

# Antimicrobial activities of marine fungi from Malaysia

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Q1: Please check Othman@-Ghazali, it cannot be part of the name

Q2: Please make sure that each name is listed as one first full name/middle name initials/last name

Q3: 'wider' than what?

Q4: Please recheck all species names for correctness; species names must be spelled out in full at 1st mention of each paragraph; thereafter, the genus name can be abbreviated

## 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*, *Caryospora rhizophorae*, *Melasporea 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 and vs. shaking cultures) and incubation time (10–25 days). Only *F. nypae* showed a wider range of antifungal and antibacterial activity. 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**) 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.