Antimicrobial activities of marine fungi from Malaysia

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Abstract

In a systematic screening effort, extracts of marine fungi from Malaysia were investigated for antimicrobial activity and potentially active secondary metabolites. In preliminary experiments, the plug assay method was employed to screen 152 strains for antimicrobial activity. Of these, 82 exhibited biological activity, with most of the active strains (90.2%) having antibacterial activity, mainly towards Gram-positive bacteria, while only 9.6% had antifungal activity. On the basis of these results, five marine fungi, *Fasciatispora nypae*, *Caryosporella rhizophorae*, *Melaspilea mangrovei*, *Leptosphaeria* sp. and ascomycete strain 19 (NF) were selected for further investigation to confirm their biological activity by the disc diffusion assay method. The selected species had various degrees of activity against the test microorganisms, depending on culture conditions (stationary vs. shaking cultures) and incubation time (10–25 days). Only *F. nypae* showed a wider range of antifungal and antibacterial activity as compared to the remaining fungal strains under investigation. Therefore, bioactivity-guided fractionation was undertaken to isolate the active principles, resulting in the characterisation of 2,2,7-trimethyl-2H-chromen-5-ol (**1**) which had antimicrobial activity towards test microorganisms. The structure of **1**, which previously had only been reported as a synthetic intermediate, but not as a natural product, was elucidated by mass spectrometry in conjunction with one- and two-dimensional NMR spectroscopy.

Keywords: antimicrobial activity; biologically active natural products; *Fasciatispora nypae*; marine fungi.

Introduction

The marine environment is an extremely diverse reservoir of life, and across the range of organisms there is a virtually untapped source of structurally unique natural products. Due to harsh, sometimes extreme and rapidly changing ambient conditions (e.g., intermittent dryness, wide ranges of salinity, pressure, or temperature), the intertidal zone in particular poses enormous challenges to marine species. Marine fungi have repeatedly been shown to synthesize or accumulate a high number of defensive compounds with intriguing structures (Liberra and Lindequist 1995, Biabani and Laatsch 1998, Lin et al. 2002, Vongvilai et al. 2004), which according to Berdy (2005), can be attributed to an intense interaction with their unique environment, which impacts on the inherent characteristics of their bioactive metabolites. A study by Cuomo et al. (1995) reported that many marine fungi have good activity profiles when compared to terrestrial fungi, making them a very promising source for the isolation of biologically active secondary metabolites.

Fungi that produce active metabolites have been obtained from various marine substrates, such as animals (predominantly sponges or fishes) but also primary producers, most importantly mangroves, sea grasses and algae. Accordingly, this group of organisms has attracted considerable attention from natural product chemists, and numerous studies dealing with diverse and unique compounds of marine fungi have been reported, with pertinent biological activities including antimicrobial, anticancer, anti-inflammatory and antiviral properties (for reviews, see Liberra and Lindequist 1995, Biabani and Laatsch 1998, Bugni and Ireland 2004, Ebel 2006, Pan et al. 2008).

Although several investigations into the diversity of marine fungi in Malaysia have been conducted so far (Alias et al. 1995, Hyde and Alias 2000, Zainuddin and Alias 2005), virtually no information is available on their biological activity. Therefore, the current study was undertaken to evaluate marine fungi from Malaysia for antimicrobial activity against selected bacteria and fungi, to test effects of culture conditions and incubation times on the production of active metabolites of selected marine fungi, and, where possible, to isolate active compounds from individual fungal strains.

Materials and methods

Identification and isolation of fungi

Marine fungi were isolated from various samples of decaying materials of mangrove wood, driftwood and *Nypa fruticans* Wurmb., collected in Morib, Port Dickson, Langkawi Island, Jarak Island, Lalang Island and Rumbia Island in Peninsular Malaysia. Identification of marine fungi followed the methods outlined by Alias et al. (1995). Initial isolation was carried out on cornmeal seawater agar (CMA) at room temperature.

