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IDENTIFICATION AND ANTIMICROBIAL ACTIVITY OF ACTINOMYCETES
FROM SOIL AT SAMED ISLAND

Miss Wijitra Anansiriwattana

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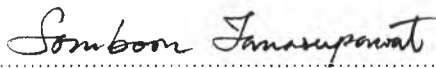
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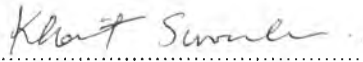
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ในการศึกษาเพื่อพิสูจน์เอกลักษณ์และคัดเลือกเชื้อที่มีฤทธิ์ต้านจุลชีพของแอคติโนมัยซีทส์ 100 ไอโซเลตที่แยกจากดิน 35 ตัวอย่างจากบริเวณชายฝั่งของเกาะเสม็ด จังหวัดระยอง โดยอาศัยลักษณะทางสัณฐานวิทยา การเจริญ สรีรวิทยา และคุณสมบัติทางชีวเคมีของเชื้อ พบว่าเชื้อที่แยกได้ 80 ไอโซเลตเป็นแบคทีเรียสกุลสเตรปโตมัยซีส และอีก 20 ไอโซเลตเป็นแบคทีเรียสกุลไมโครโมโนสปอรา การคัดเลือกขั้นต้นพบว่าสเตรปโตมัยซีส 55 ไอโซเลตและไมโครโมโนสปอรา 14 ไอโซเลตสามารถสร้างสารที่มีฤทธิ์ต้านจุลชีพได้ โดยส่วนใหญ่สามารถสร้างสารต้านการเจริญของเชื้อ *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633 และ *Candida albicans* ATCC 10231 ได้ดีและมีบางไอโซเลตสามารถสร้างสารต้านเชื้อ *Escherichia coli* ATCC 25922 และ *Pseudomonas aeruginosa* ATCC 27853 ได้ การคัดเลือกขั้นที่สองพบว่าสายพันธุ์ PC 4-3 ที่ให้ผลดีในการต้านการเจริญของเชื้อจุลชีพถูกคัดเลือกเพื่อการหมักสารทุติยภูมิ สายพันธุ์นี้มี L-diaminopimelic acid เป็นองค์ประกอบในผนังเซลล์ จากการวิเคราะห์ลำดับเบสบนสาย 16S rDNA และสายวิวัฒนาการพบว่ามีความใกล้เคียงกับสเตรปโตมัยซีสสายพันธุ์ NRRL B-1865 ซึ่งสกัดด้วยเอธิลอะซิเตทจากน้ำหมักของสายพันธุ์นี้แสดงฤทธิ์ต้านเชื้อ *S. aureus* ATCC 25923, *B. subtilis* ATCC 6633 และ *C. albicans* ATCC 10231 ได้ เมื่อทำการแยกสารให้บริสุทธิ์ด้วยวิธีทางโครมาโตกราฟีพร้อมการทดสอบฤทธิ์ต้านจุลชีพสามารถแยกได้สารในกลุ่ม ansamycins ที่เคยพบแล้ว 2 ชนิด คือ geldanamycin และ 17-O-demethylgeldanamycin การพิสูจน์โครงสร้างทางเคมีของสารนี้ใช้วิธีการวิเคราะห์ข้อมูล UV, IR, MS และ NMR spectroscopy ร่วมกับการเปรียบเทียบกับข้อมูลที่มีการรายงานมา สาร geldanamycin แสดงฤทธิ์ต้านเชื้อ *S. aureus* ATCC 25923 และ *C. albicans* ATCC 10231

ภาควิชา จุลชีววิทยา
สาขาวิชา จุลชีววิทยา
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ลายมือชื่อนิติ...วิจิตรา อนันต์ศิริวัฒนา.....
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WIJITTRA ANANSIRIWATTANA: IDENTIFICATION AND ANTIMICROBIAL ACTIVITY OF
ACTINOMYCETES FROM SOIL AT SAMED ISLAND. THESIS ADVISOR: ASSOCIATE
PROFESSOR SOMBOON TANASUPAWAT, Ph.D., THESIS CO-ADVISOR: MR. KHANIT
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In the course of identification and screening of antimicrobial activity of 100 actinomycetes isolates from 35 soil samples collected from shore of Samed Island, Rayong province based on morphological, cultural, physiological and biochemical characteristics, 80 isolates were identified as *Streptomyces* and 20 isolates were identified as *Micromonospora*. On primary screening, 55 isolates of *Streptomyces* sp. and 14 isolates of *Micromonospora* sp., most of these isolates showed the antimicrobial activities against *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633, and *Candida albicans* ATCC 10231, while the activities against *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 could be observed in few isolates. On secondary screening, the strain PC 4-3 with good antimicrobial activity was selected for secondary metabolite fermentation. This strain contained L-diaminopemelic acid in cell wall. Sequencing of 16S rDNA and phylogenetic analysis of PC 4-3 was similar to the *Streptomyces* strain NRRL B-1865. The ethyl acetate extract from fermentation broth of this strain showed antimicrobial activity against *S. aureus* ATCC 25923, *B. subtilis* ATCC 6633 and *C. albicans* ATCC 10231. Antimicrobial assay-guided fractionation of the ethyl acetate extract yielded 2 known ansamycins, including geldanamycin and 17-O-demethylgeldanamycin. The chemical structures of the isolated compounds were elucidated through extensive analyses of UV, IR, MS and NMR spectroscopic data and comparison with the literatures. Geldanamycin exhibited antimicrobial activity against *S. aureus* ATCC 25923 and *C. albicans* ATCC 10231.

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ABBREVIATIONS

$[\alpha]_D^{25}$	=	specific rotation at 25° and sodium D line (589 nm)
ATCC	=	American Type Culture Collection, Monassas,VA, USA
brs	=	broad singlet
°C	=	degree celsius
CDCl_3	=	deuterated chloroform
CHCl_3	=	chloroform
cm	=	centrimeter
$^{13}\text{C-NMR}$	=	carbon-13 nuclear magnetic resonance
d	=	doublet
dd	=	doublet of doublets (for NMR spectra)
DMSO	=	dimethylsulfoxide
$\text{DMSO}d_6$	=	deuterated dimethylsulfoxide
δ	=	chemical shift
EIMS	=	Electron Impact Mass Spectrum
EtOAc	=	Ethyl acetate
ϵ	=	molar absorptivity
g	=	gram
μg	=	microgram
μl	=	micro liter
hr.	=	hour
$^1\text{H-NMR}$	=	Proton Nuclear Magnetic Resonance
Hz	=	Hertz
IR	=	infrared
J	=	coupling constant
L	=	liter
λ_{max}	=	wavelength at maximal absorption
M^+	=	molecular ion

μg	=	microgram
mg	=	milligram
min	=	minute
μl	=	micro liter
ml	=	milliliter
MHz	=	megahertz
m/z	=	mass to charge ratio
MS	=	mass spectrometry
μm	=	micrometer
mm	=	millimeter
nm	=	nanometer
MeOH	=	methanol
NMR	=	Nuclear Magnetic Resonance
ppm	=	part per million
rpm	=	round per minute
RT	=	room temperature
s	=	singlet
TLC	=	Thin Layer Chromatography
UV	=	ultraviolet