Antimicrobial compounds produced in culture by endophytic and marine-derived fungi of selected Sri Lankan organisms

A thesis submitted for the Degree of Doctor of Philosophy

Pamoda Bhasini Ratnaweera
Faculty of Science
University of Colombo
April 2015

Abstract

There is an urgent need for new and more effective antibiotics to combat resistant pathogenic microorganisms. Secondary metabolites produced by living organisms are a proven source of clinically useful drugs. Sri Lanka has a rich and unique biodiversity with a high rate of endemic speciation. Although endophytic and marine-derived fungi are reputed as producers of antimicrobial metabolites, Sri Lankan fungal endophytes and marine-derived fungi still remains almost completely uninvestigated. The objective of the current study is to investigate endophytic and marine-derived fungi from unique ecological niches of Sri Lanka for metabolites that can serve as drug leads for new antibiotics. For this purpose five hosts, each from a different ecological setting, which consisted of an endemic orchid, a mangrove associate, an invasive cactus, a medicinal weed and a marine sponge were chosen.

Initially, ethyl acetate extracts of small-scale laboratory cultures of the endophytic and marine-derived fungi originating from the selected hosts were tested for activity against a panel of human pathogens. Next, the fungus with the most promising activity from each host was grown in large scale and the antimicrobial compounds present in their ethyl acetate extracts were isolated via a series of bioassay-guided chromatographic fractionations. The structures of the isolated active compounds were elucidated using mass spectrometry and nuclear magnetic resonance (NMR) spectroscopic data. Where necessary synthetic modifications and X-ray crystallographic studies were done to confirm the structures. The MIC values for the pure compounds were obtained using broth-micro dilution method.

In this study, an endophytic *Xylaria* species from the endemic orchid, *Anoectochilus setaceus* collected from a rainforest yielded antibacterial helvolic acid. Bioactive gliotoxin and bisdethiobismethylthiogliotoxin were isolated from the endophyte *Hypocrea virens* from the mangrove-associate *Premna serratifolia*, while bioactive equisetin was isolated from a *Fusarium* sp. of the invasive arid zone cactus, *Opuntia dillenii*. Aspochalasin B, C, D, asperphenamate and 4-OMe-asperphenamate were isolated from the marine-derived *Aspergillus flavipes* obtained from a marine Demospongiae sponge. All of the above compounds were previously known antibiotics. A completely novel structure, solanioic acid, consisting of a highly functionalized carbon skeleton that appears to be derived from fungisterol was discovered from *Rhizoctonia solani* isolated from the medicinal weed *Cyperus rotundus*. The potent antimicrobial activity of solanioic acid, against methicillin

resistant *Staphylococcus aureus* (MRSA, MIC: 1 μg mL^{-1}), *S. aureus* (MIC: 1 μg mL^{-1}), *Bacillus subtilis* (MIC: 1 μg mL^{-1}) and *Candida albicans* (MIC: 16 μg mL^{-1}) suggests that it has a high potential to be developed as a novel antibiotic.

The current investigation revealed that endophytic and marine-derived fungi from different, harsh and competitive environment settings are capable of producing a variety of bioactive compounds. While these compounds may help their hosts to overcome biotic and abiotic stresses in their environments, some of them may also serve as useful drug leads.

This pioneering study is a clear indication that the endophytes of Sri Lankan organisms from unique environmental settings is a fruitful source for isolating novel bioactive structures as useful drug leads and encourages more investigations into this potentially very productive field of study.