The marine fungi were then re-cultured onto CMA and potato dextrose agar (PDA) for 30 days prior to the antimicrobial screening.

Test microorganisms

The test microorganisms used were *Bacillus subtilis* (Ehrenberg) Cohn (ATCC 6051), *Escherichia coli* (Migula) Castellani & Chalmers MTCC 443 (ATCC 25922), *Klebsiella aerogenes* Bascome, Lapage, Willcox & Curtis (NCTC 418), *Staphylococcus aureus* Rosenbach MTCC 96 (ATCC 9144), *Candida albicans* (Robin C.P.) Berkhout MTCC 3017 (ATCC 90028), *Saccharomyces cerevisiae* Meyen ex E.C. Hansen (ATCC 18824) and *Schizosaccharomyces pombe* Lindner (JCM 8274), provided by the Microbiology Department, University of Malaya. These test microorganisms were re-cultured to prepare for the antimicrobial screening using trypticase soy agar (TSA) and Sabouraud's dextrose agar (SDA), respectively, and then incubated at 32°C for 24 h. Prior to performing the screening, the test microorganisms were inoculated onto universal bottle containing potato dextrose broth (PDB). The density of the test microorganisms was adjusted to standard McFarland 0.5 using a spectrophotometer at a wavelength of 625 nm.

Preliminary screening for antimicrobial activity

In the plug assay (adopted after Hoskisson et al. 2001, Ezra et al. 2004), PDA plates seeded with test microorganism were prepared, and a cut of 6 mm diameter was prepared at the centre of the inoculated agar plates. Plugs of active fungal mycelia (6 mm) from PDA cultures were then transferred into these wells, followed by incubation at 37°C for 24 h. The diameters of resulting zones of inhibition were recorded and measured in mm. All assays were carried out in triplicate.

Cultivation, extraction and bioassay

Five species of marine fungi namely, *Fasciatispora nypae* K.D. Hyde, *Caryosporella rhizophorae* Kohlm., *Melaspilea mangrovei* Vrijmoed, K.D. Hyde *et* E.B.G. Jones, *Leptosphaeria* sp. and ascomycete sp. 19 (NF) were cultivated on potato dextrose agar (PDA) at 25°C for 30 days. Plugs of agar with mycelial growth were transferred aseptically into 250 ml conical flasks containing 200 ml of PDB. Inoculated flasks were then incubated at 25°C, both stationary and on a shaker at 200 rpm for 10, 15, 20, 25, 30 and 35 days. Cultures were harvested by filtering off the mycelium using a filter funnel, and extracting the culture broths twice with equal volumes of ethyl acetate (EtOAc). The combined organic phases were then evaporated to dryness using a rotary evaporator.

EtOAc extracts of fungi were redissolved in EtOAc to give a concentration of 20 μg μ^{-1}, and 10 μl of this solution was transferred to 6 mm paper discs on PDA agar plates seeded with test microorganisms, as described above. The diameters (mm) of resulting zones of inhibition were recorded after 24 h. All assays were run in triplicate. Pure compound **1** was also evaluated for antimicrobial activity.

Isolation and structure elucidation of 2,2,7-trimethyl-2H-chromen-5-ol

The culture broth of *Fasciatispora nypae* (20 l) was extracted with equal volumes of EtOAc, and the combined organic layer were evaporated to dryness. The crude EtOAc extract (3.0 g) was then dissolved in CH_2Cl_2 and subjected to column chromatography (silica gel, 30.6 g) using a stepwise elution with mixtures of CH_2Cl_2 and MeOH (100% CH_2Cl_2, 99;1, 98:2, 95:5, 85:15, 80:20, and 100% MeOH). Individual fractions were combined according to their TLC profiles obtained on normal phase silica gel plates, using similar mobile phase systems as for column chromatography, and routine UV detection at 254 nm. Compound **1** (48 mg) was obtained from the fraction eluting with CH_2Cl_2:MeOH 99:1.

