อนุกรมวิธานและเมแทบอไลต์ทุติยภูมิของสายพันธุ์สเตรปโตมัยซิส, อะมัยโคลาทอปซิส และคิตะซาโตสปอราที่คัดเลือก

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TAXONOMY AND SECONDARY METABOLITES OF SELECTED *STREPTOMYCES*, *AMYCOLATOPSIS* AND *KITASATOSPORA* STRAINS

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A Dissertation Submitted in Partial Fulfillment of the Requirements
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Thesis Title	TAXONOMY AND SECONDARY METABOLITES OF SELECTED *STREPTOMYCES*, *AMYCOLATOPSIS* AND *KITASATOSPORA* STRAINS
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Field of Study	Pharmaceutical Chemistry and Natural Products
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ภรณี ศรีปรีชาศักดิ์ : อนุกรมวิธานและเมแทบอไลต์ทุติยภูมิของสายพันธุ์สเตรปโตมัยซิส, อะมัยโคลาทอปซิส และคิตะซาโตสปอราที่คัดเลือก. (TAXONOMY AND SECONDARY METABOLITES OF SELECTED *STREPTOMYCES*, *AMYCOLATOPSIS* AND *KITASATOSPORA* STRAINS) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: ศ. ดร.สมบูรณ์ ธนาศุภวัฒน์, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: อ. ดร.คณิต สุวรรณบริรักษ์, 244 หน้า.

ได้คัดแยกแอคติโนมัยสีท 79 ไอโซเลท จากตัวอย่างดิน 29 ตัวอย่าง ซึ่งเก็บจากอุทยานแห่งชาติน้ำตกกรุงชิง อุทยานแห่งชาติหมู่เกาะอ่างทอง และอุทยานแห่งชาติหมู่เกาะสิมิลัน โดยใช้อาหารวุ้น starch casein nitrate และ potato starch-glycerol จากการศึกษาลักษณะฟีโนไทป์และลักษณะจีโนไทป์โดยการวิเคราะห์ลำดับเบสในช่วง 16S rRNA gene สามารถพิสูจน์เอกลักษณ์ของแอคติโนมัยสีท จำนวน 74 ไอโซเลท เป็นสกุลสเตรปโตมัยซิส 24 สปีชีส์ จำนวน 3 ไอโซเลท เป็นสกุลคิตะซาโตสปอรา 2 สปีชีส์ และที่เหลืออีก 2 ไอโซเลท เป็นสกุลอะมัยโคลาทอปซิส 1 สปีชีส์ และสกุลโนโนมูเรีย 1 สปีชีส์ และพบว่าสเตรปโตมัยซิส 5 สายพันธุ์ เป็นสปีชีส์ใหม่ โดยเสนอตั้งชื่อสายพันธุ์ KC-031 และ KC-038 เป็น *Streptomyces siamensis* สายพันธุ์ KC-106 เป็น *S. similanensis* สายพันธุ์ KC-035 เป็น *S. krungchingensis* และสายพันธุ์ KC-112 เป็น *S. andamanensis* นอกจากนี้ยังพบว่า สายพันธุ์ KC-061 เป็นสปีชีส์ใหม่ เสนอชื่อเป็น *Nonomuraea thailandensis* พบว่าแอคติโนมัยสีทจำนวน 45 ไอโซเลท เมื่อเพาะเลี้ยงในอาหาร no. 51 และ 53 มีฤทธิ์ต้านจุลชีพ *Kocuria rhizophila* ATCC 9341, *Bacillus subtilis* ATCC 6633, *Escherichia coli* NIHJ KB213, *Xanthomonas campestris* pv. *oryzae* KB88, *Candida albicans* KF1 และ *Mucor racemosus* IFO 4581^{T}

จากการวิเคราะห์องค์ประกอบทางเคมีโดยเครื่องโครมาโทกราฟีของเหลวสมรรถนะสูงจากสารสกัดหยาบเอทิลอะซิเตทของสเตรปโตมัยซิสจำนวน 24 ไอโซเลท เมื่อเลี้ยงในอาหาร no. 54 จึงได้คัดเลือกสารสกัดหยาบจาก *S. tendae* สายพันธุ์ KC-097 และ *S. cavourensis* สายพันธุ์ KC-121 เพื่อนำไปศึกษาสารเมแทบอไลต์ทุติยภูมิ จากการศึกษาสามารถแยกสาร *N*-acetyltryptamine, phenethylacetamide, germicidin B, germicidin C, germicidin A และ isogermicidin A ได้จาก *S. tendae* สายพันธุ์ KC-097 โดยสารบริสุทธิ์ที่แยกได้ไม่แสดงฤทธิ์ทางชีวภาพที่ทดสอบ ยกเว้น *N*-acetyltryptamine แสดงฤทธิ์เป็นพิษต่อเซลล์มะเร็ง NCI-H187 (IC_{50} 46.87 µg/ml) ในขณะที่สารที่แยกได้จาก *S. cavourensis* สายพันธุ์ KC-121 คือ สาร bafilomycin D และ 21-(*O*-methyl)-bafilomycin A_1 โดยแยกได้จาก fraction KC121F2F3 ที่แสดงฤทธิ์ต้านเชื้อมาลาเรีย *Plasmodium falciparum* K1 (IC_{50} 0.21 µg/ml) ฤทธิ์เป็นพิษต่อ Vero cells (IC_{50} 0.37 µg/ml) และฤทธิ์เป็นพิษต่อเซลล์มะเร็ง (KB; IC_{50} 18.39 µg/ml, MFC-7; IC_{50} 4.18 µg/ml และ NCI-H187; IC_{50} 0.23 µg/ml)

สาขาวิชา เภสัชเคมีและผลิตภัณฑ์ธรรมชาติ ลายมือชื่อนิสิต .....

ปีการศึกษา 2556 ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก .....

ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์ร่วม .....

5176962833 : MAJOR PHARMACEUTICAL CHEMISTRY AND NATURAL PRODUCTS
KEYWORDS: ACTINOMYCETES / STREPTOMYCES / AMYCOLATOPSIS / KITASATOSPORA / TAXONOMY / SECONDARY METABOLITES

PARANEE SRIPREECHASAK: TAXONOMY AND SECONDARY METABOLITES OF SELECTED *STREPTOMYCES*, *AMYCOLATOPSIS* AND *KITASATOSPORA* STRAINS. ADVISOR: PROF. SOMBOON TANASUPAWAT, Ph.D., CO-ADVISOR: KHANIT SUWANBORIRUX, Ph.D., 244 pp.

Seventy nine isolates of actinomycetes were isolated from twenty nine soil samples collected from Krung Ching Waterfall National Park, Angthong Islands National Park and Similan Islands National Park by using starch casein nitrate agar and potato starch-glycerol agar. All isolated actinomycetes were taxonomically identified on the basis of their phenotypes and genotypes using 16S rRNA gene sequence analysis. Seventy four isolates were classified as twenty four species of *Streptomyces*. Among them, five strains were proposed as four new species, including strains KC-031 and KC-038 as *Streptomyces siamensis*, strain KC-106 as *S. similanensis*, strain KC-035 as *S. krungchingensis*, and KC-112 as *S. andamanensis*. In addition, strain KC-061 was proposed as a new *Nonomuraea thailandensis*. Moreover, three strains KC-001, KC-005 and KC-143 were identified as two species of *Kitasatospora* and strain KC-132 was identified as *Amycolatopsis*. Forty five isolates cultivated in production media no. 51 and 53 showed antimicrobial activity against *Kocuria rhizophila* ATCC 9341, *Bacillus subtilis* ATCC 6633, *Escherichia coli* NIHJ KB213, *Xanthomonas campestris* pv. *oryzae* KB88, *Candida albicans* KF1 and *Mucor racemosus* IFO 4581^{T}.

Twenty four *Streptomyces* strains were selected to cultivate in production medium no. 54 and their crude EtOAc extracts of the culture broths were analyzed for chemical profiles by HPLC. The crude extracts of *S. tendae* strain KC-097 and *S. cavourensis* strain KC-121 showed interesting chemical profiles and were selected for secondary metabolite study. The isolation of the extract from *S. tendae* strain KC-097 yielded *N*-acetyltryptamine, phenethylacetamide, germicidin B, germicidin C, germicidin A and isogermicidin A. The isolated compounds exhibited no test biological activities, except *N*-acetyltryptamine exhibited cytotoxicity against cancer cell NCI-H187 (IC_{50} 46.87 µg/ml). Meanwhile, the extract of *S. cavourensis* strain KC-121 provided bafilomycin D and 21-(*O*-methyl)-bafilomycin A_1 from fraction KC121F2F3 which exhibited antimalarial activity against *Plasmodium falciparum* K1 (IC_{50} 0.21 µg/ml), cytotoxicity against Vero cells (IC_{50} 0.37 µg/ml) and cytotoxicity against cancer cells (KB; IC_{50} 18.39 µg/ml, MFC-7; IC_{50} 4.18 µg/ml and NCI-H187; IC_{50} 0.23 µg/ml).

Field of Study: Pharmaceutical Chemistry and Natural Products

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Student's Signature .....

Advisor's Signature .....

Co-Advisor's Signature .....

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LIST OF ABBREVIATIONS AND SYMBOLS

$Ba(OH)_2$	=	Barium hydroxide
bp	=	Base pairs
BSA	=	Bovine serum albumin
ºC	=	Degree Celsius
Ca^{2+}	=	Calcium ion
CFU	=	Colony forming unit
cm	=	Centimeter
CH_3OH	=	Methanol
$CDCl_3$	=	Deuterated chloroform
CH_3CN	=	Acetonitrile
$CHCl_3$	=	Chloroform
C_5H_5N	=	Pyridine
CH_3COCH_3	=	Acetone
COSY	=	Correlation spectroscopy
^{13}C-NMR	=	Carbon-13 nuclear magnetic resonance
δ	=	Chemical shift
d	=	Doublet
dd	=	Doublet of doublets
DAP	=	Diaminopimelic acid
DEPT	=	Distortionless enhancement by polarization tranfer
DNase	=	Deoxyribonuclease
dNTP	=	Deoxyribonucreotide triphosphate
DNA	=	Deoxyribonucleic acid
DPG	=	Diphosphatidylglycerol
DON	=	2,7-Dihydroxynapthalene
EDTA	=	Ethylenediaminetetraacetic acid
EtOAc	=	Ethyl acetate

EtOH	=	Ethanol
Ex/Em	=	Excitation and emission wavelengths
F	=	Forward
FAME	=	Fatty acid methyl ester
FAB MS	=	Fast atom bombardment mass spectrometry
g	=	Gram
µg	=	Microgram
G+C	=	Guanine-plus-cytosine
h	=	Hour
HCl	=	Hydrochloric acid
HCOOH	=	Formic acid
HMBC	=	^{1}H-detected heteronuclear multiple bond correlation
HMQC	=	^{1}H-detected heteronuclear multiple quantum coherence
^{1}H-NMR	=	Proton nuclear magnetic resonance
H_2O	=	Water
HPLC	=	High performance liquid chromatography
H_2S	=	Hydrogen sulphide
H_2SO_4	=	Sulfuric acid
Hz	=	Hertz
IC	=	Inhibitory concentration
ISP	=	International *Streptomyces* Project
J	=	Coupling constant
KB	=	Human oral epidermoid carcinoma, ATCC CCL-17
K_2HPO_4	=	Dipotassium hydrogen orthophosphate
KNO_3	=	Potassium nitrate
KOH	=	Potassium hydroxide
l	=	Liter
µl	=	Microliter

m	=	Multiplet
M	=	Molar
µm	=	Micrometer
m/z	=	Mass to charge ratio
Max	=	Maximum
MCF-7	=	Human breast cancer, ATCC HTB-22
MEGA	=	Molecular Evolutionary Genetics Analysis
MeOH	=	Methanol
meso-DAP	=	*meso*-Diaminopimelic acid
mg	=	Milligram
mm	=	Millimeter
mm^3	=	Cubic millimeter
ml	=	Milliliter
mM	=	Millimole
MHz	=	Megahertz
min	=	Minute
MIC	=	Minimun Inhibitory Concentration
MK	=	Menaquinone
MS	=	Mass spectrometry
MW	=	Molecular weight
Methyl-PE	=	Methylphosphatidylethanolamine
N	=	Normal
NA	=	Nutrient agar
NaCl	=	Sodium chloride
NaOH	=	Sodium hydroxide
NCI-H187	=	Human small-cell lung cancer, ATCC CRL-5804
NPG	=	Ninhydrin-positive glycophospholipid
nm	=	Nanometer

nov.	=	Novel
NMR	=	Neclear magnetic resonance
NOESY	=	Nuclear Overhouser effect correlation spectroscopy
OD	=	Optical density
OH-PE	=	Hydroxyphosphatidylethanolamine
%	=	Percentage
PBS	=	Phosphate buffer saline
PCR	=	Polymerase chain reaction
PC	=	Phosphatidylcholine
PE	=	Phosphatidylethanolamine
Lyso-PE	=	Lyso-phosphatidylethanolamine
PG	=	Phosphatidylglycerol
PI	=	Phosphatidylinositol
ppm	=	Part per million
rRNA	=	Ribosomal ribonucleic acid
rpm	=	Round per minute
s	=	Singlet
sec	=	Second
SEM	=	Scanning electron microscope
sp.	=	Species
t	=	Triplet
TAE	=	Tris-acetate EDTA
Tm	=	Melting temperature
TLC	=	Thin layer chromatography
U	=	Units
Vero cells	=	African green monkey kidney fibroblasts; ATCC CCL-81
YS	=	Yeast extract-soluble starch agar

CHAPTER I
INTRODUCTION

Actinomycetes are filamentous Gram-positive bacteria having high G+C content in genomic DNA. They are widely distributed in natural environments and play an important role in the degradation of organic matters. They are also well known as a rich source of bioactive compounds. Actinomycetes mainly comprise *Streptomyces, Amycolatopsis, Kitasatospora*, *Microbispora, Dactylosporangium, Micromonospora, Pseudonocardia, Streptosporangium, Actinomadura, Nocardia, etc.* Actinomycetes are commonly divided into two groups namely streptomycetes and non-streptomycetes (rare actinomycetes). According to the Bergey's Manual of Systematic Bacteriology in volume 5 (2012), Gram-positive bacteria with high G+C content (50 to over 70 mol%) in genomic DNA were classified as actinobacteria. Therefore, actinomycetes are combined into a group of actinobacteria.

Actinomycetes, especially the strains of the genus S*treptomyces* are well known as important microorganisms, because these bacteria can produce novel bioactive secondary metabolites of pharmaceutical importance such as antibiotics and anticancer compounds with over 10,000 bioactive compounds representing about 45% of all bioactive microbial secondary metabolites. In addition, actinomycetes have long been reported as an important source of biotechnologically secondary metabolites such as plant growth hormones, enzymes, enzyme inhibitors and pigments (Acharyabhatta, 2013; Bérdy, 2005; Yuan *et al*., 2014). According to Bérdy (2005), the members of the genus *Streptomyces* are superior to other actinomycetes in their ability to produce large numbers and varieties of bioactive metabolites especially antibiotics. The well known antibiotics such as erythromycin, tetracycline, streptomycin, chloramphenicol, neomycin, nystatin, amphotericin, kanamycin and cycloheximide which have been used in clinical treatment for infection diseases are derived from *Streptomyces* strains. However, some important antibiotics in pharmaceutical industry such as rifamycin and vancomycin were produced from *Amycolatopsis* strains and setamycin from *Kitasatospora* strain. These two latter genera, *Amycolatopsis* and *Kitasatospora*, have a few reports for taxonomy and antibiotic research. Because of the antibiotic resistance crisis and decreasing in the rate of discovery new bioactive compounds, one of the more efficient ways of discovering the novel bioactive metabolites from microorganisms is through the search for novel microorganisms. Because of the novel microorganisms

are more possible to produce some new secondary metabolites than known microorganisms.

