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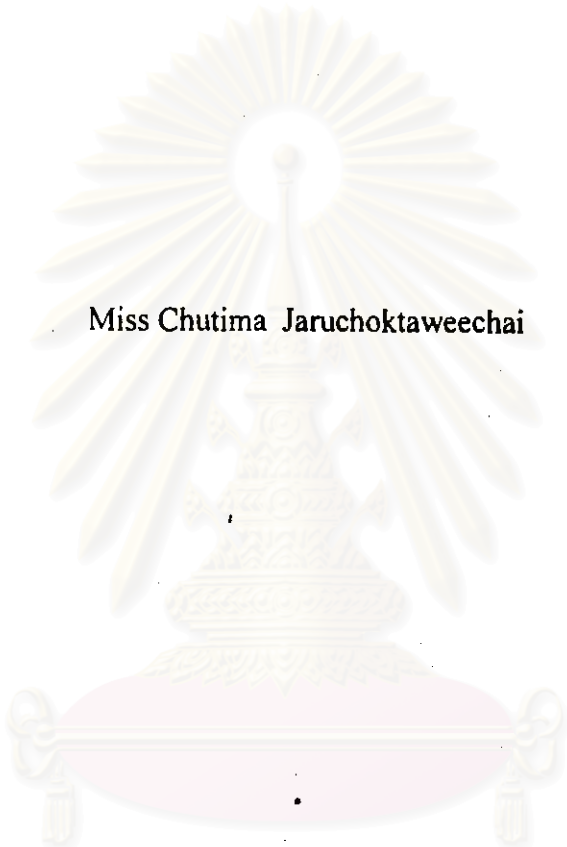
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BIOACTIVE CONSTITUENTS FROM MARINE *BACILLUS* STRAINS



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นางสาวชุติมา จารุโชคทวีชัย : สารที่มีฤทธิ์ทางชีวภาพจากสายพันธุ์บาซิลลัสทางทะเล (BIOACTIVE CONSTITUENTS FROM MARINE *BACILLUS* STRAINS)

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ในการตรวจสอบหาสารที่มีฤทธิ์ทางชีวภาพจากจุลินทรีย์ทางทะเล พบว่าแบคทีเรียแกรมบวกรูปร่างแท่ง สายพันธุ์ Sc018 และ Sc026 จากดินตะกอนใต้ทะเล และสายพันธุ์ Sc004 จากน้ำทะเล บริเวณเกาะสีชัง แสดงฤทธิ์ทางชีวภาพที่น่าสนใจ จากลักษณะทางสัณฐานวิทยา การเจริญ สรีรวิทยา และชีวเคมี สามารถพิสูจน์เอกลักษณ์ของสายพันธุ์ Sc018 และ Sc026 ได้เป็นแบคทีเรียในสกุลบาซิลลัส ในขณะที่ยังไม่สามารถพิสูจน์เอกลักษณ์ของสายพันธุ์ Sc004 จากการสกัดแยกสารควบคู่ไปกับการทดสอบฤทธิ์ยับยั้งไวรัสก่อโรคเรื้อรัง ของสิ่งสกัดในชั้นบิวทานอลจากอาหารเลี้ยงเชื้อของสายพันธุ์ Sc004 แยกได้สารที่เคยพบแล้วจำพวก diketopiperazines (DKPs) 1 ชนิดคือ *cyclo*-(L-Pro-Gly) และสารจำพวก purine nucleosides 1 ชนิด คือ 2'-deoxyadenosine ในขณะที่สิ่งสกัดในชั้นไดคลอโรมีเทนจากสายพันธุ์ Sc018 ให้สารจำพวก DKPs 3 ชนิด ประกอบด้วย สารที่เคยพบแล้ว 2 ชนิดคือ *cyclo*-(*trans*-4-OH-L-Pro-L-Phe) และ *cyclo*-(D-Pro-Leu) และสารใหม่ 1 ชนิดคือ *cyclo*-(D-Pro-Ile) การสกัดแยกสารควบคู่ไปกับการทดสอบฤทธิ์ยับยั้งแบคทีเรีย *Staphylococcus aureus* และ *Bacillus subtilis* ของสิ่งสกัดในชั้นเอทิลอะซิเตท จากอาหารเลี้ยงเชื้อของสายพันธุ์ Sc026 แยกได้สารจำพวก DKPs 5 ชนิด ประกอบด้วย สารที่เคยพบแล้ว 4 ชนิดคือ *cyclo*-(L-Pro-Gly) *cyclo*-(L-Pro-D-Leu) *cyclo*-(L-Pro-L-Trp) และ *cyclo*-(D-Pro-L-Trp) และสารใหม่อีก 1 ชนิดคือ *cyclo*-(D-Pro-L-Phe) รวมทั้งแยกได้สารใหม่จำพวก cyclic tetrapeptides 1 ชนิดคือ *cyclo*-(4-OH-Pro-4-OH-Pro-Leu-Phe) และสารจำพวก macrolactins 3 ชนิด ประกอบด้วยสารที่เคยพบแล้วคือ macrolactin F และสารใหม่อีก 2 ชนิดคือ 7-O-succinyl macrolactin F และ 7-O-succinyl macrolactin A การพิสูจน์สูตรโครงสร้างทางเคมีและ relative stereochemistry ของสาร ทำได้โดยการวิเคราะห์ข้อมูลทางสเปกโทรสโกปี จาก IR, MS, ¹H และ ¹³C NMR โดยเฉพาะอย่างยิ่ง 1D- และ 2D-NMR ร่วมกับการเปรียบเทียบข้อมูลกับสารอื่นที่มีสูตรโครงสร้างทางเคมีที่สัมพันธ์กัน สารจำพวก DKPs ทั้งหมดนี้แสดงฤทธิ์ใหม่ในการยับยั้งไวรัสก่อโรคเรื้อรัง ในขณะที่ 2'-deoxyadenosine แสดงฤทธิ์ต้านเชื้อมาเลเรียอย่างอ่อน สารจำพวก macrolactins ที่แยกได้แสดงฤทธิ์ต้านไวรัสก่อโรคเรื้อรังและฤทธิ์ต้านแบคทีเรีย *S. aureus* และ *B. subtilis* ยกเว้น 7-O-succinyl macrolactin F แสดงฤทธิ์ต้านแบคทีเรียเพียงอย่างเดียว