NMR spectra were recorded on a JEOL (JEOL Ltd., Tokyo, Japan) JNM-LA400 NMR spectrometer using standard pulse sequences, and chemical shifts were referenced to TMS. GC/MS was performed on a Finnigan (Thermo Fisher Scientific, Bremen, Germany) MAT 95 spectrometer. HR-ESI-MS was measured on a Thermo Instrument (Thermo Fisher Scientific, Bremen, Germany) LTQ XL/LTQ Orbitrap Discovery system.

Results and discussion

Preliminary screening using the plug assay method

In preliminary screening using the plug assay method, 152 strains of marine fungi belonging to 68 taxa were tested for their antimicrobial activity (Table 1). A positive response was assumed when the diameter of the resulting zone of inhibition exceeded 8 mm; the maximum diameter was 18 mm. Eighty-two strains displayed activity against bacteria or yeasts, with the majority of these marine fungi being active against *Bacillus subtilis* (64 strains) and *Staphylococcus aureus* (63 strains). Antifungal activity against *Candida albicans, Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* was detected for 6, 12 and 14 strains, respectively. A broad spectrum of antibacterial and antifungal activity was observed for 17 marine fungi, but antibacterial activity was more frequently observed than antifungal activity. The disadvantage of the plug assay method is that the concentration of active metabolites present in the extracts is unknown, and it is also not possible to determine whether active metabolites are secreted into the medium or are retained in the cell.

Antibacterial activity was determined in approximately 90% of all tested marine fungal strains, and was thus significantly more prevalent than antifungal activity. This general trend is in agreement with a similar investigation by Suay et al. (2000) who reported that about 70% fungal strains were active against bacteria. However, in contrast to our findings, Cuomo et al. (1995) and Höller et al. (2004) reported that the number of marine fungi inhibiting the growth of fungi

Table 1 Isolates active against bacteria and yeast in preliminary trials.

