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Production and purification of a bioactive substance against multi-drug resistant human pathogens from the marine-sponge-derived *Salinispora* sp.

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PEER REVIEW

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Comments

This is a good research paper in which the authors obtained rifamycin W (compound 1) from the EtOAc extract of the culture of *Salinispora* sp. FS-0034 and found that it had potent antibacterial activity. The results are meaningful and valuable and lay a solid foundation for further studies.
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ABSTRACT

Objective: To isolate, purify, characterize, elucidate structure and evaluate bioactive compounds from the sponge-derived *Salinispora* sp. FS-0034.
Methods: The symbiotic actinomycete strain FS-0034 with an interesting bioactivity profile was isolated from the Fijian marine sponge *Theonella* sp. Based on colony morphology and obligatory requirement of seawater for growth, and mycelia morphological characteristics the isolate FS-0034 was identified as a *Salinispora* sp. The bioactive compound was identified by using various spectral analysis of ultraviolet, high resolution electrospray ionization mass spectroscopy, ^{1}H nuclear magnetic resonance, correlated spectroscopy and heteronuclear multiple bond coherence spectral data. A minimum inhibitory concentration assay were performed to evaluate the biological properties of the pure compound against multi-drug resistant pathogens.
Results: Bioassay guided fractionation of the ethyl acetate extract of the culture of *Salinispora* sp. FS-0034 by different chromatographic methods yielded the isolation of an antibacterial compound, which was identified as rifamycin W (compound 1). Rifamycin W was reported for its potent antibacterial activity against methicillin-resistant *Staphylococcus aureus*, wild type *Staphylococcus aureus* and vancomycin-resistant *Enterococcus faecium* and displayed minimum inhibitory concentrations of 15.62, 7.80 and 250.00 μg/mL, respectively.
Conclusions: The present study reported the rifamycin W from sponge-associated *Salinispora* sp. and it exhibited appreciable antibacterial activity against multi-drug resistant human pathogens which indicated that sponge-associated Actinobacteria are significant sources of bioactive metabolites.

KEYWORDS
Marine actinomycetes, Sponge-derived, *Salinispora*, Multi-drug resistant, Antibacterial, Rifamycin

1. Introduction

Infectious diseases have always been serious health problems with high morbidity and mortality in humans. Even though pharmaceutical companies have produced a number of new antibiotics in the past decade, resistance to these drugs has increased and has now become a global concern[1]. The global emergence of multi-drug resistant bacteria is increasingly limiting the effectiveness of current drugs and significantly causing treatment failure[2]. *Staphylococcus aureus* (*S. aureus*) is one of the most important human pathogens associated with hospital and community-acquired infections. Over the decades, the number and proportion of drug resistant pathogens, including methicillin-resistant *S. aureus* (MRSA), vancomycin-resistant *Enterococci*, cephalosporin-resistant *Klebsiella pneumonia*, fluoroquinolone-resistant *Pseudomonas*

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aeruginosa, multi-drug resistant Gram-negative bacteria and extensively drug resistant tuberculosis infections in different countries have increased due to the rise of epidemics in humans[3-5]. Consequently, new and innovative antimicrobial agents are urgently needed to combat these life threatening infections.

New trends in drug discovery from natural sources emphasize investigation on the marine ecosystem to explore numerous complex and novel chemical entities[6]. It is noteworthy that marine sources have also demonstrated tremendous abilities as producers of anticancer compounds and bioactive secondary metabolites which act against infectious diseases and inflammation[7]. Among the marine organisms, microbial-derived natural products are playing a significant role in the drug discovery process[8]. Marine Actinobacteria represent a rich source of new molecules with pharmacological properties, which are lead compounds for the development of new drugs[9,10]. Recently, sponge-derived actinomycetes have been attracting increasingly interests as potential sources of unique and unusual bioactive secondary metabolites[11]. The isolation of secondary metabolite-producing bacteria from sponges and of microbial secondary metabolism gene clusters from the metagenome of sponges has led to the general understanding that these metabolites are, in many cases, the products of microbial symbionts and are not derived from the microbial diet of sponges[12]. In continuation of our studies on sponge-derived actinomycetes we now report on the bioactive compound against multi-drug resistant pathogens produced by *Salinispora* sp. isolated from the marine sponge *Theonella* sp. The goal of the present study is to isolate a bioactive compound showing antibacterial activities from the strain of *Salinispora* sp. FS-0034, which was isolated from the Fijian marine invertebrate.