Therefore, this research study is focused to isolate and identify the actinomycete strains in the genera *Streptomyces, Amycolatopsis*, and *Kitasatospora* and to isolate the bioactive secondary metabolites from the selected *Streptomyces, Amycolatopsis* or *Kitasatospora* strains.

The main objectives of this research study are as follows:

1. To isolate and identify the selected *Streptomyces*, *Amycolatopsis* and *Kitasatospora* strains using phenotypic, chemotaxonomic characteristics and 16S rRNA gene sequence analysis
2. To evaluate the antimicrobial activity of the *Streptomyces*, *Amycolatopsis* and *Kitasatospora* strains
3. To select the *Streptomyces*, *Amycolatopsis* or *Kitasatospora* strains showing antimicrobial activity and interested chemical profiles of secondary metabolites
4. To isolate and identify the antimicrobial secondary metabolites from the selected *Streptomyces*, *Amycolatopsis* or *Kitasatospra* strains
5. To study other biological activities of secondary metabolites from the selected strains

CHAPTER II
LITERATURE REVIEW

Actinomycetes are filamentous Gram-positive bacteria, which are a large group of aerobic microorganisms with high G+C content ranging from 50 to over 70 mol% in genomic DNA. These bacteria form branching filaments or mycelia and asexual spores which are closely resemble to fungi in overall morphology (Sharma, 2014). Actinomycestes produce branching mycelium of two kinds namely substrate mycelium and aerial mycelium that look like the fungi. Actinomycetes were originally considered to be an intermediate group between bacteria and fungi but now are recognized as prokaryotic organisms. Cell nuclei are absent in actinomycetes and hyphae are from 0.5 to 1 μm in diameter, which are much smaller than fungal hyphae with 3-8 μm in diameter. Among actinomycetes, streptomycetes are the dominant members while non-streptomycetes are called rare actinomycetes.

The life cycle of actinomycetes is quite complex which they form spores for reproduction. The spores of actinomycetes are conidia or arthrospores forming singly or in chains of various lengths or enclosed in sporangia (Lechevalier & Lechevalier, 1967). In the appropriate condition, the spore germinates the germ tube and develops the substrate mycelium toward the solid surface. Upon differentiation the aerial mycelium is formed which later develops to the chain of spores.

The majority of actinomycetes are free living, saprophytic bacteria found widely distributed in both terrestrial and aquatic ecosystems, mainly in soil, composts, sediment water and colonizing plants where they play an essential role in recycling refractory biomaterials by decomposing complex mixtures of polymers in dead plants, animals and fungal materials. They are also important in soil biodegradation and humus formation as they recycle the nutrients associated with recalcitrant polymers, such as chitin, keratin, and lignocelluloses, (Goodfellow & Williams 1983; McCarthy & Williams 1992; Stach & Bull, 2005).

Actinomycetes are important microorganisms, because these bacteria can produce novel secondary metabolites. The bioactive secondary metabolites produced by microorganisms is reported to be about 23,000 of which 10,000 are produced by actinomycetes, thus representing 45% of all bioactive microbial metabolites discovered. Among actinomycete speceis, approximately 7,600 compounds are produced by *Streptomyces* species (Berdy, 2005).

2.1 Genus *Streptomyces*

2.1.1 Taxonomy of *Streptomyces*

The genus *Streptomyces* belonging to the family *Steptomycetaceae* was proposed by Waksman & Henrici (1943) to accommodate aerobic, Gram-positive, spore-forming actinomycetes. Nowadays, the genus comprises more than 649 recognized species with validly published names, some new species have been described in the past few years as listed in Table 2.1. *Streptomyces* are widely distributed in natural environments, especially in soils.

Members of *Streptomyces* are able to form an extensively branched substrate mycelium and are also able to produce aerial mycelium that typically differentiate into spore chains. Chemotaxonomically, *Streptomyces* strains contain LL-diaminopimelic acid in the cell-wall peptidoglycan but lack diagnostic sugars in whole-cell hydrolysates (wall chemotype I) (Lechevalier & Lechevalier, 1970). The predominant components of menaquinones are hexadrogenated and octahydrogenated menaquinones with nine isoprene units [MK-9(H_6), MK-9(H_8)]. The phospholipid profile is phosphatidylethanolamine (PE) as a diagnostic phospholipid (Type PII phospholipid pattern) (Lechevalier *et al*., 1977). The mycolic acid is absence. The members of this genus have DNA G+C contents in the range of 69-78 mol%.

The hierarchic taxonomy of *Streptomyces* based on 16S and 23S rRNA gene sequence comparison is indicated below:

Phylum: *Actinobacteria*

Class: *Actinobacteria*

Order: *Streptomycetales*

Family: *Streptomycetaceae*

Genus: *Streptomyces*

Table 2.1 Novel *Streptomyces* species and sources (2013-present)

Species	Sources	References
S. abietis	Pine forest soil	Fujii *et al*., 2013
S. aidingensis	Lake sediment	Xia *et al*., 2013
S. amritsarensis	Soil	Sharma *et al*., 2014
S. araujoniae	Potato tubercle	da Silva *et al*., 2013
S. barkulensis	Fish dumping yard sediment	Ray *et al*., 2014
S. bullii	Hyper-arid Atacama Desert soil	Santhanam *et al*., 2013
S. catbensis	Soil	Sakiyama *et al*., 2014
S. chiangmaiensis	South-East Asian stingless bee (*Tetragonilla collina*)	Promnuan *et al*., 2013
S. chilikensis	Brackish water sediment	Ray *et al*., 2013
S. chlorus	Soil	Kim *et al*., 2013
S. endophyticus	*Artemisia annua* L. root	Li *et al*., 2013
S. erringtonii	Hay meadow soil	Santhanam *et al*., 2013
S. fukangensis	Saline-alkaline soil	Zhang *et al*., 2013
S. graminilatus	Bamboo litter	Lee & Whang, 2014
S. graminisoli	Bamboo rhizosphere soil	Lee & Whang, 2014
S. halophytocola	Surface-sterilized stems of a coastal halophyte *Tamarix chinensis* Lour	Qin *et al*., 2013
S. harbinensis	Soybean root	Liu *et al*., 2013
S. heilongjiangensis	Root surface of soybean	Liu *et al*., 2013
S. hokutonensis	Strawberry root rhizosphere	Yamamura *et al*., 2014
S. hoynatensis	Deep marine sediment	Veyisoglu & Sahin, 2014
S. hundungensis	Limestone quarry soil	Nimaichand *et al*., 2013
S. jiujiangensis	Soil	Zhang *et al*., 2014
S. kaempferi	Hay meadow soil	Santhanam *et al*., 2013
S. karpasiensis	Soil	Veyisoglu *et al*., 2014
S. kebangsaanensis	Ethnomedicinal plant	Sarmin *et al*., 2013
S. lannensis	South-East Asian stingless bee (*Tetragonilla collina*)	Promnuan *et al*., 2013
S. leeuwenhoekii	Hyper-arid soil	Busarakam *et al*., 2014
S. muensis	Soil	Ningthoujam *et al*., 2013
S. polyrhachii	Edible Chinese black ant (*Polyrhachis vicina* Roger)	Yu *et al*., 2013
S. pratensis	Soil	Rong *et al*., 2013
S. rhizophilus	Bamboo rhizosphere soil	Lee & Whang, 2014
S. siamensis	Soil	Sripreechasak *et al*., 2013
S. similanensis	Soil	Sripreechasak *et al*., 2013
S. tsukubensis	Soil	Muramatsu & Nagai, 2013
S. tunisiensis	Soil	Slama *et al*., 2014
S. viridis	Soil	Kim *et al*., 2013
S. wuyuanensis	Soil	Zhang *et al*., 2013
S. yaanensis	Soil	Zheng *et al*., 2013
S. zhaozhouensis	Leaf of candelabra aloe (*Aloe arborescens* Mill)	He *et al*., 2014
S. ziwulingensis	Grassland soil	Lin *et al*., 2013

2.1.2 Secondary metabolites from *Streptomyces*

The *Streptomyces* represents a group of microorganisms widely distributed in nature. The genus *Streptomyces* remains a focus of systematic research, because of not only being still a rich source of commercially significant compounds such as antibiotics, enzymes, enzyme inhibitors and pharmacologically active agents but also taxonomic difficulties within the genus caused by a large number of isolates and insufficient species definition (Berdy, 2005, Rong & Huang, 2010).

The *Streptomyces* are valuable because they produce most of commercial antibiotics including erythromycin, tetracycline, streptomycin, chloramphenicol, neomycin, nystatin, amphotericin, kanamycin and cycloheximide. Some examples of bioactive secondary metabolites produced by *Streptomyces* strains are shown in Table 2.2.

Table 2.2 Bioactive secondary metabolites from *Streptomyces*

Compounds	Strains	Sources	Biological activity	References
Acarviostatins	*S. coelicoflavus* ZG0656	Soil	Inhibitors of porcine pancreatic α-amylase	Geng & Bai, 2008
Albidopyrone	*Streptomyces* sp. NTK 227	Marine sediment	Inhibitory activity against protein-tyrosin phosphatase β.	Hohmann *et al*., 2009
Amino-oligosaccharide SF638-1	*Streptomyces* sp. PW638	Soil	Antidiabetic activity	Meng *et al*., 2011
Angumycinones	*Streptomyces* sp. KMC004	Acidic mine drainage	Antimicrobial activity against *Micrococcus luteus*, *Enterococcus hirae*, and methicillin-resistant *Staphylococcus aureus* (MRSA)	Park *et al*., 2014
Bafilomycin derivatives	*Streptomyces* sp. CS	*Maytenus hookeri* callus	Cytotoxicity against MDA-MB-435 cell	Li *et al*., 2010
Caboxamycin	*Streptomyces* sp. NTK 937	Deep-sea sediment	Antimicrobial activity against Gram-positive bacteria Antitumor Inhibitor of phosphodiesterase	Hohmann *et al*., 2009
Campechic acids	*Streptomyces* sp. CHI93	soil	Antibacterial activity against *Micrococcus luteus* Cytotoxicity against murine colon carcinoma 26-L5 cells (anti-invasive)	Yu *et al*., 2014
Carpatamides	*Streptomyces* sp. SNE-011	Marine sediment	Cytotoxicity against NSCLC cell lines HCC366	Fu *et al*., 2014
Chaxamycins	*Streptomyces* sp. C34	Hyper-arid Desert Soil	Antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*	Rateb *et al*., 2011
Chromomycin B	*Streptomyces* sp. WBF16	Marine sediments	Cytotoxicity against SGC7901, HepG2, A549, HCT116 and COC1 and HUVEC.	Lu *et al*., 2012
Coprismycins	*Streptomyces* sp.	Dung beetle	Neuroprotective activity	Kim *et al*., 2011
Cyclic hexapeptides	*S. alboflavus* 313	Soil	Antibacterial activity against Gram-positive bacteria	Ji *et al*., 2012
Cyslabdans	*Streptomyces* sp. K04-0144	Soil	Imipenem activity against methicillin-resistant *Staphylococcus aureus*	Koyama *et al*., 2011

Table 2.2 (continued)

Compounds	Strains	Sources	Biological activity	References
Dehydroxyaquayamycin	*Streptomyces* sp. BCC45596	Marine sponge, *Xestospongia* sp.	Antimalarial act activity against *Plasmodium palcifarum* K1 Antitubercular against *Mycobacterium tuberculosis* Cytotoxicity against KB, MCF-7, NCI-H187 and Vero cells	Supong *et al*., 2012
Elaiomycins	*Streptomyces* sp. strain HKI0708	Soil	Antimycobacterial activity Anti-*Aspergillus* activity Cytotoxic activities	Ding *et al*., 2012
Farneside A	*Streptomyces* sp. CNT-372	Marine sediment	Antimalarial activity against parasite *Plasmodium falciparum*	Zafrir Ilan *et al*., 2013
Fijimycins	*Streptomyces* sp. CNS-575	Marine sediment	Antibacterial activity against three methicillin-resistant *Staphylococcus aureus* (MRSA) strains	Sun *et al*., 2011
Frenolicins	*Streptomyces* sp. RM-4-15	coal fire site	Anticoccidial activity	Wang *et al*., 2013
Furaquinocin derivative (JBIR-136)	*Streptomyces* sp. 4963H2	Soil	Cytotoxicity	Kawahara *et al*., 2012
Gephyromycin	*S. griseus* NTK 14	Soil	Glutaminergic activity towards neuronal cells (neuroprotective properties)	Bringmann *et al*., 2005
Gombapyrones	*S. griseoruber* Acta 3662	Soil	Antibacterial and Antifungal activity Inhibitor of glycogen synthase kinase-3β (GSK-3β) and human recombinant protein tyrosine phosphatase 1B (PTPN1)	Helaly *et al*., 2009
Halichoblelides	*S. hygroscopicus* OUPS-N92	Marine fish *Halichoeres bleekeri*	Cytotoxicity against human cancer cell lines	Yamada *et al*., 2012
Herbimycins	*Streptomyces* sp. RM-7-15		Nontoxic ansamycin-based Hsp90 inhibitors for the treatment of neurodegenerative disease	Shaaban *et al*., 2013
Heronamycin A	*Streptomyces* sp. CMB-M0392	Marine sediments	Antimicrobial activity against Bacillus subtilis	Raju *et al*., 2012
Hyaluromycin	*Streptomyces* sp. MB-PO13	Sea squirt specimem (Molgula manhattensis)	Hyaluronidase Inhibitor	Harunari *et al*., 2014
Hydrazidomycins	*S. atratus*	Soil	Cytotoxic activity	Ueberschaar *et.al*., 2011
Izuminosides	*Streptomyces* sp. IFM 11260	Soil	Activity in overcoming tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) resistance in human gastric adenocarcinoma cells Synergistic activity in sensitizing TRAIL-resistance AGS cells	Abdelfattah *et.al*., 2011
Kettapeptin	*Streptomyces* sp. GW99/1572	Soil	Anti-Gram-positive bacteria	Maskey *et al*., 2006
Langkocyclines	*Streptomyces* sp. Acta 3034	Soil	Antimicrobial activity against Gram-positive bacteria Antiproliferative activity against various human tumor cell lines	Kalyon *et al*., 2013
Lansai A–D	*Streptomyces* sp. SUC1	Aerial roots of *Ficus benjamina*	Anticancer Antifungal	Tuntiwachwuttikul *et al*., 2008
Linearolides	*Streptomyces* sp. RK95-74	Soil	Cytotoxicity against HL-60 cells	Ueki *et al*., 2013

Table 2.2 (continued)