Species	Number of strains		Main activity
	Tested	Active	
Acrocardiopsis patilii Borse *et* K.D. Hyde	1	1	BS, SA, CA
Aigialus mangrovei Borse	4	0	–
Aigialus grandis Kohlm. *et* S. Schatz	4	1	BS, SA
Aigialus sp. 1	2	2	BS, SA, CA, SC, SP
Aigialus sp. 2	1	0	–
Aniptodera mangrovei K.D. Hyde	1	0	–
Aniptodera longispora K.D. Hyde	1	0	–
Anthostomella nypensis K.D. Hyde, Alias *et* B.S. Lu	1	0	–
Ascocratera manglicola Kohlm.	1	1	BS, SA
Caryosporella rhizophorae Kohlm.	2	2	BS, SA
Cuculosporella sp.	1	0	–
Dactylospora haliotrepha (Kohlm. *et* E. Kohlm.) Hafellner	6	0	–
Eutypa sp.	2	1	BS
Eutypa bathurstensis K.D. Hyde *et* Rappaz	1	1	BS, SC, SP
Fasciatispora nypae K.D. Hyde	3	3	AE, BS, SC, KA, SC, SP
Halomassarina thalassiae (Kohlm. *et* Volkm.-Kohlm.) Suetrong, Sakarayoj, E.B.G. Jones, Kohlm., Volkm-Kohlm. *et* C.L. Schoch	7	3	BS, SA, SP
Halorosellinia oceanica (S. Schatz) Whalley, E.B.G. Jones, K.D. Hyde *et* Læssøe	5	5	BS, SA
Halosarpheia sp.	2	2	BS, SA, KA
Hypoxylon sp. 1	3	0	–
Hypoxylon sp. 2	2	1	CA, SC
Julella avicenniae (Borse) K.D. Hyde	3	2	BS, SA, SC
Kallichroma glabrum (Kohlm.) Kohlm. *et* Volkm.-Kohlm.	1	1	BS, SA
Kallichroma tethys (Kohlm. *et*. E. Kohlm.) Kohlm. *et* Volkm.-Kohlm.	5	2	BS
Lautospora sp.	2	2	SA
Leptosphaeria sp. (7se)	1	1	BS, SA, CA, SC
Lignincola laevis Höhnk	1	0	–
Lophiostoma sp.	3	3	BS, SA
Lulworthia sp.	2	1	BS, SA
Lulworthia grandispora Meyers	2	0	–
Marinosphaera sp.	1	1	BS, SA
Mauritiana rhizophorae Poonyth, K.D. Hyde, Aptroot *et* Peerally	1	1	BS, SA, KA
Melaspilea mangrovei Vrijmoed, K.D. Hyde *et* E.B.G. Jones	1	1	BS, SA, CA, SC, SP
Morosphaeria ramunculicola (K.D. Hyde) Suetrong, Sakayaroj, E.B.G. Jones *et* Schoch	4	3	BS, SA, SC, SP
Morosphaeria velatospora (K.D. Hyde *et* Borse) Suetrong, Sakarayoj, E.B.G. Jones *et* C.L. Schoch	1	1	BS, SA
Natantispora retorquens (Shearer *et* J.L. Crane) J. Campb., J.L. Anderson *et* Shearer	3	0	–
Neolinocarpon globosicarpum K.D. Hyde	6	4	BS, SA
Neolinocarpon sp. 1	1	0	–
Phaeosphaeria sp.	2	1	BS, SA, KA
Pyrenographa xylographoides Aptroot	6	2	BS, SA, SC
Quintaria lignatilis (Kohlm.) Kohlm. *et* Volkm.-Kohlm.	4	3	BS, SA, SC
Rhizophila marina K.D. Hyde *et* E.B.G. Jones	4	3	BS, SA, SC, SP
Saagaromyces abonnis (Kohlm.) K.L. Pang *et* E.B.G. Jones	2	0	–
Saccardoella mangrovei K.D. Hyde	1	1	BS, SA, KA
Salsuginea ramicola K.D. Hyde	3	3	BS, SA
Saccardoella mangrovei K.D. Hyde	1	1	BS, SA, KA
Torpedospora radiata Meyers	7	0	–
Verruculina enalia (Kohlm.) Kohlm. *et* Volkm.-Kohlm.	12	12	EA, BS, SA, KA
Ascomycete sp. 1	1	1	BS, SA, SC, SP
Ascomycete sp. 2	1	1	BS, SA, CA, SC, SP
Ascomycete sp. 3	1	0	–
Ascomycete sp. 4	1	1	BS, SA
Ascomycete sp. 5	1	1	BS, SA
Ascomycete sp. 17	1	0	–
Ascomycete sp. 21	1	0	–
Ascomycete sp. 46	1	1	BS, SA

(Table 1 continued)

Species	Number of strains		Main activity
	Tested	Active	
Ascomycete sp. 49	1	1	SA, KA
Ascomycete sp. 52	1	0	–
Ascomycete sp. 1 (NF)	1	0	–
Ascomycete sp. 5 (NF)	1	0	–
Ascomycete sp. 11 (NF)	1	1	BS, SA
Ascomycete sp. 12 (NF)	2	0	–
Ascomycete sp. 15 (NF)	1	1	BS, SA
Ascomycete sp. 16 (NF)	1	1	BS
Ascomycete sp. 17 (NF)	1	0	–
Ascomycete sp. 19 (NF)	1	1	BS, SP
Ascomycete sp. 21 (NF)	1	0	–
Ascomycete sp. 22 (NF)	1	0	–
Ascomycete sp. (p)	1	0	–
Total	152	82	

BS, *Bacillus subtilis*; CA, *Candida albicans*; EC, *Escherichia coli*; KA, *Klebsiella aerogenes*; SA, *Staphylococcus aureus*; SC, *Saccharomyces cerevisiae*; SP, *Schizosaccharomyces pombe*.

was higher than those displaying antibacterial properties. Differences in activity profiles have been shown to depend on both the carbon source used for fermentation, and the amount of mycelial biomass produced, with the latter obviously being time-dependent (Cuomo et al. 1995). However, due to the large numbers of isolates in our study, we did not aim for a systematic investigation of how the activity profiles of marine fungi can be affected by growth conditions, i.e., composition of media, salinity, pH or temperature.

