversity and evolution of the green fluorescent protein family. Proc Natl Acad Sci U S A 99: 4256–4261

Li ZY, Liu Y (2006) Marine sponge *Craniella austrialiensis* associated bacterial diversity revelation based on 16S rDNA library and biologically active actinomycetes screening, phylogenetic analysis. Lett Appl Microbiol 43:410–416

Moore RE, Bartolini G (1981) Structure of palytoxin. J Am Chem Soc 103:2491–2494

Moore RE, Scheuer PJ (1971) Palytoxin: a new marine toxin from a coelenterate. Science 172:495–498

Newman DJ, Cragg GM (2012) Natural products as sources of new drugs over the 30 years from 1981 to 2010. J Nat Prod 75:311–335

Nithyanand P, Pandian SK (2009) Phylogenetic characterization of culturable bacterial diversity associated with the mucus and tissue of the coral *Acropora digitifera* from Gulf of Mannar. FEMS Microbiol Ecol 69:384–394

Nithyanand P, Indhumathi T, Ravi AV, Pandian SK (2011) Culture independent characterization of bacteria associated with the mucus of the coral *Acropora digitifera* from the Gulf of Mannar. World J Microbiol Biotechnol 27:1399–1406

Paul WB, Jonathan K, Alan WD, Julian RM (2009) Phylogenetic diversity and antimicrobial activities of fungi associated with *Haliclona simulans* isolated from Irish Coastal Waters. Mar Biotechnol 11: 540–547

Priess K, Le Campion-Alsumard T, Golubic S, Gadel F, Thomassin BA (2000) Fungi in corals: black bands and density-banding of *Porites lutea* and *P. lobata* skeleton. Mar Biol 136:19–27

Reimer JD, Hirano S, Fujiwara Y, Sinniger F, Maruyama T (2007) Morphological and molecular characterization of *Abyssoanthus nankaiensis*, a new family, new genus and new species of deep-sea zoanthid (Anthozoa: Hexacorallia: Zoantharia) from a northwest Pacific methane cold seep. Invert Syst 21:255–262

Rohwer F, Breitbart M, Jara MJ, Azam F, Knowlton N (2001) Diversity of the bacteria associated with Caribbean coral *Montastraea franksi*. Coral Reefs 20:85–91

Rohwer F, Seguritan V, Azam F, Knowlton N (2002) Diversity and distribution of coral-associated bacteria. Mar Ecol Prog Ser 243:1–10

Schupp P, Proksch P, Wray V (2002) Further new staurosporine derivatives from the ascidian *Eudistoma toealensis* and its predatory flatworm *Pseudoceros* sp. J Nat Prod 65:295–298

Shao CL, Wu HX, Wang CY, Liu QA, Xu Y, Wei MY, Qian PY, Gu YC, Zheng CJ, She ZG, Lin YC (2011) Potent antifouling resorcylic acid lactones from the gorgonian-derived fungus *Cochliobolus lunatus*. J Nat Prod 74:629–633

Shnit-Orland M, Kushmaro A (2009) Coral mucus-associated bacteria: a possible first line of defense. FEMS Microbiol Ecol 67:371–380

Simmons TL, Andrianasolo E, McPhail K, Flatt P, Gerwick WH (2005) Marine natural products as anticancer drugs. Mol Cancer Ther 4: 333–342

Strobel GA (2003) Endophytes as sources of bioactive products. Microbes Infect 5:535–544

Sun W, Zhang FL, He LM, Li ZY (2014) Pyrosequencing reveals diverse microbial community associated with the zoanthid *Palythoa australiae* from the South China Sea. Microbial Ecol 67:942–950

Thompson JD, Gibson, TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The Clustal X Windows interface: Flexible strategies for multible sequence alignment aided by quality analysis tools. Nucleic Acids Res 24:4876–4882

Wang GY, Li QZ, Zhu P (2008) Phylogenetic diversity of culturable fungi associated with the Hawaiian sponges *Suberites zeteki* and *Gelliodes fibrosa*. Antonie Van Leeuwenhoek 93:163–174

Wang YN, Shao CL, Zheng CJ, Chen YY, Wang CY (2011) Diversity and antibacterial activities of fungi derived from the gorgonian *Echinogorgia rebekka* from the South China Sea. Mar Drugs 9: 1379–1390

White TJ, Bruns TD, Lee SB (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds) PCR protocols: A guide to methods and applications. Academic Press, New York, pp 315–322

Wicke C, Hüners M, Wray V, Nimtz M, Bilitewski U, Lang S (2000) Production and structure elucidation of glycoglycerolipids from a marine sponge-associated *Microbacterium* species. J Nat Prod 63: 621–626

Xu J, Chen B, Lei XL, She ZG, Xiao BH (2011) Phylogenetic diversity analysis of cultured symbiotic fungi of *Galaxea fascicularis* L. Microbiol China 38:1193–1198

Yang KL, Wei MY, Shao CL, Fu XM, Guo ZY, Xu RF, Zheng CJ, She ZG, Lin YC, Wang CY (2012) Antibacterial anthraquinone derivatives from a sea anemone-derived fungus *Nigrospora* sp. J Nat Prod 75:935–941

Zhang D, Satake M, Fukuzawa S, Sugahara K, Niitsu A, Shirai T, Tachibana K (2012a) Two new indole alkaloids, 2-(3,3-dimethylprop-1-ene)-costaclavine and 2-(3,3-dimethylprop-1-ene)-epicostaclavine, from the marine-derived fungus *Aspergillus fumigatus*. J Nat Med 66:222–226

Zhang XY, Sun YL, Bao J, He F, Xu XY, Qi SH (2012b) Phylogenetic survey and antimicrobial activity of culturable microorganisms associated with the South China Sea black coral *Antipathes dichotoma*. FEMS Microbiol Lett 336:122–130

Zuluaga-Montero A, Toledo-Hernandez C, Rodriguez JA, Sabat AM, Bayman P (2010) Spatial variation in fungal communities isolated from healthy and diseased sea fans *Gorgonia ventalina* and seawater. Aquat Biol 8:151–160