Compounds	Strains	Sources	Biological activity	References
Lydiamycins	*S. lydicus* HKI0343	Soil	Antibacterial activity Antifungal activity	Huang *et al*., 2006
Macrocyclic lactone	*S. microflavus* neau3	Soil	Insecticidal activity against adult mites Nematocidal activity against *Caenorhabditis elegans*	Wang *al*., 2011
Mollemycin A	*Streptomyces* sp. CMB-M0244	Marine sediment	Antimicrobial activity Antimalarial activity against *Plasmodium falciparum*	Raju *et al*., 2014
Naphthomycin L	*Streptomyces* sp. CS	Tissue cultures of *M. hookeri*	Antifungal activity	Yang et al., 2012
Nivetetracyclates	*S. niveus* LS2151	Soil	Cytotoxicity against human HeLa cells	Chen *et al*., 2013
Nosokophic acid	*Streptomyces* sp. K04-0144		Antimicrobial activity against MRSA	Koyama *et al*., 2012
Novclobiocin 101	*Streptomyces* sp. BCC33756	Soil	Cytotoxicity against NCI-H187 cell Antibacterial activity against *Bacillus subtilis*	Dramae *et al*., 2013
Phenylpyridineylbutenol	*Streptomyces* sp. KACC91010	Soil	Antiproliferative activity	Shin *et al*., 2006
Phoslactomycins	*Streptomyces* sp. MLA1839	Soil	Antifungal activity against multiple plant pathogenic fungi	Fotso *et al*., 2013
Promomycin	*Streptomyces* sp. 153	Soil	Antibiotic activity	Amano *et al*., 2010
Pterocidin	*Streptomyces* sp. 153	Soil	Antibiotic activity	Amano *et al*., 2010
Pyrrolosesquiterpenes	*Streptomyces* sp. Hd7-21	Soil	Cytotoxic activity against a panel of human cancer cell lines	Liu & Liang, 2014
Rubimycinone A	*Streptomyces* sp. Lv-6-8	Terrestrial soil	Antibacterial activity against Gram positive bacteria and cytotoxic activity against various cancer cell lines	Raju *et al*., 2013
Ruthmycin	*Streptomyces* sp. RM-4-15	Soil	Antifungal activity	Wang *et al*., 2014
Samroiyotmycins	*Streptomyces* sp. BCC33756	Soil	Antimalarial activity against *Plasmodium falciparum* K1, multi-drug resistant strain Antifungal activity against *C. albicans*	Dramae *et al*., 2013
Sannastatin	*S. sannanensis* KC-7038	Feces of *Ailuropoda melanoleuca*	Growth inhibitory activity against the brine shrimp (*Artemia salina*) larvae	Yang *et al*., 2011
Strepnonesides	*Streptomyces* sp. WBF-16	Soil	Cytotoxic activity against HCT116 cell lines	Lu *et al*., 2012
Streptokordin	*Streptomyces* sp. KORDI-3238	Deep-sea Sediments	Cytotoxicity against seven human cancer cell lines	Jeong *et al*., 2006
Streptopyrrolidine	*Streptomyces* sp. KORDI-3973	Deep sea sediment	Anti-angiogenesis activity (angiogenesis inhibitor)	Shin *et al*., 2008
Tumescenamide C	*Streptomyces* sp. KUSC_F05	Soil	Antimicrobial activity against *Streptomyces* species.	Kishimoto *et al*., 2012
Venturicidin C	*Streptomyces* sp. TS-2-2	Soil	Antifungal activity against *Cladosporium cucumerinum*	Shaaban *et al*., 2014
Warkmycin	*Streptomyces* sp. Acta 2930	Alkaline soil	Anti-Gram-positive bacteria Antiproliferative activity against mouse fibroblast cell line NIH-3T3 and human cancer cell lines HepG2 and HT29	Helaly *et al*., 2013
Xiamycin	*Streptomyces* sp. GT2002/1503	Mangrove plant *Bruguiera gymnorrhiza*	Anti-HIV activity	Ding *et al*., 2010

2.2 Genus *Amycolatopsis*

2.2.1 Taxonomy of *Amycolatopsis*

The genus *Amycolatopsis* belonging to the family *Pseudonocardiaceae* was first proposed by Lechevalier *et al*. (1986) for aerobic, nocardioform actinomycetes which also encompasses the genera *Actinobispora*, *Actinopolyspora*, *Prauserella*, *Kibdelosporangium*, *Pseudonocardia*, *Saccharomonospora*, *Saccharopolyspora* and *Thermocrispum*. This genus currently comprises 65 recognized species, some new species have been described in the past few years which are shown in Table 2.3. Members of the genus *Amycolatopsis* have been isolated from diverse environments such as soil, vegetation, human and animal clinical sources, fresh water, rock and subterranean sites.

Members of *Amycolatopsis* are Gram-positive, non-acid-fast, non-motile actinomycetes that form branched vegetative hyphae undergoing fragmentation into rod-like and squarish elements. Aerial mycelium may or may not be present. Aerial hyphae, when formed, may be sterile or break down into chains of squarish to oval fragments or spore-like structures. Chemotaxonomically, *Amycolatopsis* strains have been found to contain *meso*-diaminopimelic acid, glutamic acid, alanine, muramic acid, glucosamine, arabinose and galactose in cell-wall hydrolysates (wall chemotype IV; type A whole-cell sugar pattern). The predominant components of menaquinones are dihydrogenated and tetrahydrogenated menaquinone with nine isoprene units [MK-9(H_2), MK-9(H_4)]. The phospholipid profiles are phosphatidylethanolamine (PE) as a diagnostic phospholipid with or without phosphatidylmethylethanolamine (PME) (Type PII phospholipid pattern) (Lechevalier *et al*., 1977). Fatty acid profiles include complex mixtures of saturated and branched-chain acids. The mycolic acid is absence. The members of this genus have DNA G+C contents in the range of 66-69 mol%.

The hierarchic taxonomy of *Amycolatopsis* based on 16S and 23S rRNA gene sequence comparison is indicated below:

Phylum:	*Actinobacteria*
Class:	*Actinobacteria*
Order:	*Pseudonocardiales*
Family:	*Pseudonocardiaceae*
Genus:	*Amycolatopsis*

Table 2.3 Novel *Amycolatopsis* species and sources (2007-present)

Species	Sources	References
A. bartoniae	Arid soils	Zucchi *et al*., 2012
A. bullii	Arid soils	Zucchi *et al*., 2012
A. endophytica	Oil-seed plant *Jatropha curcas* L.	Miao *et al*., 2011
A. halophila	Salt lake soil	Tang *et al*., 2010
A. helveola	Soil	Tamura *et al*., 2010
A. jiangsuensis	Coastal plant *Dendranthema indicum* (Linn.)	Xing *et al*., 2013
A. magusensis	Soil	Camas *et al*., 2013
A. marina	Ocean sediment	Bian *et al*., 2009
A. nigrescens	Stone from the wall of a Roman catacomb	Groth *et al*., 2007
A. pigmentata	Soil	Tamura *et al*., 2010
A. regifaucium	Soil	Tan *et al*., 2007
A. saalfeldensis	Rocks	Carlsohn *et al*., 2007
A. salitolerans	Hypersaline habitat	Guan *et al*., 2012
A. samaneae	Roots of *Samanea saman* (Jacq.) Merr	Duangma *et al*., 2011
A. thailandensis	Soil	Chomchoei *et al*., 2011
A. thermophila	Arid soil	Zucchi *et al*., 2012
A. tucumanensis	Copper-polluted sediments	Albarracin *et al*., 2010
A. ultiminotia	Rhizosphere soil	Lee, 2009
A. umgeniensis	Soil	Everest *et al*., 2013
A. viridis	Arid soil	Zucchi *et al*., 2012
A. xylanica	Soil	Chen *et al*., 2012

2.2.2 Secondary metabolites from *Amycolatopsis*

Amycolatopsis strains are a rich source of secondary metabolites, including ansamycins (rifamycins and tolypomycins) and glycopeptides (balhimycins and vancomycins). Furthermore, *Amycolatopsis* strains have been thoroughly studied because of their important secondary metabolism and applications in medicine and industry. Their commercial significance has led to an intensive search of *Amycolatopsis* strains for sources of novel pharmaceutical activities. The secondary metabolites produced by *Streptomyces* strains are shown in Table 2.4.

Table 2.4 Bioactive secondary metabolites from *Amycolatopsis*

Compounds	Strains	Sources	Biological activity	References
(2R, 3R)-2-hydroxy-5-*O*-methyltetrangomycin	*Amycolatopsis* sp. HCa1	Grasshoppers (*Oxya chinensis*)	Cytotoxic activity against human cervical cancer cell line (HeLa), human gastric adenocarcinoma cell line (SGC-7901), human lung adenocarcinoma cell line (SPC-A1) and mouse macrophage cell line (RAW264.7)	Guo *et al*., 2011
(2R, 3R)-2-hydroxy-8-*O*-methyltetrangomycin	*Amycolatopsis* sp. HCa1	Grasshoppers (*Oxya chinensis*)	Cytotoxic activity against human cervical cancer cell line (HeLa), human gastric adenocarcinoma cell line (SGC-7901), human lung adenocarcinoma cell line (SPC-A1) and mouse macrophage cell line (RAW264.7)	Guo *et al*., 2011
A-102395	*Amycolatopsis* sp. SANK 60206	Soil	Inhibitor of Bacterial Translocase I	Murakami *et al*., 2007
Actinotetraose E	*Amycolatopsis* sp. HCa1	Grasshoppers (*Oxya chinensis*)	Immunosuppressive activity	Guo *et al*., 2013
Amycolactam	*Amycolatopsis* sp.	Marine sponge	Cytotoxicity against gastric cancer cell line SNU638 and colon cancer cell line HCT116.	Kwon *et al*., 2014
Angucyclines	*Amycolatopsis* sp. HCa1	Grasshopper (*Oxya chinensis*)	Cytotoxic activity	Guo *et al*., 2012
Pargamicin A	*Amycolatopsis* sp. ML1-hF4	Soil	Antibacterial activity against *Staphylococcus aureus* including MRSA, *Enterococcus faecalis* and *Enterococcus faecium*	Igarashi *et al*., 2008
Pyridinium	*A. alba DVR D4*	Marine sediment	Cytotoxic activity against cancer cell lines of cervix (HeLa), breast (MCF-7) and brain (U87MG) Antibacterial activity	Dasari *et al*., 2012
Thiazomycin A	*A. fastidiosa*	Soil	Anti-Gram positive bacteria	Zhang *et al*., 2008
Vancoresmycin	*Amycolatopsis* sp. ST101170	Soil	Anti-Gram positive bacteria	Hopmann *et al*., 2002
Wuxistatin	*Amycolatopsis* sp.	Soil	HMG–CoA reductase inhibitor	Zhuge *et al*., 2008

2.3 Genus *Kitasatospora*

2.3.1 Taxonomy of *Kitasatospora*

The genus *Kitasatospora* belonging to the family *Streptomycetaceae* was firstly proposed by Omura *et al*. (1982) which also encompasses the genus *Streptomyces*. Nowadays, members of the genus Kitasatospora comprise 23 recognized species, some new species have been described in the past few years that are shown in the Table 2.5.

Kitasatospora is aerobic, Gram-positive, non-motile, chemoorganotrophic actinomycetes that form an extensively branched substrate mycelium, aerial hyphae that differentiate into long spore chains of more than 20 spores. *Kitasatospora* strains are phenotypically similar to *Streptomyces* strains. Chemotaxonomically, *Kitasatospora* strains contain *N*-acetylglucosamine, *N*-acetyl muramic acid, glycine and galactose in cell-wall hydrolysates and contain both LL-DAP and *meso*-DAP in cell wall (cell wall chemotype I/III). The presence of two isomers of DAP in cell wall depend on stages of differentiation and growth. Aerial spores on solid culture and submerged spores in liquid media contain LL-DAP whereas mycelia grown under both cultural conditions have mainly *meso*-DAP (Groth *et al*., 2003; Omura *et al*.,1982; Takahashi *et al*., 1984). These properties are unique characteristics of *Kitasatospora* strains. The predominant components of menaquinones are hexahydrogenated and octahydrogenated menaquinone with nine isoprene units [MK-9(H_6), MK-9(H_8)]. The phospholipid profiles are diphosphatidylglycerol (DPG), phosphatidylethanolamine (PE), phosphatidylglycerol (PG), phosphatidylinositol (PI) and phosphatidylinositol mannosides (PIMs), corresponding to type PII phospholipid pattern (Lechevalier *et al*., 1977). Fatty acid profiles include major amounts of iso and anteiso components. The mycolic acid is absence. The members of this genus have DNA G+C contents in the range of 66-73 mol%

The hierarchic taxonomy of *Kitasatospora* based on 16S and 23S rRNA gene sequence comparison is indicated below:

Phylum: *Actinobacteria*

Class: *Actinobacteria*

Order: *Streptomycetales*

Family: *Streptomycetaceae*

Genus: *Kitasatospora*

Table 2.5 Novel *Kitasatospora* species and sources (2001-present)

Species	Sources	References
K. arboriphila	Rhizosphere soil	Groth *et al*., 2004
K. cineracea	Soil	Tajima *et al*., 2001
K. gansuensis	Soil	Groth *et al*., 2004
K. kazusanensis	Soil	Li *et al*., 2009
K. niigatensis	Soil	Tajima *et al*., 2001
K. nipponensis	Soil	Groth *et al*., 2004
K. putterlickiae	Rhizosphere soil	Groth *et al*., 2003
K. paranensis	Soil	Groth *et al*., 2004
K. saccharophila	Soil	Li *et al*., 2009
K. sampliensis	Soil	Mayilraj *et al*., 2006
K. viridis	Soil	Liu *et al*., 2005
K. terrestris	Soil	Groth *et al*., 2004

2.3.2 Secondary metabolites from *Kitasatospora*

The reports on bioactive compounds produced by *Kitasatospora* seem to be rare when compared with the bioactive compounds produced by *Streptomyces* strains. The isolation and screening of *Kitasatospora* strains from natural sources are still limited. The secondary metabolites produced by *Kitasatospora* strains are shown in Table 2.6.

Table 2.6 Bioactive secondary metabolites from *Kitasatospora*

Compounds	Strains	Sources	Biological activity	References
Bafilomycin C1	*K. cheerisanensis* YC75	Soil	Cytotoxicity against human tumor cell lines	Moon & Hwang, 2003
Fuzanins	*Kitasatospora* sp. IFM10917	Soil	Cytotoxicity against human colon carcinoma DLD-1 cells Inhibition of Wnt signal transcription	Aida *et al*., 2009
Kitastatin 1	*Kitasatospora* sp.	Soil	Antineoplastic agent inhibited cancer cell growth	Pettit et al., 2007
Kitasatodine	*Kitasatospora* sp. H6549	Soil	Cytotoxicity against HeLa and HepG-2 cell lines	Shi *et al*., 2013
Kitasatopenoid	*Kitasatospora* sp. H6549	Soil	Cytotoxicity against HeLa and HepG-2 cell lines	Shi *et al*., 2013
Respirantin	*Kitasatospora* sp.	Soil	Antineoplastic agent inhibited cancer cell growth	Pettit et al., 2007
Sch 725424	*Kitasatospora* sp. SPRI-0408	Soil	Inhibitory activity against *Staphylococcus aureus* Antifungal activity against *Saccharomyces cerevisiae* (PM503)	Yang *et al*., 2005
Setamycin	*K. setae* NBRC 14216T	Soil	Antibiotic activity against trichomonads, some fungi and Gram-positive bacteria	Otoguro *et al*., 1988
Talosins	*K. kifunensis MJM341*	Soil	Antifungal activity	Yoon *et al*., 2006
Terpentecin	*Kitasatospora* sp. MF730-N6	Soil	Antitumor against leukemia L-1210, P388 and *Ehrlichascites carcinoma* Antibiotic activity against Gram-positive and Gram-negative bacteria.	Tamamura *et al*., 1985
Tyropeptins	*Kitasatospora* sp. MK993-dF2	Soil	New proteasome inhibitors	Momose *et al*., 2001

2.4 Identification techniques of actinomycetes

Phenotypic, chemotaxonomic and genotypic characteristics have been used for classification and identification the actinomycetes.

2.4.1 Phenotypic characteristics

Phenotypic characteristics are classical approaches for classification including morphological, physiological and biochemical characteristics. These approaches described in identification key by Nonomura (1974) and Bergey's Manual of Determinative Bacteriology (Buchanan & Gibbons, 1974) are very useful in the identification of *Streptomyces* strains. These methods are quite useful in routine identification which consisted of aerial mass color, spore chain morphology, spore surface, soluble and melanoid pigment production, reverse side of colony, assimilation of carbon source.