หลักสูตร วิทยาศาสตร์คชภูมิต

สาขาวิชา เกษตรเคมีและผลิตภัณฑ์ธรรมชาติ

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ลายมือชื่ออาจารย์ที่ปรึกษา

ลายมือชื่ออาจารย์ที่ปรึกษาร่วม

ลายมือชื่ออาจารย์ที่ปรึกษาร่วม

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KEY WORD : *BACILLUS* SP./ MARINE *BACILLUS*/ ANTIBACTERIAL ACTIVITY/
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ACTIVITY/ CYTOTOXIC ACTIVITY/ DIKETOPIPERAZINES/
MACROLACTINS/ NUCLEOSIDE/ CYCLIC TETRAPEPTIDE

MISS CHUTIMA JARUCHOKTAWEECHAI : BIOACTIVE
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In the course of our investigation on bioactive substances from marine microorganisms, the strains of gram-positive rod-shaped bacteria, Sc018 and Sc026 from marine sediment and Sc004 from seawater around Sichang Island showed interesting biological activity. Based on morphological, cultural, physiological and biochemical characteristic studies, the strains Sc018 and Sc026 were identified as belonging to the genus *Bacillus*, while the strain Sc004 was still unable to be identified. Directed by anti-herpes simplex virus activity, fractionation of the butanol extract from the broth of the strain Sc004 yielded a known diketopiperazines (DKPs), *cyclo*-(L-Pro-Gly) and a known purine nucleoside, 2'-deoxyadenosine; meanwhile the dichloromethane extract from the strain Sc018 yielded three DKPs including two known compounds, *cyclo*-(*trans*-4-OH-L-Pro-L-Phe) and *cyclo*-(D-Pro-Leu), and one new compound, *cyclo*-(D-Pro-Ile). The bioassay-directed fractionation, using antibacterial activity against both *Staphylococcus aureus* and *Bacillus subtilis*, of the ethylacetate extract from the fermentation broth of the strain Sc026 led to the isolation of five DKPs including four known compounds, *cyclo*-(L-Pro-Gly), *cyclo*-(L-Pro-D-Leu), *cyclo*-(L-Pro-L-Trp), and *cyclo*-(D-Pro-L-Trp) and a new compound, *cyclo*-(D-Pro-L-Phe), together with a new cyclic tetrapeptide, *cyclo*-(4-OH-Pro-4-OH-Pro-Leu-Phe). Three macrolactins including the known macrolactin F, along with two new compounds, 7-*O*-succinyl macrolactin F and 7-*O*-succinyl macrolactin A were also obtained from the strain Sc026. The chemical structures and relative stereochemistry of the compounds were elucidated through extensive analyses of their IR, MS, ¹H, and ¹³C NMR spectral data, especially 1D- and 2D-NMR, as well as comparison with known related compounds. All of these DKPs showed new activity against *Herpes simplex* virus while 2'-deoxyadenosine exhibited weak antimalarial activity. The isolated macrolactins exhibited both anti-herpes simplex virus activity and antibacterial activity against *S. aureus* and *B. subtilis* except for 7-*O*-succinyl macrolactin F showing only antibacterial activity.