We also found antibacterial activity to be more common towards Gram-positive bacteria (86.7%) than Gram-negative bacteria (13.3%). The greater resistance of Gram-negative bacteria compared to Gram positive-bacteria was also reported by Christophersen et al. (1999), Höller et al. (2000) and Suay et al. (2000). These differences in susceptibility towards antibiotically active secondary metabolites have been repeatedly attributed to differences in cell wall structure of Gram-positive bacteria compared to Gram-negative bacteria. The cell walls of Gram-positive bacteria are less complex and lack the natural sieve effect against large molecules (Hawkey 1998), whereas the outer membrane and the periplasmic space that is present in Gram-negative bacteria is thought to provide an additional degree of protection against antibiotics targeting the cell wall (Basile et al. 1998).

Some of the fungal strains investigated in the current study have previously been investigated for their secondary metabolite profiles and their biological activities. For example, the marine fungus *Verruculina enalia* produces two phenolic compounds, enalin A and B, together with hydroxymethyl furfural and three cyclodipeptides (Lin et al. 2002). Enalin A is a coumaranone-type compound, and representatives of this class of natural products are widely distributed from microorganisms to higher plants and have been shown to possess antibacterial, antifungal, and phytotoxic properties (Furumoto et al. 1997) as well as antidiabetic activity (Manickam et al. 1997). The secondary metabolite chemistry of *Halorosellinia oceanica* has been extensively studied, and besides displaying antibiotic properties, which were also encountered in the present study, natural products from this fungus have antifungal, antimalarial and cytotoxic properties (Chinworrungsee et al. 2001, 2002).

Antimicrobial activity of extracts of selected marine fungi using the disc diffusion assay method

Among the five strains tested, *Fasciatispora nypae* had the widest range of antimicrobial activity (Table 2), while the ascomycete strain 19 (NF) and *Caryosporella rhizophorae*

Table 2   Marine fungi with antimicrobial activities in disc diffusion assays.

Species	Extract (200 μg disc⁻¹)	Time producing active metabolites (days)	Culture incubation	Main activity
Fasciatispora nypae		15	Shaken/static	EC, BS, SA, CA, SC, SP
Leptosphaeria sp.		15	Static	BS, SA, CA
Caryosporella rhizophorae		15	Shaken	EC, BS, SA
Ascomycete strain 19 (NF)		15	Static	BS, SA
Melaspilea mangrovei		15	Shaken/static	EC, BS, SA

BS, *Bacillus subtilis*; CA, *Candida albicans*; EC, *Escherichia coli*; SA, *Staphylococcus aureus*; SC, *Saccharomyces cerevisiae*; SP, *Schizosaccharomyces pombe*.

Table 3 NMR data for 2,2,7-trimethyl-2H-chromen-5-ol (**1**) in $CDCl_3$ (400 and 100 MHz, respectively).

Position	δ_H (mult, J Hz)	δ_c	HMBC (H→C)
2		76.1	
3	5.50 (d, 9.8)	128.3	2, 4a
4	6.57 (d, 9.9)	116.6	2, 5
4a		107.2	
5		153.9	
6	6.22 (s)	110.0	4a, 5, 8, 7-Me
7		139.7	
8	6.11 (s)	108.8	4a, 6, 8a, 7-Me
8a		151.5	
2-Me	1.39 (6H, s)	27.9	2, 3, 2-Me
7-Me	2.18 (3H, s)	21.6	6, 7, 8
5-OH	4.63 (br s)		

did not inhibit the growth of yeasts. Subsequently, culture conditions and incubation times were varied for these fungal strains in order to investigate possible effects on their antimicrobial profiles.