2.4.2 Chemotaxonomic characteristics

Chemotaxonomy is the study of chemical variation in organisms and also the use of chemical characters within the classification and identification. The chemotaxonomic methods are one of the precious strategies to distinguish actinomycetes from other bacteria (Lechevalier & Lechevalier, 1970). The development of reliable, rapid and sensitive analytical methods such as electrophoresis, chromatography and spectroscopy has led to the development of alternative chemotaxonomic approaches for actinomycete classification (Goodfellow & O'Donnell, 1993). The chemical characters usually used as key markers in chemotaxonomic identification of the actinomycetes are as follows.

(1) Cell wall composition

The composition of cell wall in actinomycetes varies greatly among different groups and is of considerable taxonomic significance. Four major cell wall types are distinguished in these filamentous bacteria on the basis of the three features of peptidoglycan composition and structure. Major constituents of cell wall chemotypes of actinomycetes are shown in Table 2.7. These features are diaminopimelic acid (DAP) isomer on tetrapeptide side chain position 3 (Figure 2.1) including LL-DAP, *meso*-DAP or 3-hydroxyl-DAP, sugar content of peptidoglycan and

the presence of glycine in interpeptide bridges. Characteristic sugar patterns are present only in cell wall chemotype IV of those actinomycetes with *meso*-diaminopimelic acid (Lechevalier & Lechevalier, 1970; Sharma, 2014).

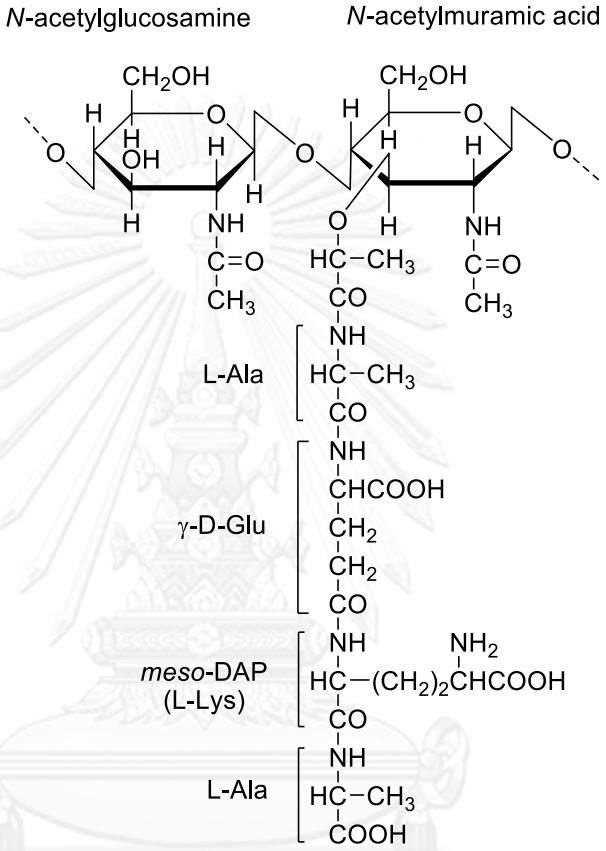


Figure 2.1 Structure of peptidoglycan in actinomycete cell wall

Table 2.7 Cell wall chemotypes of actinomycetes in cell wall peptidoglycan

Constituents	Cell wall chemotypes							
	I	II	III	IV	V	VI	VII	VIII
LL-DAP	+	-	-	-	-	-	-	-
meso-DAP	-	+**	+	+	-	-	-	-
Diaminobutyric acid	-	-	-	-	-	-	+	-
Aspartic acid	-	-	-	-	-	v	-	-
Glycine	+	+	-	-	-	-	+	-
Lysine	-	-	-	-	+	-	v	-
Ornithine	-	-	-	-	+	-	-	+
Arabinose	-	-	-	+	-	-	-	-
Galactose	-	-	-	+	-	v	-	-

** 3-Hydroxyl-DAP (may also present), v = variable amounts, + = present, - = absent

(2) Whole cell sugar composition

The whole cell sugar patterns play a key role in the identification of actinomycetes which have *meso*-DAP in the cell wall. This sugar patterns contribute to the cell wall chemotypes of actinomycetes which proposed by Lechevalier and Lechevalier (1970). The whole cell sugar patterns of actinomycetes have been used to identify in the genus level which are shown in Table 2.8.

Table 2.8 Whole cell sugar pattern of actinomycetes

Patterns	Diagnostic sugar				
	Arabinose	Fructose	Galactose	Madurose*	Xylose
A	+	-	+	-	-
B	-	-	-	+	-
C	-	-	-	-	-
D	+	-	-	-	+
E	-	+	-	-	-

*3-*O*-methyl-D-galactose, + = Present, - = Absent

(3) Phospholipid composition

Phospholipids or polar lipids are the major components of bacterial cell membranes. These lipid compounds are common polar lipid types because they possess closely related and complex structures classes according to the polar head group linked to the phosphate moiety. The diversity of the polar head groups is important. The main types of polar lipids are phosphatidylethanolamine (PE) and phosphatidylmethylethanolamine (PME), phosphatidylglycerol (PG), acylphosphatidyl-glycerol (APG), Diphosphatidylglycerol (DPG), phosphatidylinositol (PI), phosphatidyl-choline (PC), and phosphatidylserine (PS). Furthermore, some actinomycetes also have unknown phospholipids containing glucosamine (GluNu) or phosphatidylinositol mannosides (PIMs). Polar lipids in cell membrane of actinomycetes which are useful for identification in the genus level are divided into types I–IV as listed in Table 2.9 (Lechevalier, 1997; Mazzella *et al*., 2004)

Table 2.9 Phospholipid types of actinomycetes

Types	PIMs	PI	PC	PG	PE	PME	GluNu	APG	DPG
I	+	+	-	v	-	-	-	v	v
II	+	+	-	v	+	-	-	v	+
III	v	+	+	v	v	+	-	v	v
IV	ND	+	-	-	v	v	+	-	+
V	ND	+	-	v	v	-	+	v	+

+ = Present, - = Absent, v = Variable, ND = No data

(4) Isoprenoid quinones

Isoprenoid quinones are constituents of bacterial plasma membranes which play important roles in electron transport, oxidative phosphorylation, and, possibly, active transport (Collins & Jones, 1981). Isoprenoid quinones are structurally divided as ubiquinone, menaquinone, rhodoquinone, caldariellaquinone, chlorobiquinone, thermoplasmaquinone, methionaquinone and expoxyuquinone. Menaquinone was normally found in Gram-positive bacteria especially in actinomycetes and ubiquinone was normally found in Gram-negative bacteria and yeasts (Collins *et al*., 1977). The number of isoprene units and the degree of hydrogenation with double bonds in the isoprene chain are used as the important

key for identification of actinomycetes (Komakata & Suzuki, 1987). For example, MK-9(H_6) and MK-9(H_8) are the major menaquinones in *Streptomyces* strains.

(5) Cellular fatty acids

Fatty acid analysis is used to distinguish bacterial genera. For fatty acid analysis to be of use below the genus level, standardized growth and analytical conditions are required to ensure reproducible fatty acid profiles that are suitable for multivariate statistical analysis (Saddler *et al*., 1986). Fatty acid methyl esters (FAMEs) were characterized using gas chromatography and FAME patterns obtained using standardized conditions are still of high value for the rapid characterization of large numbers of wild-type actinomycetes isolated from the environment, independently from the taxonomic status of each isolate (Anderson and Wellington, 2001).

(6) DNA base composition

DNA molecules consist of nitrogenous bases including adenine (A), thymine (T), guanine (G) and cytosine (C). For double stranded DNA, adenine pairs with thymine and cytosine pairs with guanine by hydrogen bonds. The DNA base compositions are expressed as the G+C content (mol%) which is calculated from (G+C)/(A+T+C+G) x 100. The G+C content is usefulness for identification of actinomyces. Moreover, the G+C content could be analyzed from melting temperature (T_m) of double stranded DNA molecules.

2.4.3 Genotypic characteristics

The application of molecular techniques to the analysis of bacterial genomes has contributed considerably to the knowledge of bacterial taxonomy. In addition to clustering organisms taxonomically, some of these methods, such as the sequencing of 16S rRNA gene and the DNA-DNA hybridization have also provided an insight into the phylogenetic relationships of the prokaryotes at the genus, species and subspecies levels. (Anderson & Wellington, 2001)

(1) 16S rRNA gene sequence and phylogenetic tree

The 16S rRNA gene has become the standard for determining phylogenetic and taxonomic relationships among diverse bacteria. However, several cases have been documented in which prokaryotic organisms contain divergent 16S rRNA genes, suggesting the potential for horizontal exchange of rRNA genes, which pose a challenge for reconstructing the evolutionary history of a species and complicates efforts to classify microorganisms. Therefore, 16S rRNA gene analysis is used for actinomycete identification upto the genus level only (Rong *et al*., 2013).

(2) DNA-DNA hybridization

DNA-DNA hybridizations of total chromosomal DNA have been used to determine species identity within the genus. This is performed by monitoring the reassociation of single-stranded DNA from different organisms. The degree of relatedness is expressed as % homology and the genomic definition of species is considered to encompass strains with ≥70% DNA-DNA relatedness and ≤5°C difference in the melting temperature (ΔT_m) between the homologous and heterologous hybrids formed using the standard stepwise denaturation conditions (Wayne *et al*., 1987).

CHAPTER III
RESEARCH METHODOLOGY

3.1 Soil sample collection and isolation of *Streptomyces, Amycolatopsis* and *Kitasatospora* strains

3.1.1 Soil sample collection

Twenty nine soil samples were collected from three National Parks in the south of Thailand including Krung Ching Waterfall National Park, Nakhon Si Thammarat province; Angthong Islands National Park, Surat Thani province; and Similan Islands National Park, Phanga province.

3.1.2 Isolation of *Streptomyces*, *Amycolatopsis* and *Kitasatospora* strains

The soil samples were dried at room temperature until constant weight and then ground and preserved at 4°C until the process of actinobacterial isolation. One gram of each dried soil sample was suspended in 9 ml of sterile distilled water, mixed well and heated at 55°C for 5 min, then 1 ml of the suspension was subsequently transferred to 9 ml of sterile distilled water to make 10-fold dilution series of 10^{-2}, 10^{-3} and 10^{-4}. One hundred µl of the serial dilutions of 10^{-2}, 10^{-3} and 10^{-4} suspensions were spread on surface of starch casein nitrate agar plates and potato starch-glycerol agar plates supplemented with 25 µg/mL of nystatin for antifungal. Each agar plate was supplemented with 10 µg/ml of tetracycline for selection of *Streptomyces* or supplemented with 50 µg/ml of novobiocin for selection of *Amycolatopsis* and *Kitasatospora* strains (Tajima *et al*., 2001; Takahashi & Omura, 2003). Agar plates were incubated at 28°C for 14 days depended on the growth of each isolate. The different colonies were picked up, streaked for further purification on yeast extract-malt extract agar plates (YMA, ISP 2) and incubated at 28°C for 14 days. The pure isolates were transferred to ISP2 slants, incubated at 28°C for 14 days and then kept in a cold room at 4°C as stock cultures. They were also preserved by lyophilization using 10% skim milk as a cryoprotectant solution.

3.2 Identification methods

The phenotypic characteristics including cultural, morphological, physiochemical and biochemical characteristics of *Streptomyces*, *Amycolatopsis* and *Kitasatospora* strains were determined by the method of Shirling and Gottlieb (1996).

Each actinomycete strain was prepared as the inoculum by cultivation into yeast extract-glucose broth (Appendix A) at 28°C for 3-5 days. The cell culture (1 ml) of each isolate was centrifuged at 10,000 rpm, 4°C for 5 min and discarded the supernatant. The cells were washed with 1 ml of sterile distilled water (3 times) for removing the culture broth and re-suspension with 1 ml of sterile distilled water using as the inoculum for phenotypic characteristic determination.

3.2.1 Morphological and cultural characteristics

The cultural characteristics were determined using 14-day cultures grown at 28°C on various agar media including yeast extract-malt extract agar (ISP 2), oatmeal agar (ISP 3), inorganic salts-starch agar (ISP 4), glycerol-asparagine agar (ISP 5), peptone-yeast extract iron agar (ISP 6), tyrosine agar (ISP 7), yeast extract-soluble starch agar (YS) and nutrient agar (NA) (Appendix A). The colors of aerial and substrate mycelia and soluble pigments were determined using of the NBS/IBCC color system (Kelly, 1964). The morphological characteristics of the selected strains were observed by light and scanning electron microscopy (model JSM-5410LV; JEOL, Tokyo, Japan). Samples for scanning electron microscopy were prepared as follows: the culture agar media were cut to 3-5 mm^2 and the specimens were fixed with 1% osmium in 0.1 M phosphate buffer pH 7.2 for 1-2 h and then washed three times with phosphate buffer. They were dehydrated through a gradient ethanol series.

3.2.2 Physiological and biochemical characteristics

3.2.2.1 pH for growth

Each strain was inoculated with 10 µl of the inoculum by dropping on the surface of ISP 2 adjusted pH at 4, 5, 6, 7, 8, 9, 10, 11 and 12 and incubated at 28°C for 14 days. The minimum and maximum pH for growth of each strain were recorded.

3.2.2.2 Temperature for growth

Each strain was inoculated by streaking on the surface of ISP 2 slant agar and incubated at 15, 20, 30, 37, 40 and 45°C for 14 days. The minimum and maximum temperatures for growth of each strain were recorded.

3.2.2.3 Sodium chloride (NaCl) tolerance

Each strain was inoculated with 10 µl of the inoculum by dropping on the surface of ISP 2 medium agar supplemented with 0%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9% and 10% NaCl and incubated at 28°C for 14 days. The maximum of NaCl concentration for growth was recorded.

3.2.2.4 Carbon utilization

Carbon sources were included L-arabinose, D-fructose, D-glucose, *myo*-inositol, D-mannitol, melezitose, D-melibiose, raffinose, L-rhamnose, D-sorbitol, sucrose and D-xylose. They were dissolved with distilled water and sterilized by filtration with 0.2 µm filter papers. After that each sterile carbon source was supplemented into the basal agar medium (ISP 9) (Appendix A) to give 1% final concentration of the carbon source, the mixture was mixed well and 25 ml of the mixture was poured into a petri dish. ISP 9 medium (no carbon source) and D-glucose were used as negative and positive controls, respectively.

The basal agar medium plates were inoculated with 10 µl of the inoculum by dropping on the agar surface in duplicate and incubated at 28°C for 14 days.

The results were recorded as follows:

1. Positive utilization (+), when growth on the tested carbon source in the basal medium is significantly better than the negative control, equal or greater than the positive control.

2. Doubtful utilization (±), when growth on the tested carbon source in the basal medium is only slightly better than the negative control and significantly less than the positive control.

3. Negative utilization (-), when growth on the tested carbon in the basal medium is similar to or less than the negative control.

3.2.2.5 Starch hydrolysis

Each strain was inoculated with 10 µl of the inoculum by dropping on the surface of inorganic salt-starch plate (ISP 4) and incubated at 28°C for 14 days. After that, Gram's iodine solution was flooded on the surface the agar plate. If starch was hydrolyzed by the strain, the clear zone would be appeared.

3.2.2.6 Gelatin liquefaction

Each strain was inoculated into Bouillon broth (Arai, 1975, Appendix A) and incubated at 28°C for 14 days. After that the incubated tube was compared with the control (the uninoculated tube) when observed at 4°C for 30 min. The gelatin became liquid if it was hydrolyzed.