จุฬาลงกรณ์มหาวิทยาลัย

หลักสูตร วิทยาศาสตร์ดุสิตบัณฑิต

สาขาวิชา เกษษเคมีและผลิตภัณฑ์ธรรมชาติ

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ลายมือชื่อนิติ

ลายมือชื่ออาจารย์ที่ปรึกษา

ลายมือชื่ออาจารย์ที่ปรึกษาร่วม

ลายมือชื่ออาจารย์ที่ปรึกษาร่วม

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ABBREVIATIONS

$[\alpha]^{20}_D$	=	specific rotation at 20° and sodium D line (589 nm)
AcOH	=	acetic acid
ATCC	=	American Type Culture Collection, Maryland, USA
ax	=	axial
BC	=	breast cancer cells
br s	=	broad singlet
BuOH	=	butanol
°C	=	degree Celsius
^{13}C NMR	=	carbon-13 nuclear magnetic resonance
CCRF-CEM	=	human leukemia cells
CDCl_3	=	deuterated chloroform
CHCl_3	=	chloroform
CH_2Cl_2	=	dichloromethane
CH_3CN	=	acetonitrile
$(\text{CH}_3)_2\text{CO}$	=	acetone
cm	=	centimeter
CIMS	=	chemical ionization mass spectroscopy
COSY	=	^1H - ^1H correlation spectroscopy
δ	=	chemical shift
CV1	=	monkey kidney fibroblast cells
DEPT	=	distortionless enhancement by polarization transfer

d	=	doublet
dd	=	doublet of doublets
ddd	=	doublet of doublet of doublets
dddd	=	doublet of doublet of doublet of doublets
DMSO- <i>d</i> ₆	=	deuterated dimethylsulphoxide
dt	=	doublet of triplets
ED ₅₀	=	50% effective dose
EtOAc	=	ethylacetate
ε	=	molar absorptivity
EIMS	=	electron impact mass spectroscopy
ESITOF	=	electrospray ionization time of flight
eq	=	equatorial
g	=	gram
μg	=	microgram
HCT 116	=	human colorectal carcinoma cell lines
HETCOR	=	¹ H- ¹³ C heteronuclear correlation (one bond)
HMBC	=	¹ H-detected heteronuclear multiple bond correlation
HMQC	=	¹ H-detected heteronuclear multiple quantum coherence
¹ H NMR	=	proton nuclear magnetic resonance
H ₂ O	=	water
HPLC	=	high performance liquid chromatography
HRFABMS	=	high resolution fast atom bombardment mass spectroscopy
HT 29	=	human colon carcinoma cell lines

Hz	=	hertz
IC ₅₀	=	50% inhibition concentration
IR	=	infrared
<i>J</i>	=	coupling constant
KB	=	human epidermoid carcinoma cells of the nasopharynx
L1210	=	murine lymphoma cells or murine leukemia cells
L5178Y	=	murine leukemia cells
μl	=	microliter
L	=	liter
λ _{max}	=	wavelength at maximal absorption
LoVo	=	human colon adenocarcinoma cells
M ⁺	=	molecular ion
m	=	multiplet
μm	=	micrometer
MeOH	=	methanol
μg	=	microgram
mg	=	milligram
MHz	=	megahertz
MCF-7	=	various human breast tumor cells
MIC	=	minimum inhibition concentration
min	=	minute
ml	=	milliliter
mm	=	millimeter

MRC5CV1	=	SV40-transformed fibroblast cells
ν_{\max}	=	wave number at maximum absorption
nm	=	nanometer
NMR	=	nuclear magnetic resonance
NOE	=	nuclear overhauser effect
NOESY	=	nuclear overhauser effect correlated spectroscopy
NSCLC-N6	=	human bronchopulmonary non-small-cell-lung carcinoma cells
P388	=	murine leukemia cells
ppm	=	part per million
s	=	singlet
SDA	=	Sabouraud dextrose agar
sp.	=	species
t	=	triplet
T24	=	transitional bladder carcinoma cells
TFA	=	trifluoroacetic acid
THF	=	tetrahydrofuran
TLC	=	thin layer chromatography
TSA	=	Trypticase soy agar
UV	=	ultraviolet
Vero cell line	=	African green monkey kidney cell line