Effect of culture conditions on the production of bioactive metabolites As evident from the disc diffusion assay (Table 2), *Caryosporella rhizophorae* produced active metabolites when cultured under shaking conditions. In comparison, extracts of *Melaspilea mangrovei, Leptosphaeria* sp. and ascomycete strain 19 (NF) developed activity upon fermentation under static conditions. Interestingly, *Fasciatispora nypae* displayed activity after cultivation in both conditions, and subsequently also produced compound **1** under static conditions. Crude extracts of *C. rhizophorae, M. mangrovei* and ascomycete strain 19 (NF) only exhibited antibacterial activities, but no antifungal activity. Results observed for *C. rhizophorae* were consistent with the results of the preliminary screening, but were not for *M. mangrovei* and ascomycete strain 19 (NF) as they also had activity against selected fungi, which they did not in the previous preliminary screening. Newman et al. (1998) reported that different activities were expressed by various fungi when cultivated in shaken and static conditions. Their findings are similar to the present study, indicating that variation in activity profiles can occur when fungi are cultured under different conditions.

Effect of incubation time on the production of bioactive metabolites The antimicrobial activities of the extracts of the five selected fungal strains were also monitored through the cultivation period. While no activity was detected for any fungal extracts at 10 days incubation (data

not shown), in all cases, maximum activity was observed after 15 days (Table 2). After a longer incubation period (up to a total period of 35 days, data not shown), only marginal changes in activity profiles were observed against certain microorganisms, but most of these changes were not statistically significant. In the chemical literature there are various reports indicating that incubation time can be an important factor in the development of secondary metabolite profiles (Isaka et al. 2002, Vongvilai et al. 2004).

Characterization of a secondary metabolite from *Fasciatispora nypae* In the ESI-MS, a pseudomolecular ion was observed at *m/z* 191.1068 ([M+H]$^+$, calc for $C_{12}H_{15}O_2$, 191.1067, Δ=+0.5 ppm), which was consistent with a molecular formula of $C_{12}H_{14}O_2$. In the ^{1}H NMR spectrum (Table 3), singlet peaks assigned to two equivalent aliphatic methyl groups (δ 1.39, 6H, 2×2-CH_3) and a further aromatic methyl group (δ 2.18, 7-CH_3) were detected, together with an AB system (δ 6.57 and δ 5.5, H-4 and H-3, J=9.8 Hz), two slightly broadened singlets (δ 6.22 and 6.11, H-6 and H-8) and a broad singlet (δ 5.40, OH-5) in the aromatic region. Key correlations in the HMBC spectrum of **1** from H-4 to C-5 (δ 153.9) as well as the aromatic methyl 7-CH_3 to both protonated carbons C-6 (δ 110.0) and C-8 (δ 108.8) were consistent with the phenolic OH in **1** residing at C-5 instead of C-6, as in **2**. Further evidence for the identification of **1** as 2,2,7-trimethyl-2H-chromen-5-ol was obtained from the COSY spectrum which had long range correlations of 7-CH_3 to both H-6 and H-8. Interestingly, compound **1** has been synthesized as an intermediate by two independent groups (Shen et al. 2003, Lee et al. 2005), and the reported spectral data closely match those obtained for **1** in the present study, but to the best of our knowledge, this compound has hitherto never been reported from nature.