3.2.2.7 Nitrate reduction

Each strain was inoculated into 5 ml of peptone potassium nitrate (KNO_3) broth (Appendix A) and incubated at 28°C, 200 rpm for 14 days. After that, the culture broth was added with 1 ml of sulfanilic acid and 1 ml of *N*,*N*-dimethyl-1-naphtylamine solution and mixed well. If nitrate was present, the mixture would become pink to red color as a positive result for nitrate reduction. The mixture remained light yellow color, the mixture was added with few amount of zinc powder. If nitrate was reduced to nitrite (over reduction), the mixture would be remained light yellow color as a positive result and the mixture would become pink to red color as a negative result.

3.2.2.8 Milk coagulation and peptonization

Each strain was inoculated in 5 ml of 10% skim milk (Appendix A) and incubated at 28°C for 14 days. If the skim milk solution could be coagulated and peptonized by the strain, the solution would be precipitated and clear, respectively.

3.2.2.9 Enzyme activity

The enzyme activities were determined using the API ZYM system (bioMérieux, Lyon, France), according to the manufacturer's instructions. Briefly, fresh

actinomycete cells were washed with distilled water for three times, suspended in distilled water and adjusted the turbidity of cell suspension equal to a McFarland No.4. Then, the cell suspension was added into API ZYM strips (75 µl/strip) and incubated at 37°C for 18 h. After that, Zym A and Zym B reagents (1 drop of each reagent) were added into each strip. The reactions were developed for 5 min, observed colors and recorded on the result sheet. The enzyme activities, including alkaline phosphatase, acid phosphatase, α-chromotrypsin, cystine allylamidase, esterase C4, esterase lipase C8, α-frucosidase, lipase C14, leucine allylamidase, naphthol-AS-BI-phosphohydrase, α-galactosidase, ß-galactosidase, α-glucosidase, ß-glucosidase, ß-glucoromidase, *N*-acetyl-ß-glucosaminidase, α-mannosidase, trypsin and valine allylamidase, were determined.

3.2.3 Chemotypic characteristics

The biomass (dried cells) of each isolate for determination of the chemotypic characteristics were obtained after the strain cultivation in yeast extract-glucose broth on a rotary shaker at 200 rpm, 28°C for 3-7 days. The cells were collected by centrifugation at 10,000 rpm for 10 min, 4°C and then washed 3 times with sterile distilled water and dried by a lyophilizer.

3.2.3.1 Isomers of diaminopimelic acid analysis

Dried cells (10 mg) of each strain were hydrolyzed with 1 ml of 6N HCl at 100°C for 16 h. The hydrolyzed solution was filtered with cotton and evaporated by using an evaporator until dryness. The extract was dissolved with 300 µl of water. This sample was applied onto a TLC cellulose plate (No. 1.05716.0001; Merk, Germany) and developed with methanol-water-10 N HCl-pyridine (32:7:1:4 v/v) for 16 h. The plate was dried for 30 min and then developed again with the same solvent system for 10 h. The spots were visualized by spraying with ninhydrin solution followed by heating at 100°C for 3-5 min. DL-diaminopimelic acid (DAP) which contains *meso*-DAP and LL-DAP isomers was used as the standard. The DAP spot appeared as grayish green color and other amino acids as purple to red colors. Spots will gradually disappear in a few minutes (Becker *et al*., 1965).

3.2.3.2 Whole cell sugar analysis

Whole cell sugar composition will be analyzed according to the methods of Becker *et al*. (1965). Dried cells (250 mg) were hydrolyzed with 1 ml of 1 N H_2SO_4 and heated at 100°C for 2 h. The whole-cell hydrolysate was immediately cooled to room temperature, adjusted pH to 5-7 with saturated $Ba(OH)_2$. The debris cells were removed by centrifugation at 5,000 rpm for 10 min. The supernatant was transferred into the new tube, added 2 ml of methanol and 1 ml of chloroform, mixed strongly, left and centrifuged at 5,000 rpm for 10 min to separate into two layers. The aqueous supernatant was evaporated at 45°C until dryness. The whole cell sugar extract was dissolved with 200 µl of distill water. The solution (3 µl) was applied into TLC aluminium sheets (No. 5552; Merk, Germany) and developed with mixture of *n*-butanol-water-pyridine-toluene (10:6:6:1) for twice times. Whole cell sugar composition was detected by spraying with aniline-butanol-phthalate reagent followed by heating at 100°C for 3-5 min. Glucose, galactose, mannose, arabinose, xylose, ribose, rhamnose and madurose (2 mg/ml) were used as the standard sugars.

3.2.3.3 Cell wall acyl type of muramic acid

Dried cells (10 mg) were hydrolyzed with 100 µl of 6 N HCl at 100°C for 3 h. The hydrolyzed solution was added with 100 µl of distilled water and 2 ml of saturated diethyl ether (Appendix B), mixed by a vortex mixer for 1 min and then centrifuged at 3,000 rpm for 10 min. The upper layer (ether layer) was transferred into a new tube (upper layer tube 1). The lower layer was added with 2 ml of saturated diethyl ether, mixed by a vortex mixer for 1 min and then centrifuged at 3000 rpm for 10 min. The upper layer was transferred into a new tube (upper layer tube 2). The lower layer was extracted muramic acid again with 2 ml of saturated diethyl ether, mixed by a vortex mixer for 1 min and then centrifuged at 3,000 rpm. The upper layer was transferred into a new tube (upper layer tube 3). The upper layers in tubes 1, 2 and 3 were dried by an evaporator. The dried extracts of all tubes were added with DON reagent (Appendix B), boiled at 100°C for 10 min and then detected the color of the solution. Acetyl and glycolyl types of muramic acid were presented as colorless and reddish purple, respectively (Uchida & Aida, 1984).

3.2.3.4 Polar lipid analysis

Polar lipids in cells were extracted and identified by the method of Minnikin *et al*. (1977).

Polar lipid extraction: Dried cells (150 mg) were put into a test tube with a screw cap, added with 3 ml of methanol-0.3% NaCl solution (100:10) and 3 ml of petroleum ether, mixed strongly by a vortex mixer for 15 min and then centrifuged at 3,000 rpm for 10 min. The upper layer was removed and the lower layer was added with 1 ml of petroleum ether and mixed well again for 5 min. The mixture was centrifuged at 3,000 rpm for 10 min and removed the upper layer. The lower layer was heated at 100°C for 5 min and cooled immediately at 37°C for 5 min. The suspension was added with 2.3 ml of chloroform-methanol-water (90:100:30), mixed well for 15 min and then centrifuged at 3,000 rpm for 10 min. The aqueous supernatant was transferred into a new tube (supernatant tube) and the lower layer was extracted polar lipid again with 2.3 ml of chloroform-methanol-water (90:100:30). The mixture was mixed well for 15 min and then centrifuged at 3,000 rpm for 10 min. The aqueous supernatant was combined into the supernatant tube. The aqueous supernatant tube was added with 1.3 ml of chloroform and water, mixed well, centrifuged at 3,000 rpm for 10 min and then remove upper layer. The lower layer was dried with nitrogen gas.

Polar lipid analysis: The polar lipid extract was dissolved with 60 µl of chloroform-methanol (2:1) and applied 10 µl of the solution onto two-dimensional silica gel TLC (No. 5633; Merk, Germany). The TLC plate was developed with two following solvent systems. The first solvent system was chloroform-methanol-water (65:25:4). The second solvent system was chloroform-acetic acid-methanol-water (40:7.5:6:2).

Polar lipid detection: The polar lipids on TLC plates were separately visualized by using five spraying reagents including anisaldehyde reagent, Dittmer & Lester reagent, Dragendroff's reagent, ninhydrin reagent, and phosphomolybdic acid reagent.

Anisaldehyde reagent was used for glycolipid detection. After spraying with the reagent and heating at 110°C for 10 min, glycolipids were presented as green yellow spots.

Dittmer & Lester reagent was used for all phospholipid detection. After spraying with the reagent, all phospholipids were presented as blue spots.

Dragendroff's reagent was used for choline-containing phospholipids (phosphatidyl choline, PC) detection. After spraying with the reagent, phosphatidyl choline was presented as a brown spot.

Ninhydrin reagent was used for phosphatidylethanolamine (PE) and its derivative detection. After spraying with the reagent and heating at 110°C for 10 min, PE and its derivatives were presented as purple spots.

Phosphomolybdic acid reagent was used for all polar lipid detection. After spraying with the reagent and heating at 130°C for 10 min, all polar lipids were presented as dark pots.

3.2.3.5 Isopreniod quinone analysis

About 100 mg of dried cells were extracted isoprenoid quinones with 20 ml of chloroform:methanol (2:1) on a magnetic stirrer for overnight and protected from light. After extraction, the suspension was filtered and the filtrate was evaporated to dryness. The dried extract was dissolved with acetone, applied onto a silica gel TLC plate (no. 5774) and then developed with benzene. Vitamin K1 was used as a standard, the R_f value of menaquinone band is almost similar to the vitamin K1 band. The isopreniod quinone band was shortly detected under a UV lamp (254 nm). The band was scraped and extracted the isopreniod quinone from silica gel with acetone, centrifuged at 3,000 rpm for 5 min, filtered through membrane (0.5 µm) and dried the filtrate by an evaporator (Collins *et al*., 1977). The isopreniod quinones were analyzed by LC/MS (JMS-T 100LP, JEOL) using a CAPCELL PAK C18 UG120 column (Shiseido, Tokyo, Japan) with mathanol-2-propanol (7:3).

3.2.3.6 Cellular fatty acids

The cellular fatty acids were determined by four steps involving, saponification, methylation, extraction, and base washing.

Saponification: Dried cells (40 mg) were put into the screw-cap tube, added with 1 ml of a sponification reagent (Appendix B) and mixed well by shaking. After that, the suspension was heated at 100°C for 30 min and cooled to room temperature in water.

Methylation: The suspension was added with a methylation reagent (Appendix B), mixed well for 5-10 sec with a vortex mixer, heated at 80°C for 10 min and then cooled to room temperature in water.

Extraction: The suspension was added with an extraction solvent (Appendix B), mixed well for 10 min and transferred the upper layer into a new tube.

Base washing: The upper layer was added with a base washing reagent (Appendix B) and mixed for 5 min, if it became to an emulsion form, added the saturated sodium chloride solution (Appendix B). The upper layer was transferred into a new vial. Fatty acid methyl ester was analyzed by gas chromatography according to the instructions of the Microbial Indentification System (MIDI, version 6.0) (Sasser, 1990; Kämpher & Kroppenstedt, 1996) with ACTIN1 MIDI database.

3.2.3.7 DNA base composition analysis

Genomic DNA extraction and purification: Chromosomal DNA was prepared following the procedure of Saito and Miura (1963). Dried cells (200 mg) were added with 6 ml of saline-EDTA pH 8.0 (Appendix B), 10 mg of lysozyme, 6-8 mg of achromopeptidase and 30 µl *N*-acetylmuramidase (1mg/100 µl) and then incubated by shaking at 37 °C for overnight. After incubation, the suspension was freezed in dried ice/EtOH and thawed at 60 °C for 10 min (repeated 4-5 times). The suspension was added 500 µl of 20% SDS (Appendix B), shaken at 60°C for 2 h and then cooled at room temperature. The equal volume of tris-phenol (Appendix B) was added into the suspension, mixed by a vortex mixer and centrifuged at 10,000 rpm, 4°C for 15 min. After centrifugation, the supernatant was transferred into a beaker, added with 1/10 volume of 3 M sodium acetate pH 5.2 (Appendix B) and cold absolute ethanol and then swirled clean glass rod gently to pool DNA. The glass rod with DNA was washed in 5 ml of absolute ethanol for twice times and dried. After air-drying, DNA was dissolved with 3 ml of 0.1X SSC (Appendix B) and kept at 4°C for overnight. The DNA solution was treated with 1/40 volume of RNase A (Appendix B) and 1/40 volume of RNase T (Appendix B) by shaking at 37°C for overnight. The equal volume of tris-phenol was added into the solution, mixed by a vortex mixer and centrifuged at 10,000 rpm, 4°C for 15 min. After centrifugation, the supernatant was transferred into a beaker, added with 1/10 volume of 3 M sodium acetate pH 5.2 and cold absolute ethanol and then swirled clean glass rod gently to pool DNA. The glass rod with DNA was washed in 5 ml of absolute ethanol for twice and dried. After air-

drying for, DNA was dissolved with 3 ml of 0.1X SSC and kept at 4°C for overnight and measured purity of the DNA solution at OD_{260}/OD_{280} ($1.8 < OD_{260}/OD_{280} < 2.0$).

DNA base composition analysis: DNA solution (100 µl) corresponding to 20 µg was put into an appendorf tube, heated at 100°C for 10 min and then immediately cooled in ice. The denatured DNA was added with 100 µl of nuclease P1 solution (Appendix B) and incubated at 60°C for 30 min and then the solution was added with 100 µl of alkaline phosphatase solution (Appendix B) and incubated at 37°C for 1 h. The nucleoside suspension was determined by using the HPLC method (Tamaoka & Komagata, 1984). An equimolar mixture of nucleosides was used as the quantitative standard for DNA base composition as shown below.

$$\text{Mol\% G + C} = (G_S/G_R + C_S/C_R)/(A_S/A_R + G_S/G_R + C_S/C_R + T_S/T_R)$$

When, A_R is the peak area of adenine (standard)

A_S is the peak area of adenine (sample)

C_R is the peak area of cytosine (standard)

C_S is the peak area of cytosine (sample)

G_R is the peak area of guanine (standard)

G_S is the peak area of guanine (sample)

T_R is the peak area of thymine (standard)

T_S is the peak area of thymine (sample)

3.2.4 Genotypic characteristics

3.2.4.1 16S rRNA gene sequencing and phylogenetic analysis

Genomic DNA extraction: Actinobacterial strain was grown in yeast extract-glucose broth. The cell culture (1 ml) was centrifuged at 12,000 rpm, 4°C for 10 min. After centrifugation, the supernatant was removed and the cells were washed with 1 ml of sterile distilled water twice and then washed with 1 ml of 0.1 M Tris-HCl pH 8.0 and then centrifuged at 12,000 rpm, 4°C for 5 min. The supernatant was removed and the cells were added with 300 µl of TE buffer and 80-100 µg of sterile aluminium oxide. The cells were lysed by using a micro mixer for 90 sec. The

suspension was added with 300 µl of phenol-chloroform (1:1), gently mixed and centrifuged at 12,000 rpm, 4°C for 10 min. The supernatant was transferred into a new eppendorf tube, added with 30 µl of 3 M sodium acetate pH 5.2 and 600 µl of absolute ethanol. The solution was kept at 80°C for 30 min and centrifuged at centrifuged at 12,000 rpm, 4°C for 10 min. the solution was discarded and the precipitate DNA was washed with 500 µl of absolute ethanol and then centrifuged at centrifuged at 12,000 rpm, 4°C for 10 min. The precipitate DNA was air-dried, dissolved with 500 µl of TE buffer and then used as the DNA template for PCR.

16S rRNA gene amplification: The PCR was performed in a total volume of 100 µl containing 4.0 µl of the DNA template, 67.5 µl of sterile MilliQ water, 10 µl of 10X Taq buffer, 2 µl of dNTP, 4.0 µl of 10 µM forward (20F, 5' AAGGAGGTGATCCAGCC 3') and reverse (1530R, 5' AAG GAG GTG ATC CAG CC 3') primers, 8 µl of 25 mM $MgCl_2$ and 0.5 µl of *Taq* DNA polymerase. A DNA thermal cycler (Gene Amp® PCR System 2400; Perkin Elmer) was used for 16S rRNA gene amplification with a temperature profile of initial denaturation at 94°C for 3 min, followed by 30 cycles of DNA denaturation at 94°C for 1 min, primer annealing at 50°C for 1 min, DNA extension at 72°C for 2 min, and a final extension at 72°C for 3 min. The PCR product was analyzed by agarose gel electrophoresis. The PCR product (5 µl) was mixed with 2 µl of loading dye, loaded in well of 0.8% agarose gel (Appendix B) and then run in 50X Tris-acetate (TAE) buffer (Appendix B). The agarose gel was stained in an ethidium bromide solution and visualized the amplified 16S rRNA gene band under the UV transilluminator (UVP Inc.). The PCR products were purified by a GenepHlow™ Gel/PCR Kit.

