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BIOLOGICAL AND CHEMICAL CHARACTERISATION OF MARINE STREPTOMYCETES ACTIVE AGAINST SELECTED PATHOGENIC YEASTS

Field of Study: **MICROBIOLOGY**

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ABSTRACT

Streptomyces spp. have been the most abundant sources of all types of antibiotics. The success of marine natural products has promoted the marine environment as a source of novel chemical diversity for drug discovery. In Malaysia, the marine actinomycetes niche remains virtually unexplored and is a promising resource for the biotechnological applications including drug discovery. Screening for antifungal activity revealed that six out of 44 putative *Streptomyces* spp. isolated from sponges from Tioman Island showed strong antifungal activities against *Candida albicans* and *Schizosaccharomyces pombe*. Some physiological characterisation was done to these six potential strains to facilitate the strain identification including temperature range and optimum temperature range for growth, formation of melanoid pigment, liquefaction of gelatin, hydrolysis of starch, hydrogen sulfide production, sodium chloride tolerance, carbon sources utilization, nitrate reduction test and pH sensitivity. Fermentation of these strains was done using ISP 2 broth as growth medium prior to extraction of their bioactive components using ethyl acetate in ratio of 3:1 (filtrate broth: ethyl acetate). The crude extract of the strains were analysed using high performance liquid chromatography (HPLC) for chemical profiling. Based on the HPLC analysis, interesting compounds in three strains (X34, X42 and X77) were detected. 16S rRNA gene sequence analysis of these three strains showed 100%, 99.70% and 99.76% similarity to *Streptomyces rochei* (strain X34), *Streptomyces albidoflavus* (strain X42) and *Streptomyces cavourensis* (strain X77), respectively. When crude extracts of these three strains were tested against *S. pombe*, only strain X34 was positive and was further profiled, purified and isolated by column chromatography, thin layer chromatography (TLC), HPLC and preparative thin layer chromatography (PTLC). Six fractions (F1-F6) were obtained after column chromatography was done to strain X34, and were tested against *S. pombe*. As only

fraction F5 was positive, this fraction was subjected to PTLC. Another six sub-fractions (F5.1-F5.6) obtained after PTLC (of fraction F5) which were then tested against *S. pombe.* As a result, two out of six sub-fractions (F5.1 and F5.4) showed antifungal activity against the tested fungi but based on their ^{1}H-NMR chromatogram, only sub-fraction F5.1 was further analysed by nuclear magnetic resonance (NMR) and fourier transformation infrared spectroscopy (FTIR) for structure identification. Both NMR and FTIR analyses showed that the active sub-fraction (F5.1) was belonging to 2-(3-hydroxybutan-2-yloxy) propanoic acid. This is the first report of antifungal activity of this compound isolated from *Streptomyces rochei.*

ABSTRAK

Streptomyces spp. merupakan sumber utama bagi kebanyakan antibiotik. Keberkesanan produk semula jadi marin telah memperkenalkan persekitaran marin sebagai suatu sumber yang kaya dengan pelbagai penemuan baru. Di Malaysia, nic aktinomiset marin masih belum diteroka sepenuhnya dan merupakan sumber aplikasi bioteknologi termasuk penemuan ubatan. Ujian antikulat menunjukkan enam daripada 44 strain Streptomyces spp. yang diperolehi daripada span dari Pulau Tioman yang diuji mempunyai aktiviti antikulat terhadap Candida albicans dan Schizosaccharomyces pombe. Beberapa pencirian fizikal telah dilakukan terhadap keenam-enam strain ini bagi memudahkan proses identifikasi termasuklah lingkungan suhu dan lingkungan suhu optimum bagi pertumbuhan, pembentukan pigmen melanin, pencairan gelatin, hidrolisis kanji, penghasilan hidrogen sulfida, toleransi terhadap natrium klorida, penggunaan sumber karbon, ujian penurunan nitrat dan sensitiviti terhadap pH. Seterusnya, proses fermentasi dilakukan terhadap strain-strain ini menggunakan media cair ISP 2 sebagai media pertumbuhan sebelum mengekstrak komponen bioaktif menggunakan etil asetat dalam nisbah 3:1 (cairan fermentasi yang ditapis: etil asetat). Ekstrak mentah strain ini telah dianalisis menggunakan kromatografi cecair berpencapaian tinggi (HPLC) untuk pemprofilan kimia. Berdasarkan analisis HPLC tersebut, beberapa sebatian penting telah dikesan terdapat dalam ekstrak tiga strain (X34, X42 dan X77). Analisis jujukan gen 16S rRNA bagi ketiga-tiga strain ini menunjukkan persamaan 100%, 99.7% dan 99.8% dengan Streptomyces rochei (strain X34), Streptomyces albidoflavus (strain X42) and Streptomyces cavourensis (strain X77). Apabila ekstrak ketiga-tiga strain ini diuji terhadap S. pombe, hanya strain X34 didapati positif dan seterusnya diprofilkan, ditulenkan dan dipencilkan menggunakan kolum kromatografi, kromatografi lapisan nipis (TLC), HPLC dan kromatografi lapisan