2. Materials and methods

2.1. General experimental procedures

The ultraviolet (UV) spectrum was acquired in spectroscopy grade methanol using a PerkinElmer Lambda 35 spectrophotometer. Nuclear magnetic resonance (NMR) experiments were performed on Varian Inova spectrometer 600 MHz. The chemical shifts were expressed in δ (ppm) and referred to the residual solvent (δ_H 2.50 ppm for dimethylsulfoxide-d_6). High resolution electrospray ionization mass spectra (HRESIMS) were acquired by using an Agilent 1 100 series separations module equipped with an Agilent G1969A MSD (mass spectroscopy detector) in positive ion mode. Purification was done on a Zorbax ODS 5 μm 9.4 mm×250 mm column. Analytical and semi-preparative high performance liquid chromatography (HPLC) was performed using a Waters 515 pump connected to a 2487 UV-visible detector. Thin-layer chromatography (TLC) analyses were carried out by using glass plate pre-coated silica gel 60 reversed phase (RP)-18 $F_{254}S$ (Merck, Darmstadt, Germany). Analytical grade solvents were utilized for TLC analysis. Burdick and Jackson high purity solvents were used for HPLC while Riedel-de Haen, Chromasolv liquid chromatography-mass spectrometry (LC-MS) grade solvents were used for LC-MS.

2.2. Sample collection and processing

The sponge *Theonella* sp. was collected by hand using selfcontained underwater breathing apparatus at a depth of 10 m from Cicia, Lau group, Fiji Islands (17°47′33″ S, 179°23′94″ W) in September 2008 during a three week biodiversity expedition in the central Lau Group. The sponge material was transferred into a sterilized bag immediately after harvesting and was transported cooled to the nearby laboratory. The isolation of bacteria was subsequently carried out. The sponge was identified by Dr. Paco Cardenas, Uppsala University, Sweden. A voucher specimen (G-0634) has been preserved at the Marine Reference Collection, The University of the South Pacific, Fiji Islands and at Georgia Institute of Technology, USA.

The sponge sample was rinsed three times with sterile seawater to eliminate nonspecific microbial propagules that stick to the sponge surface from the seawater, and the surface of the sponge tissue was subjected to surface-sterilization by using 70% ethanol under aseptic conditions. The surface sterilized sponge tissue was then cut into small pieces of approximately 0.1 cm^3, homogenized and diluted with autoclaved membrane-filtered seawater. The resulting homogenate was diluted with sterile seawater at three dilutions (1:10, 1:100, and 1:1 000). Hundred microliters of each dilution was plated onto A1 agar (10 g of starch, 4 g of yeast extract, 2 g of peptone, 18 g of agar and 1 L of filtered natural seawater) medium. The isolation medium was amended with cycloheximide (100 μg/mL) and polymixin B (5 μg/mL) after autoclaving to avoid unwanted Gram negative bacteria and fungi contamination. The plates were incubated at 28 °C for 3-4 weeks until the morphology of actinomycetes could be distinguished.

2.3. Isolation of Salinispora strains

Actinomycetes were evaluated on each plate by eye and with the aid of an Olympus SZ51 binocular zoom stereomicroscope (8-40×). Actinomycetes were recognized by the presence of filamentous hyphae, a characteristic that was just within the range of detection at the highest magnification used, and/or by the formation of tough, leathery colonies that adhered to the agar surface. Colonies were assigned to the genera *Salinispora* if, for larger colonies, they produced orange pigment, black spores that darkened the colony surface, and lacked areal hyphae. Smaller colonies, viewed microscopically, could be ascribed to the *Salinispora* group if they possessed finely branched, scattered hyphae that formed a moderately developed substrate mycelium as

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