16S rRNA gene sequence and phylogenetic tree analysis: The purified 16S rRNA gene product was used as a template for sequencing with a big dye terminator sequencing Kit (Perkin Elmer) and analyzed by a ABI377 automated DNA sequencer (Perkin Elmer). The sequencing reaction of each sample was performed in a DNA thermal cycler (Gene Amp® PCR System 2400; Perkin Elmer) with a temperature profile of 30 sec at 96°C followed by 25 cycles of 10 sec at 96°C (DNA denaturation), 5 sec at 50°C (primer annealing) and 4 min at 60°C (DNA extension). Sequencing of each sample was carried out in both forward and reverse directions with the following primers (Universal primers): 27F (5' AGA GTT TGA TCA TGG CTC AG 3'), 357F (5' CTG CTG CCT CCC GTA G 3'), 518F (5' GTA TTA CCG CGG CTG CTG G 3'), 800R (5' TAC CAG GGT ATC TAA TCC 3'), 920R (5' TGT TCA TGA ATT GGG TTG GA 3'), 1492R (5' CGG TTA CCT TGT TAC GAC TT 3'). The 16S rRNA gene sequence

similarity of each strain was analyzed by blast search with all recognized species in EzTaxon-e databases (Kim *et al*., 2011). The sequence was multiply aligned the selected sequences of some closely type strians obtained from EzTaxon-e databases by using the CLUSTAL X version 1.81 (Kimura, 1980) of BioEdit software. The alignment was manually verified and adjusted prior to the construct a phylogenetic tree. The phylogenetic tree was constructed by using the neighbor-joining (Saito & Nei, 1987), maximum-likelihood (Felsenstein, 1981) and maximum parsimony method (Kluge & Farris, 1969) in the MEGA 5 software (Tamura *et al*. 2011). The confident values of branches of the phylogenetic tree were determined by using the 1,000 bootstrap replications (Felsenstein, 1985). The values for sequence similarity among the closest strains were calculated manually after pairwise alignments obtained by using the CLUSTAL X program (Thompson *et al*., 1997) that gap and ambiguous nucleotides were eliminated from the calculations.

3.2.4.2 DNA-DNA hybridization and %DNA homology analysis

DNA-DNA hybridization was performed by fluorometric DNA-DNA hybridization in the micro-dilution well method (Ezaki *et al*., 1989). DNA-DNA hybridization was determined by five steps containing DNA sample fixation, DNA probe labeling, pre-hybridization, hybridization and detection.

DNA sample fixation: The double stranded DNAs of an unknown strain, type strains and reference strain (Calf thymus) were separately diluted to 10 µg/ml in PBSMG solution (Appendix B), boiled at 100°C for 10 min and then cooled immediately on an ice bath. The solution (100 µl/well) of each strain was loaded into a black microtitre plate. The microtitre plate was tightly sealed and incubated at 37°C for overnight.

DNA probe labeling: 100 µl of each DNA solution (100 µg/ml) was added with 10 µl of 3 M sodium acetate pH 5.2 and 200 µl of cold absolute ethanol. The solution was kept at -80°C for 15 min and centrifuged at 15,000 rpm, 4°C for 15 min. The precipitate DNA was washed with 500 µl of absolute ethanol and then centrifuged at 15,000 rpm, 4°C for 15 min. The DNA pellet was dried in vacuum desiccator for 45 min. The dried DNA was dissolved with 10 µl of distilled water and sonicated for 2 min. The solution was added with 7 µl of photobiotin, placed on an ice bath and exposed to light (500 W) for 30 min. Then, the solution was added 16 µl of 0.1 M Tris-HCl, 160 µl of *n*-butanol and 127 µl of distilled water. The solution was mixed by a vortex mixer, centrifuged at 5,000 rpm, 4°C for 1 min and then taken the

lower layer (~160 µl) to a new eppendorf tube. The lower layer was added with 160 µl of *n*-butanol, mixed by a vortex mixer, centrifuged at 5,000 rpm, 4°C for 1 min and then taken the lower layer (~20-30 µl) to a new eppendorf tube. The lower layer was boiled at 100°C for 10 min and then cooled immediately on an ice bath. The labelling DNA probe was mixed with a hybridization solution (Appendix B).

Pre-hybridization: After incubation of the DNA sample fixation, the microtitre plate was discarded the solution, washed with 250 µl/well of 1X PBS (Appendix B) and discarded the solution. The single stranded DNA was added with 200 µl/well of a pre-hybridization solution (Appendix B), tightly sealed and incubated at 37°C for 30 min.

Hybridization: After pre-hybridization, the fixed single stranded DNA was discarded the pre-hybridization solution, added with 100 µl/well of the hybridization solution containing labeling DNA probe, tightly sealed and incubated at 50-54°C for overnight.

Detection: The hybridization plate was discarded the hybridization solution and added 100 µl/well of PBS-BSA-Triton solution, tightly sealed and incubated at 37°C for 45 min. After incubation, the PBS-BSA-Triton solution was discarded. The plate was washed with 250 µl/well of 1X PBS for three times, added with 100 µl/well of 4 MUF solution (Appendix B) and then detected fluorescent intensity. The fluorescent intensity was measured at excitation and emission wavelengths (Ex/Em) at 360/450 nm by a fluorescent microplate reader (Microplate Reader Wallac 1420, PerkinElmer™) and calculated the values of percentage DNA homology as shown below.

%DNA-DNA homology - 100 x (Sample DNA - Calf thymus DNA)/(Type strain DNA - Calf thymus DNA)

3.3 Primary screening for antimicrobial activity

The antimicrobial activity of the supernatants was examined by the paper disc diffusion method. The 8-mm paper discs were soaked in the supernatant and dried at room temperature for 4 h. Six test microorganisms were *Kocuria rhizophila* ATCC 9341, *Bacillus subtilis* ATCC 6633, *Escherichia coli* NIHJ KB213, *Xanthomonas campestris* pv. *oryzae* KB88, *Candida albicans* KF1 and *Mucor racemosus* IFO 4581^{T}. *K. rhizophila* ATCC 9341, *B. subtilis* ATCC 6633, *E. coli* NIHJ KB213 and *X. campestris* pv. *oryzae* KB88 were cultivated on Muller-Hinton agar (MHA) plates at 37°C for 24 h. *C. albicans* KF1 and *M. racemosus* IFO 4581^{T} were cultivated on Sabouraud's dextrose agar (SDA) plates at 30°C for 24 h. The cell cultures on agar surface were suspended with normal saline solution and standardized to match a turbidity of McFarland standard No. 0.5, providing approximately $1x10^{8}$ CFU/ml (colony forming unit/ml) for bacteria and $1x10^{6}$ CFU/ml for fungi. Each test microorganism solution (1% of inoculum) was inoculated into the MHA for bacteria and SDA for fungi, and then mixed well. Each inoculated medium (20 ml) was put in a petri dish. The dried paper discs were placed on the surface of the inoculated agar plate and incubated at 37°C, 24 h for bacteria and 30°C, 48 h for fungi. The diameters of inhibition zones were measured. Antimicrobial activity against each test microorganism was determined for duplication.

3.4 Biological activity determination

The isolated compounds with enough amounts were further evaluated for other biological activities. The biological activities were conducted by Bioassay Laboratory at National Center for Genetics Engineering and Biotechnology (BIOTEC), Thailand.

3.4.1 Antibacterial activity

The isolated compounds were determined antibacterial activity against *Bacillus cereus* ATCC 11778. The antimicrobial activity was tested by the resazurin microplate assay (REMA) (Sarker *et al*., 2007). *B. cereus* ATCC 11778 was inoculated in 5 ml of tryptic soy broth at 37°C for 30 min on a shaker at 200 rpm (OD_{600}~0.1), and then diluted 30 folds in tryptic soy broth. To assay in a 384-well plate, each well containing 5 µl of *B. cereus* ($5x10^{4}$ CFU/well), 7.5 µl of sample, and 25 µl of 0.25 mM rezazurin was cultured in Mueller-Hinton broth (MHB) at the final volume of 75

µl/well and incubated at 37°C for 3 h. The fluorescent intensity at Ex/Em 530/590 nm was measured by a microplate reader (Molecular device, SpectraMax M5) and data were analyzed. Minimum inhibitory concentration (MIC) represents the lowest concentration of the compound that inhibits the growth of the bacteria. Vancomycin and 0.5% DMSO were used as positive and negative controls, respectively.

3.4.2 Antifungal activity

The isolated compounds were determined antifungal activity against *Candida albicans* ATCC 90028. The antifungal activity was tested by the resazurin microplate assay (REMA) (Sarker *et al*., 2007). *C. albicans* ATCC 90028 was grown on potato dextrose agar (PDA) at 30°C for 3 days and then transferred to RPMI-1640 until cell density reaches $5x10^5$ CFU/ml. The yeast cell suspension was added to the 384-well plate; each containing 45 µl of cell suspension and 5 µl of the test compounds and then incubated at 37°C for 4 h. After that, 10 µl of 62.5 µg/ml resazurin solution was added to each well and incubated at 37°C for 30 min. Inhibition concentration (IC_{50}) represents the concentration of the compound which causes 50% growth reduction of the fungus. Amphotericin B and 0.5% DMSO were used as positive and negative controls, respectively.

3.4.3 Antimalarial activity

The isolated compounds were determined antimalarial activity against *Plasmodium falciparum* (K1, multidrug resistant strain). *P. falciparum* were cultured according to the method of Trager and Jensen (1976) using continuous cultures (*in vitro*) of asexual erythrocytic stages. Quantitative assessment of antimalarial activity (*in vitro*) was determined by mean of the microculture radioisotope technique based upon the method described by Desjardins *et al*. (1979). Inhibition concentration (IC_{50}) represents the concentration of the compound which causes 50% reduction in parasite growth as indicated by the *in vitro* uptake of [^{3}H]-Hypoxanthine by *P. falciparum.* Dihydroartemisinin and mefloquine were used as standard references.

3.4.4 Antitubercular activity

The isolated compounds were tested antitubercular activity against *Mycobacterium tuberculosis* strain H37Ra. The activity was determined by the green fluorescent protein microplate assay (GFPMA) (Changsen *et al*., 2003). Minimum inhibitory concentration (MIC) represents the lowest concentration of the compound that inhibits the growth of the bacteria. Isoniazid, ofloxacin, rifampicin, streptomycin and ethambutol were used as standard references.

3.4.5 Cytotoxicity against cancer cells

The isolated compounds were determined cytotoxicity against KB (human oral epidermoid carcinoma, ATCC CCL-17), MCF-7 (human breast cancer, ATCC HTB-22), and NCI-H187 cells (human small-cell lung cancer, ATCC CRL-5804). The cytotoxicity was performed by using the resazurin microplate assay (REMA) (O'Brien *et al*., 2000). Inhibition concentration (IC_{50}) represents the concentration of the compound which causes 50% reduction of the tested cells. Ellipticine was used as a reference for cytotoxicity to KB and NCI-H187 and doxorubicin was used as a reference for cytotoxicity to KB, MCF-7 and NCI-H187. Tamooxifen was also used as a reference for cytotoxicity to MCF-7.

3.4.5 Cytotoxicity against Vero cells

The isolated compounds were determined cytotoxicity activity against a Vero cells (African green monkey kidney fibroblasts; ATCC CCL-81). The cytotoxicity was performed by the green fluorescent protein microplate assay (GFPMA) (Changsen *et al*., 2003). Inhibition concentration (IC_{50}) represents the concentration of the compound which causes 50% reduction of the Vero cells. Ellipticine was used as a reference for cytotoxicity against Vero cell assay.

3.5 Chemical profile analysis

The crude EtOAc extracts were dissolved in methanol (10 mg/ml) and the solutions were analyzed UV spectra and retention time (RT) by HPLC (UltiMate 3000, DIONEX), with linear gradient system (0-100% CH_3CN in H_2O + 0.05% formic acid) at the flow rate 0.5 ml/min for 18 min, using a C-18 column (5 µm), 2.1x50 mm (Puropher®STAR; Merck). Detection was used a UV/UV-VIS detector.

3.6 Fermentation, isolation and characterization for the secondary metabolites from the selected strains

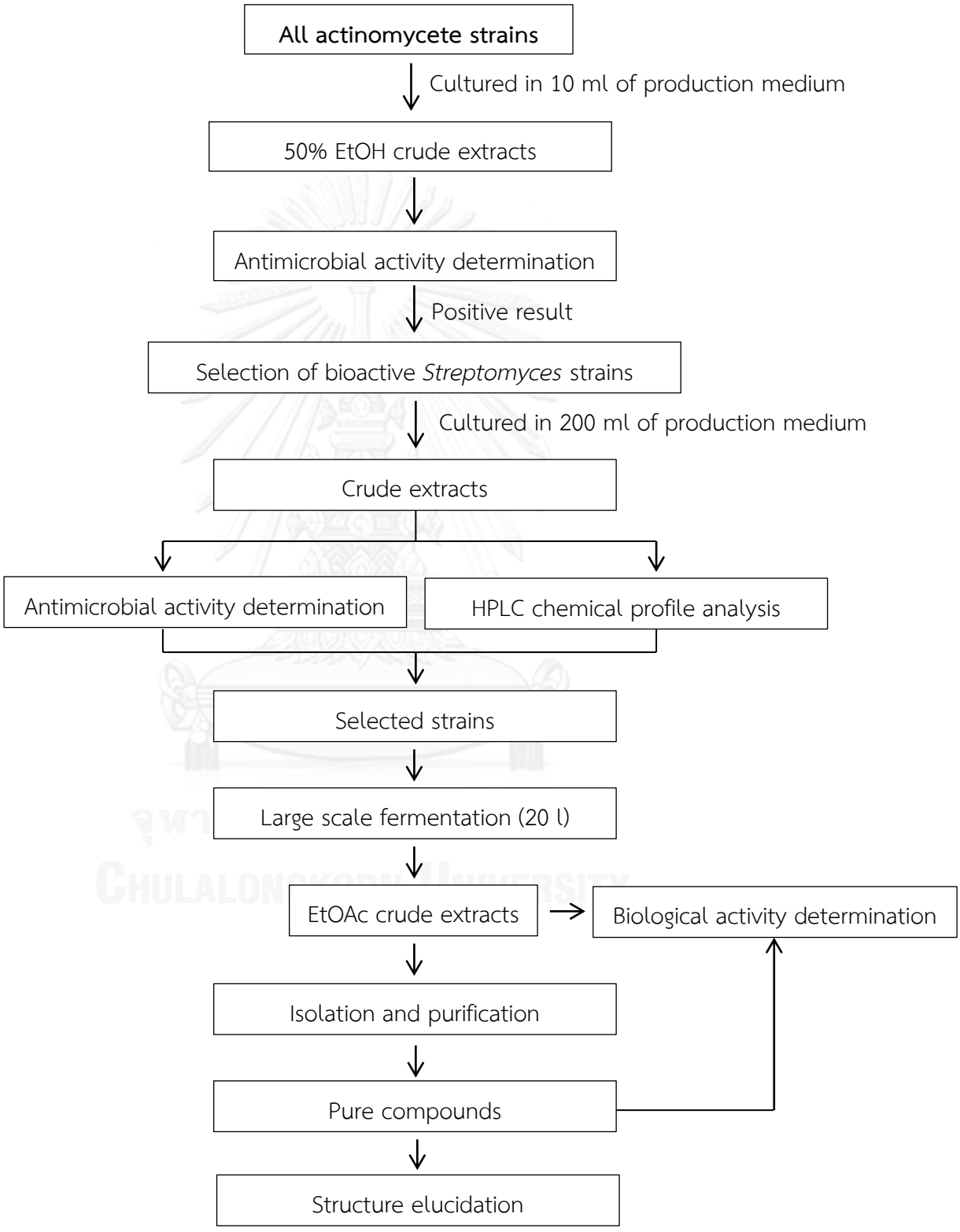
3.6.1 Screening of antimicrobial activity of *Streptomyces*, *Amycolatopsis* and *Kitasatospora* strains

Each isolate of *Streptomyces*, *Amycolatopsis* and *Kitasatospora* strains was inoculated in 10 ml of the seed medium (Appendix A) and cultivated on a rotary shaker at 200 rpm, 28°C for 3-5 days and used as an inoculum. Each inoculum (1%) was transferred into 10 ml of production media no. 51 and 53 and cultivated on a rotary shaker at 200 rpm, 28°C for 6 days. The cell culture was extracted with 10 ml of ethanol on a rotary shaker at 200 rpm for 30 min, mixed well by a vortex mixer and centrifuged at 3,000 rpm for 5 min. The supernatants (50%-EtOH extracts) were determined antimicrobial activity as described in section 3.3. The 50%-EtOH extract of each production medium was used as a negative control.