From a structural point of view, **1** is closely related to acremine C (**2**) (Figure 1), which was originally described from the endophytic fungal strain *Acremonium* sp. A20 (Assante et al. 2005), and from which it differs by the position of the hydroxyl function at C-5 instead of C-6. Interestingly, a series of C-8 prenylated congeners of **1** have been reported from the plant *Peperomia serpens* (Sw.) Loudon (Piperaceae), and their biosynthetic origin was assumed to being derived from orsellinic acid by prenylation and decarboxylation (Salazar et al. 2005, Kitamura et al. 2006). Since orsellinic acid is a typical fungal-derived polyketide, horizontal gene transfer of the PKS gene cluster has been considered as a likely explanation for the occurrence of 2,2,7-trimethylchromenols in *P. serpens* (Salazar et al. 2005). In this context, our detection of **1** in *Fasciatispora nypae* as

Table 4 Antimicrobial activity of compound **1** isolated from *Fasciatispora nypae* against various test microorganisms.

Escherichia coli	*Bacillus subtilis*	*Staphylococcus aureus*	*Candida albicans*	*Saccharomyces cerevisiae*	*Schizosaccharomyces pombe*
No activity	10 mm	10 mm	9 mm	10 mm	14 mm

Disc diameter: 6 mm; concentration: 200 μg per disc.
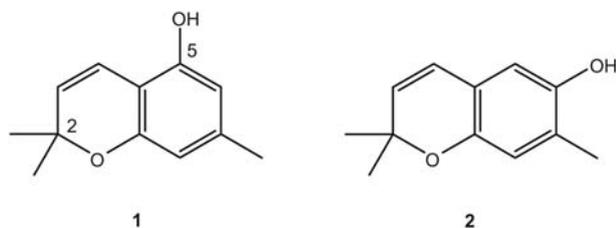Activities were classified as: 7–9 mm: very weak activity, 10–11 mm: weak activity; and >11 mm: moderate activity.

Figure 1 Structures of 2,2,7-trimethyl-2H-chromen-5-ol (**1**) and acremine C (**2**).

an associate of the palm *Nypa fruticans* is remarkable, since it may hint at an alternative explanation, i.e., the possible production of similarly substituted chromenols in higher plants by endophytic fungi.

The antimicrobial activity of 2,2,7-trimethyl-2H-chromen-5-ol (**1**) was assessed in the disc diffusion assay (Table 4). Overall, compound **1** had weak antimicrobial activities against *Bacillus subtilis, Candida albicans* and *Saccharomyces cerevisiae*, and moderate activity towards *Schizosaccharomyces pombe* at a concentration of 200 μg disc^{-1}, but was inactive against *Escherichia coli*. This is the first report of biological activity for this compound.

In conclusion, the biological activity of marine fungi from Malaysia has been investigated for the first time, focussing on the fungal diversity of a single habitat, i.e., decaying driftwood, mangrove wood and *Nypa* palm. More than half of all 152 strains we investigated had either antibacterial or antifungal activity, or both. In the present study, we also demonstrated that the activity profiles of the five most active fungi clearly depend on culture conditions (static and shaken culture) and incubation time. Static conditions appeared to be slightly more favorable in this study in comparison to shaken cultures. With regard to incubation time, we consistently observed no biological activity after 10 days, while antibacterial activity was in most cases fully developed after 15 days, and thereafter varied little up to 30 days. Our results thus indicate that production of active compounds can easily be overlooked, and of course, an even more pronounced influence on secondary metabolite production is to be expected if media composition is varied (Helmholz et al. 1999; Miao et al. 2006). Chemical characterization of one strain, *Fasciatispora nypae*, resulted in the isolation of compound **1**, which was previously only known as a synthetic intermediate, and which apparently represents the first active metabolite recorded from fungi of the Malaysian marine ecosystem. Further studies need to be conducted to obtain an understanding of the potential role of this compound (and of other secondary metabolites) in the marine ecosystem. Given their enormous fungal diversity, it is to be expected that marine habitats in Malaysia hold considerable potential for future discovery of other fungi capable of producing new secondary metabolites with interesting biological activities.

Acknowledgements

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