nipis preparative (PTLC). Enam pecahan ekstrak (F1-F6) yang diperolehi selepas kolum kromatografi dijalankan ke atas strain X34 kemudiannya diuji terhadap S. pombe. Keputusannya, hanya pecahan ekstrak F5 yang didapati positif, maka hanya pecahan ekstrak ini akan dilakukan PTLC. Hasilnya, enam sub-pecahan ekstrak (F5.1-F5.6) diperolehi selepas PTLC dijalankan, yang kemudiannya diuji lagi terhadap kulat yang sama, S. pombe. Dua daripada enam sub-pecahan ekstrak ini iaitu F5.1 dan F5.4 menunjukkan aktiviti anti-kulat terhadap kulat tersebut, namun berdasarkan kromatogram ^{1}H-NMR kedua-dua sub-pecahan ekstrak ini, hanya sub-pecahan ekstrak F5.1 dianalisis selanjutnya dengan NMR dan FTIR bagi menentukan strukturnya. Analisis tersebut menunjukkan sub-pecahan ekstrak F5.1 ini merupakan asid propanoik 2-(3-hydroxybutan-2-yloxy). Kajian ini merupakan kajian yang pertama melaporkan aktiviti anti-kulat bagi sebatian asid propanoik 2-(3-hydroxybutan-2-yloxy) yang dipencilkan daripada Streptomyces rochei.

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LIST OF SYMBOLS AND ABBREVATIONS

%	Percent
˚C	Degree Celsius
µl	Micro liter
µg	Microgram
^{13}C-NMR	Carbon-13 nuclear magnetic resonance
^{1}H-NMR	Proton nuclear magnetic resonance
AA	Antibiotic Assay
Approx.	Approximately
BLAST	Basic Local Alignment Search Tool
bp	Base pairs
$CDCl_3$	duterated chloroform
cm	Centimeter
COSY	Correlation Spectroscopy
DEPT	Distortionless Enhancement by Polarisation Transfer
dH_2O	Distilled water
DNA	Deoxyribonucleic acid
dNTPs	Deoxyribonucleotide triphosphate
e.g	*Exempli gratia*
EDTA	Ethylenediaminetetraacetic acid
et al.	*Et alia / et alii*
Etc.	*Et cetera*
EtOAc	Ethyl acetate
FASTA	FAST-all
FeS	Ferric sulfate
FTIR	Fourier Transform Infrared Spectroscopy
H_2S	Hydrogen sulfide
HMBC	Heteronuclear Multiple-Bond Correlation
HSQC	Heteronuclear Single Quantum Correlation
i.e	*Id est*
ISP	International *Streptomyces* Project
kb	Kilobyte

MEGA	Molecular Evolutionary Genetics Analysis
MeOH	Methanol
mg	Milligram
$MgCl_2$	Magnesium chloride
MHz	Mega Hertz
min	Minute
ml	Milliliter
mM	Milimol
N	Normality
NaCl	Sodium chloride
nm	Nanometer
NOESY	Nuclear Overhauser Effect Spectroscopy
PCR	Polymerase chain reaction
PTLC	Preparative Thin Layer Chromatography
RDP	Ribosomal Databases Project
RNA	Ribonucleic acid
rpm	Rotation per minute
rRNA	Ribosomal ribonucleic acid
TBE	Tris-borate-EDTA
TLC	Thin Layer Chromatography
TOCSY	Total Correlation Spectroscopy
UV	Ultraviolet
V	Voltan
v/v	Volume-in-volume
w/v	Weight-in-volume