3.6.2 Selection of target strains for fermentation

Twenty four isolates of *Streptomyces* showing antimicrobial activity were selected and cultured in two 500-ml Erlemenyer flasks, each flask containing 200 ml of production medium no. 54 on a rotary shaker at 200 rpm, 28 °C for 6 days. Then, each culture was extracted with an equal volume of ethanol on a rotary shaker for 1 h, centrifuged at 3,000 rpm for 10 min and then evaporated to remove ethanol. The supernatant was partitioned with equal volumes of ethyl acetate for three times. The EtOAc layers were combined and evaporated to dryness. After that, the crude EtOAc extracts were analyzed by HPLC to examine their chemical profiles with the BIOTEC in-house database and determined antimicrobial activity. The strains showing interesting chemical profiles were selected for further studies in 3.6.3. The stepwise for selection the target strains and chemical studies such as extraction, isolation,

purification and structure elucidation of secondary metabolites are shown in Scheme 3.1.



Scheme 3.1 The overview of secondary metabolite study

3.6.3 Fermentation and extraction of antimicrobial metabolites from the selected strains

Two *Streptomyces* strains (KC-097 and KC-121) were selected by their antimicrobial activities or HPLC chemical profiles. The selected strains were cultured in a large scale fermentation (20 l). For fermentation, each selected strain was cultivated in 250-ml Erlenmeyer flask, each contained 100 ml of 301 seed medium (Appendix A). Seed culture was cultivated on a rotary shaker at 200 rpm, 28°C for 3 days and then 1% of the seed culture was transferred into 1000-ml Erlenmeyer flasks, contained 250 ml of production medium no. 54 for 80 flasks (20 l). The strain was cultivated on a rotary shaker at 200 rpm, 28°C for 6 days. The 20 l-cultures were extracted with equal volume of EtOAc for three times. The EtOAc layers were combined and evaporated to dryness. After that, the crude EtOAc extracts were purified by chromatographic techniques.

3.6.3.1 Isolation, purification and structure elucidation of secondary metabolites from *Streptomyces tendae* strain KC-097

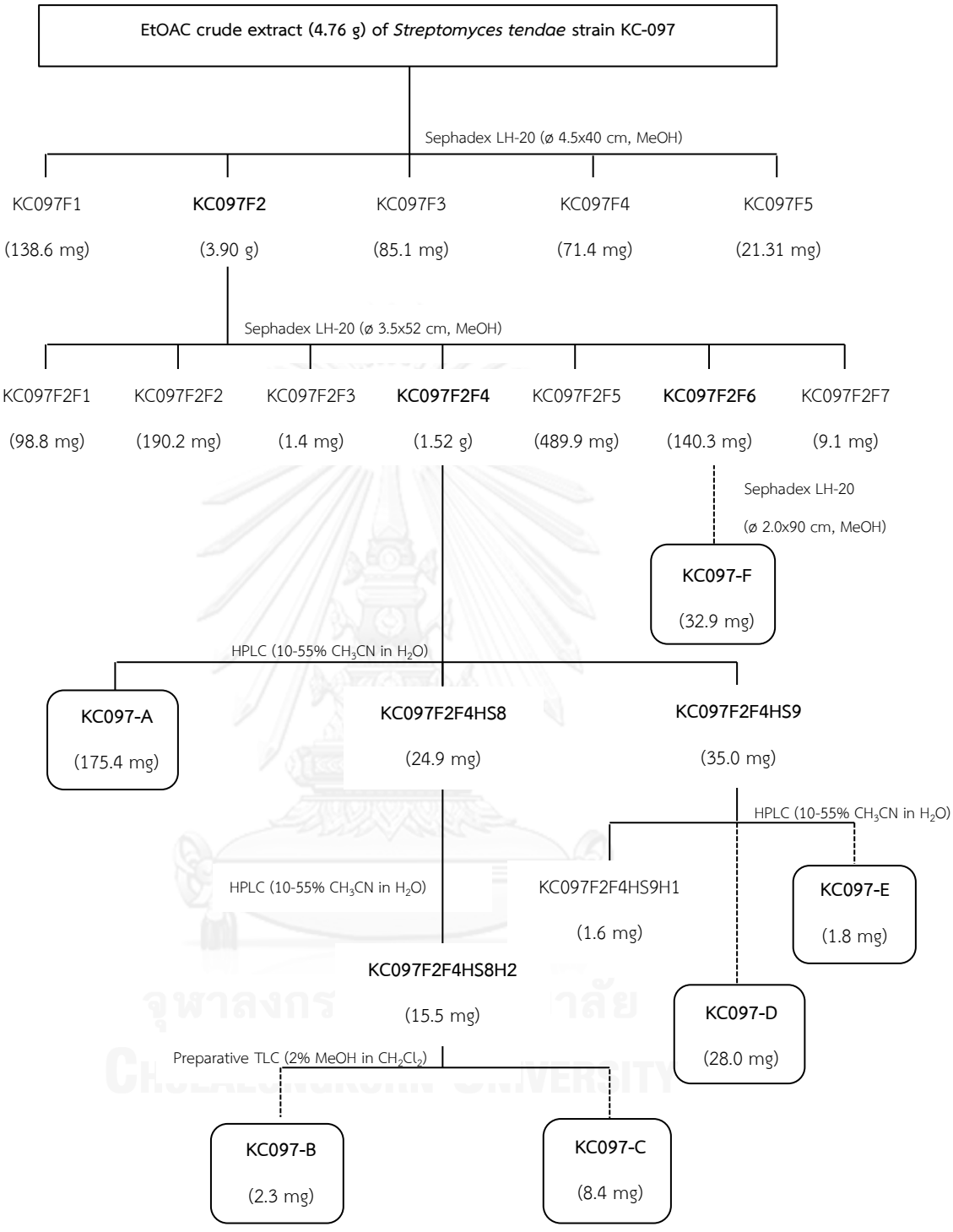
The crude EtOAc extract (4.76 g) was isolated by Sephadex LH-20 column chromatography (column: 4.5x40 cm), eluted with methanol (MeOH) to obtain five fractions (KC097F1-KC097F5). All fractions were analyzed by HPLC for chromatogram comparison with the crude extract. The chromatograms showed that only fraction KC097F2 composed of interesting peaks. Therefore, fraction KC097F2 was carried out for further purification.

Fraction KC097F2 (3.90 g) was repeatedly separated by Sephadex LH-20 column chromatography (column: 3.5x52 cm), eluted with MeOH to give eight fractions (KC097F2F1-KC097F2F8). The chemical profiles of all fractions indicated that fraction KC097F2F4 contained the interesting peaks and fraction KC097F2F6 contained a major peak of the crude extract. Fractions KC097F2F4 and KC097F2F6 were investigated for further purification in the next step.

Fraction KC097F2F4 (1.52 g) was separated by semi-preparative HPLC (Shiseido C-18 column, 5 µm, column: 20x150 mm, flow rate 9 ml/min). The column was eluted with a gradient system of 10-55% acetonitrile (CH_3CN) in H_2O to obtain nine fractions (KC097F2F4HS1-KC097F2F4HS9). The interesting peaks were in fractions KC097F2F4HS7 (**KC097-A**; 175.4 mg), KC097F2F4HS8 (24.9 mg) and KC097F2F4HS9 (35.0 mg). For ^{1}H NMR analysis, fraction KC097F2F4HS7 was a pure compound and

each fraction of KC097F2F4HS8 and KC097F2F4HS9 contained 2 compounds. Fractions KC097F2F4HS8 and KC097F2F4HS9 were purified again by semi-preparative HPLC. The columns were eluted with a gradient system of 10-45% CH_3CN in H_2O to obtain three fractions (KC097F2F4HS8H1-KC097F2F4HS8H3) and KC097F2F4HS9 (KC097F2F4HS9H1-KC097F2F4HS9H3). Then, all fractions were subjected to chemical profiles and ^1H NMR analyses, indicating KC097F2F4HS9H2 (**KC097-D**; 28.0 mg) and KC097F2F4HS9H3 (**KC097-E**; 1.8 mg) were interesting pure compounds but other interesting compounds were together in fraction KC097F2F4HS8H2 (15.5 mg). Fraction KC097F2F4HS8H2 was further purified by preparative TLC which developed with 2% MeOH in dichloromethane (CH_2Cl_2) for three times to obtain two pure compounds KC097F2F4HS8H2B1 (**KC097-B**; 2.3 mg) and KC097F2F4HS8H2B1 (**KC097-C**; 8.4 mg).

Fraction KC097F2F6 were purified by Sephadex LH-20 column chromatography (column: 2.0x90 cm), eluted with MeOH to obtain seven fractions (KC097F2F6F1-KC097F2F6F7). The major peak of the crude extract was in fraction KC097F2F6F3 (**KC097F**, 32.9 mg). The isolation and purification processes of secondary metabolites from *Streptomyces tendae* strain KC-097 are summarized in Scheme 3.2.



Scheme 3.2 Isolation and purification processes of secondary metabolites from *Streptomyces tendae* strain KC-097

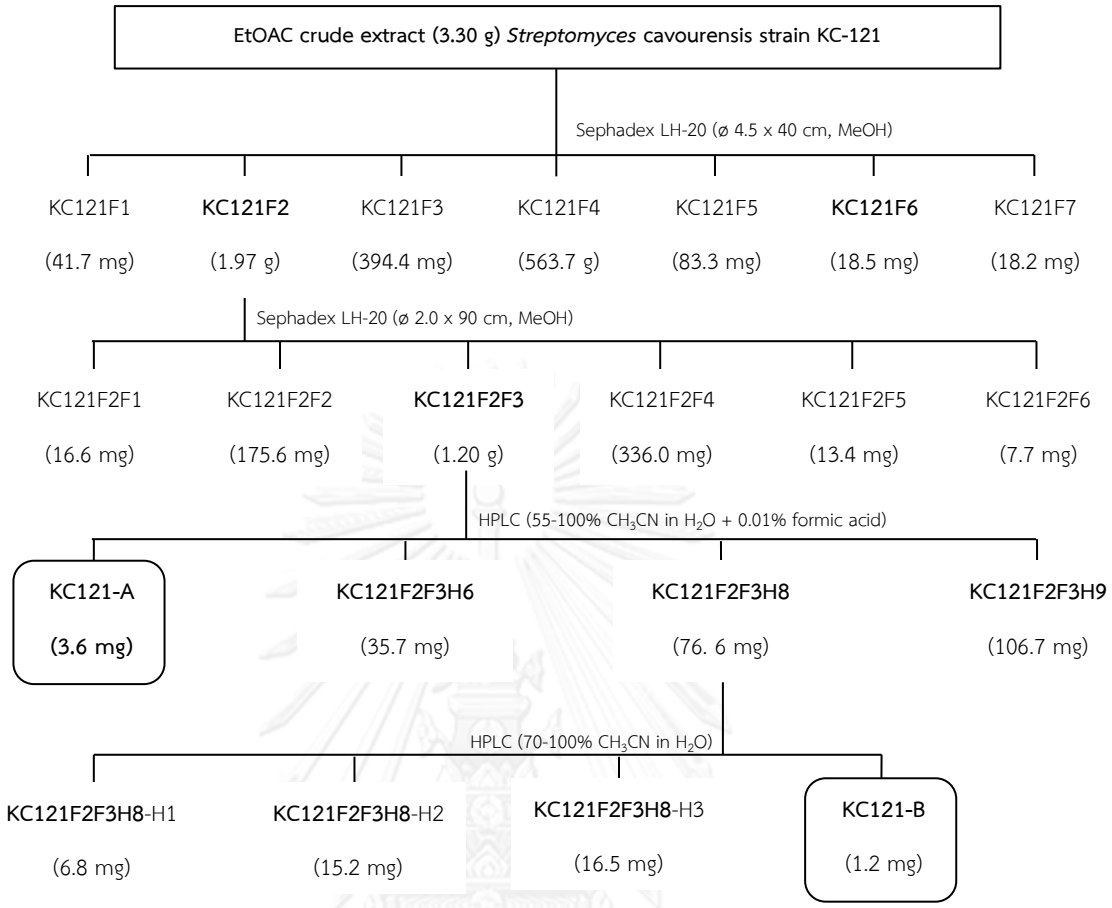
3.6.3.2 Isolation, purification and structure elucidation of secondary metabolites from *Streptomyces cavourensis* strain KC-121

The crude EtOAc extract (3.30 g) was separated by fractionation with Sephadex LH-20 column chromatography (column: 4.5x40 cm), eluted with MeOH to obtain seven fractions (KC121F1-KC121F7). All fractions were subjected chemical profile analysis by HPLC. The chromatograms showed that only fraction KC121F2 composed of interesting peaks. Therefore, fraction KC121F2 was carried out for further purification.

Fraction KC121F2 (1.97 g) was separated by Sephadex LH-20 column chromatography (column: 2.0x90 cm), eluted with MeOH to obtain six fractions (KC121F2F1-KC121F2F6). The chemical profiles of all fractions indicated that fraction KC121F2F3 contained the interesting peaks, so fraction KC121F2F3 was investigated for further purification.

Fraction KC121F2F3 (1.20 g) was purified by preparative HPLC (Shiseido C-18 column, 10 µm, column: 19x250 mm, flow rate 15 ml/min). The column was eluted with a gradient system of 10-45% CH_3CN in H_2O + 0.01% formic acid to obtain nine fractions (KC121F2F3F1-KC121F2F3F9). Then, all fractions were subjected to chemical profiles and ^1H NMR analyses, indicating fractions KC121F2F3H1 and KC121F2F3H8 contained interesting peaks. Fraction KC121F2F3H1 was a pure compound (**KC121-A**; 3.6 mg) but fraction KC121F2F3H8 was not pure compounds. Therefore, fraction KC121F2F3H8 was further purified.

Fractions KC121F2F3H8 (76.6 mg) was continued purification by semi-preparative HPLC (Shiseido C-18 column, 5 µm, column: 20x150 mm, flow rate 9 ml/min) which the column was eluted with a gradient system of 70-100% CH_3CN in H_2O to obtain four fractions (KC121F2F3H8H1-KC121F2F3H8H4) for fraction KC121F2F3H8. The interesting peak was in fraction KC121F2F3H8H4 (**KC121-B**; 1.2 mg). For ^1H NMR analysis, compound KC121-B was pure. The isolation process of the EtOAc crude extract of *Streptomyces cavourensis* strain KC-121 is summarized in Scheme 3.3.



Scheme 3.3 Isolation and purification processes of secondary metabolites from *Streptomyces* strain KC-121

CHAPTER IV
RESULTS AND DISCUSSION

4.1 Soil sample collection and isolation of *Streptomyces*, *Amycolatopsis* and *Kitasatospora* strains

Seventy nine actinomycete strains were isolated from twenty nine soil samples by using starch casein nitrate agar and potato starch-glycerol agar. Twenty isolates were isolated from six soil samples collected from Krung Ching Waterfall National Park, twenty eight isolates from eleven soil samples collected from Angthong Islands National Park and thirty one isolates from twelve soil samples collected from Similan Islands National Park (Table 4.1).

4.2 Identification of *Streptomyces*, *Amycolatopsis* and *Kitasatospora* strains

Seventy nine actinomycete isolates were preliminary identified by using partial 16S rRNA gene sequence analysis including phylogenetic tree construction. for Seventy four isolates were identified as *Streptomyces* and were divided into twenty four groups (Groups I to XXIV), one as *Amycolatopsis*, three as *Kitasatospora* and they were divided into two groups (Groups 1 to 2) and one as *Nonomuraea* (Table 4.2, Figure 4.1).

The representative strains of each group were selected for the identification by using a full 16S rRNA gene sequence analysis. A strain in each group showed lowest 16S rRNA gene sequence similarity compared with closely related strains in EzTaxon database was selected as a representative strain. On full 16S rRNA gene sequence analysis, the twenty four groups in genus *Streptomyces* were identified as the different twenty four *Streptomyces* species and two groups of *Kitasatospora* were identified as the different two *Kitasatospora* species (Table 4.2 and Figure 4.2).

The identification of the seventy nine strains was carried out on the basis of the morphological, cultural, physiological and biochemical characteristics and the almost complete 16S rRNA gene sequence analysis of the selected strains. The complete taxonomic studies based on phenotypic and chemotaxonomic characteristics including 16S rRNA gene sequence analysis and DNA-DNA hybridization were determined for the novel species in the genus *Streptomyces*.

Table 4.1 Location, soil sample number, and isolate number

Location	Soil sample No.	Isolate no.
Krung Ching Waterfall National Park, Nakhon Si Thammarat Province	1	KC-001, KC-003, KC-004
	2	KC-005, KC-017
	3	KC-020, KC-150, KC-152, KC-157
	4	KC-031, KC-032, KC-033, KC-034
	5	KC-035, KC-036, KC-037, KC-038
	6	KC-047, KC-132, KC-143,
Angthong Islands National Park, Surat Thani Province	7	KC-054, KC-055, KC-058, KC-060
	8	KC-061, KC-062, KC-063, KC-066
	9	KC-070, KC-141
	10	KC-072
	11	KC-73, KC-142, KC-155
	12	KC-074, KC-075, KC-076, KC-156
	13	KC-079, KC-080
	14	KC-085, KC-151
	15	KC-087
	16	KC-088, KC-090
	17	KC-093, KC-094, KC-138
Similan Islands National Park, Phang Nga Province	18	KC-095, KC-096, KC-097, KC-098
	19	KC-100, KC-101, KC-102, KC-103
	20	KC-104, KC-105
	21	KC-106
	22	KC-110, KC-111, KC-112, KC-136
	23	KC-135, KC-140
	24	KC-115
	25	KC-117, KC-118
	26	KC-119, KC-134
	27	KC-120, KC-121, KC-122, KC-133
	28	KC-124, KC-125, KC-145
	29	KC-128, KC-130

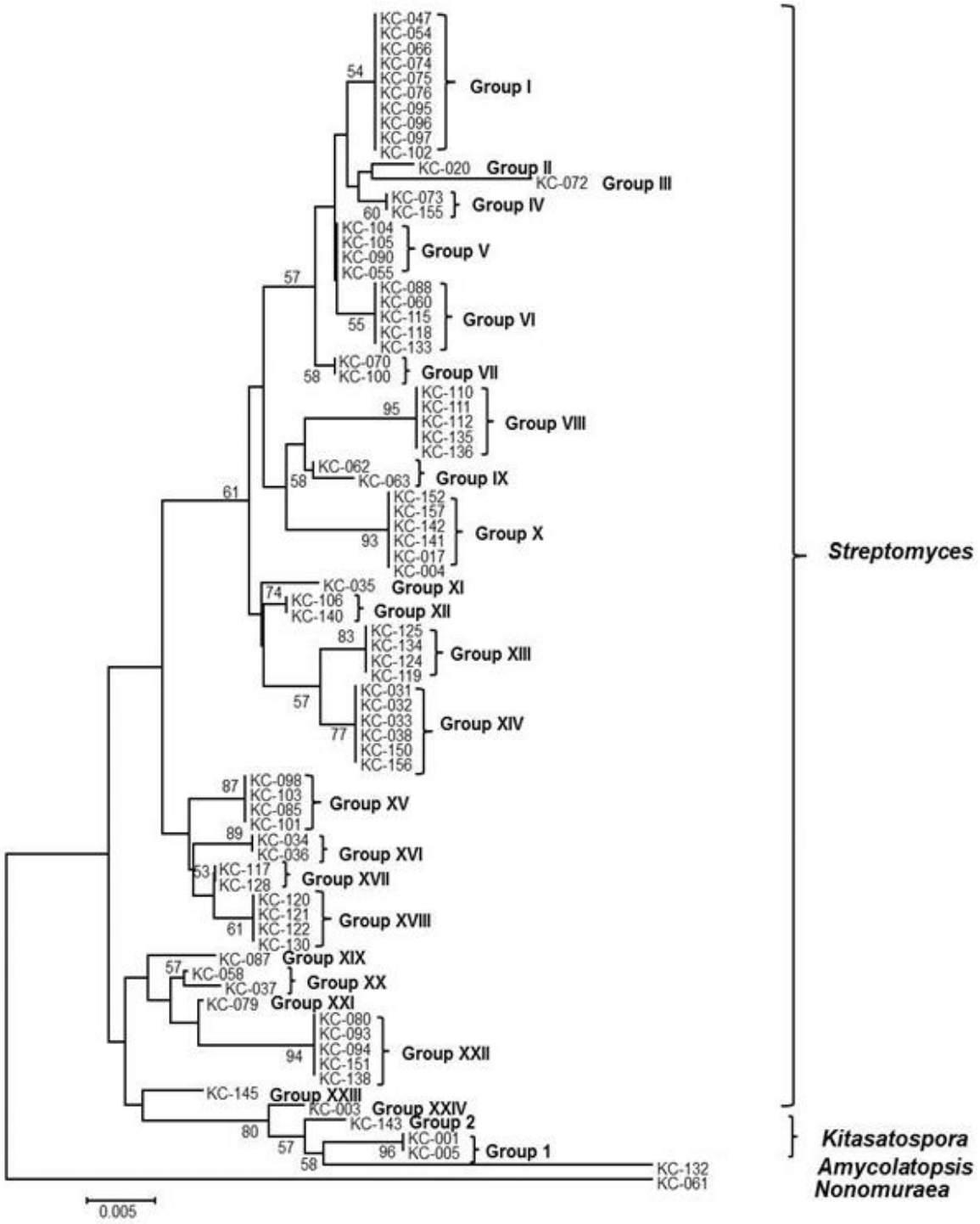


Figure 4.1 Neighbor-joining phylogenetic tree based on the partial 16S rRNA gene sequences of *Streptomyces*, *Amycolatopsis* and *Kitasatospora* strains

Table 4.2 Identity between *Streptomyces*, *Amycolatopsis* and *Kitasatospora* strains and closely related strains

Groups	Isolate no.	Closely related type strains	16S rRNA gene Similarity (%)	Length of sequences (bp)
***Streptomyces* strains**				
I	KC-097	*S. tendae* ATCC 19812^T (D63873)	100.0	1334
II	KC-020	*S. enissocaesilis* NRRL B-16365^T (DQ026641)	100.0	1340
III	KC-072	*S. fragilis* NRRL 2424^T (AY999917)	99.3	1357
IV	KC-155	*S. marokkonensis* Ap1^T (AJ965470)	99.5	1453
V	KC-055	*S. parvulus* NBRC 13193^T (AB184326)	100.0	1350
VI	KC-088	*S. malachitospinus* NBRC 101004^T (AB249954)	99.9	1357
VII	KC-070	*S. diastaticus* subsp. *ardesiacus* NRRL B-1773^T (DQ026631)	99.7	1342
VIII	KC-112	*S. anandii* NRRL B-3590^T (AY999803)	98.8	1500
IX	KC-062	*S. spiralis* NBRC 14215^T (AB184575)	99.4	1479
X	KC-017	*S. aureus* NBRC 100912^T (AB249976)	99.8	1329
XI	KC-035	*S. siamensis* KC-038^T (AB773848)	98.4	1494
XII	KC-106	*S. seoulensis* NBRC 16668^T (AB249970)	98.9	1508
XIII	KC-134	*S. violarus* NBRC 13104^T (AB184316)	99.6	1347
XIV	KC-031	*S. olivochromogenes* NBRC 3178^T (AB184737)	98.1	1496
	KC-038	*S. olivochromogenes* NBRC 3178^T (AB184737)	98.1	1497
XV	KC-101	*S. drozdowiczii* NBRC 101007^T (AB249957)	99.7	1473
XVI	KC-036	*S. exfoliatus* NBRC 13191^T (AB184324)	99.9	1354
XVII	KC-117	*S. sindenensis* NBRC 3399^T (AB184759)	100.0	1359
XVIII	KC-121	*S. cavourensis* NBRC 13026^T (AB184264)	99.9	1383
XX	KC-058	*S. rapamycinicus* NRRL B-5491^T (EF408733)	99.3	1358
XXI	KC-079	*S. yatensis* NBRC 101000^T (AB249962)	99.4	1359
XXII	KC-138	*S. samsunensis* M1463^T (EU077190)	99.8	1346
XXIII	KC-145	*S. cinereoruber* subsp. *cinereoruber* NBRC 12756^T (AB184121)	99.8	1352
XXIV	KC-003	*S. misakiensis* NBRC 12891^T (AB184223)	99.8	1326
***Amycolatopsis* strain**				
	KC-132	*A. keratiniphila* subsp. *keratiniphila* DSM 44409^T (AJ278496)	99.3	1297
***Kitasatospora* strains**				
1	KC-001	*K. saccharophila* SK15^T (AB278568)	100	1256
	KC-005	*K. saccharophila* SK15^T (AB278568)	100	1280
2	KC-143	*K. putterlickiae* F18-98^T (AY189976)	99.3	1330
***Nonomuraea* strain**				
	KC-061	*N. monospora* PT708^T (FJ347524)	99.3	1495

Figure 4.2 Neighbor-joining tree based on almost full 16S rRNA gene sequences of the representative strains in each group of *Streptomyces*, *Amycolatopsis* and *Kitasatospora* strains

4.2.1 Characteristics of *Streptomyces* strains

Group I consisted of ten strains, including KC-047, KC-054, KC-66, KC-074, KC-075, KC-076, KC-095, KC-096, KC-097 and KC-102. These strains were observed to form extensively branched substrate mycelia and abundant aerial mycelia on most of agar media tested, except ISP 6. The aerial mycelia consisted of long spiral spore chains with smooth surface spores (Figure 4.3). On the basis of morphological characteristics, these strains were identified as members of the genus *Streptomyces*. They produced white to bluish gray aerial mycelia on various agar media tested. The colors of substrate mycelium ranged from pale yellow to dark grayish brown (Appendix C, Table 1).

They grew at 15-40°C, pH 5-12 and tolerated up to 7-10% (w/v) NaCl. All strains hydrolyzed starch but did not hydrolyze casein and gelatin. Most of strains could reduce nitrate, except strains KC-095 and KC-097. They utilized L-arabinose, D-fructose, D-glucose, *myo*-inositol, D-mannitol, L-rhamnose and D-xylose as carbon sources but did not utilize melezitose, melibiose, raffinose, D-sorbitol and sucrose (Appendix C, Table 2).

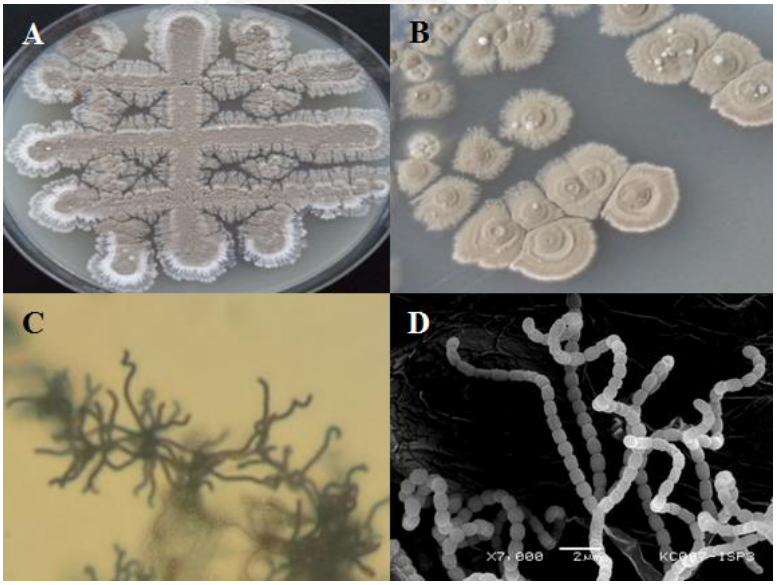


Figure 4.3 The morphological characters of strain KC-097 representing *Streptomyces* group I. A and B) the colonial appearance on ISP 3, C) light micrograph on nutrient agar, and D) scanning electron micrograph on ISP3.

Strain KC-097, the representative strain in this group had the highest sequence similarity (100%) to *S. tendae* ATCC 19812^{T} (Table 4.2). The phylogenetic tree based on 16S rRNA gene sequence showed that strain KC-097 was from a cluster with *S. tendae* (Figure 4.2). Therefore, strain KC-097 was identified as *S. tendae*.

Group II contained one strain, KC-020. Morphological observation of a 14-day culture, strain KC-020 grew well and formed a highly branched substrate mycelium on any test media. The white aerial mycelia were formed abundant on ISP 2, ISP 3, ISP 5, ISP 6 and nutrient agar and bluish gray were formed abundant on ISP 4. The aerial mycelia were not produced on YS agar. The aerial mycelia consisted of long straight to flexuous (*Rectiflexibile*) spore chains with smooth surface spores (Figure 4.4). The substrate mycelia colors ranged from pale yellow to olive brown on agar media tested. It produced only grayish yellow soluble pigment on ISP 4. The cultural characteristics of *Streptomyces* strain in group II are shown in Table 3 (Appendix C).

Strain KC-020 reduced nitrate, hydrolyzed starch and peptonized milk but did not liquefy gelatin and coagulate milk. It utilized L-arabinose, D-fructose, D-glucose, D-mannitol, *myo*-inositol, melibiose, raffinose, L-rhamnose and D-xylose as carbon sources but did not utilize melezitose, D-sorbitol and sucrose. Growth of strain KC-020 occurred at pH 5-12 and with 0-7% (w/v) NaCl. The temperature range for growth was 15-40°C. Detailed physiological and biochemical characteristics are presented in the Table 4 (Appendix C).

Sequence analysis of the 16S rRNA gene sequence showed that strain KC-020 was affiliated to the genus *Streptomyces*. Based on EzTaxon-e analysis, strain KC-020 was related the most closely to *S. enissocaesilis* NRRL B-16365^{T} with 100% 16S rRNA gene sequence similarity (Table 4.2 and Figure 4.2). On the basis of phenotypic and 16S rRNA gene sequence analyses, strain KC-020 was identified as *S. enissocaesilis*.

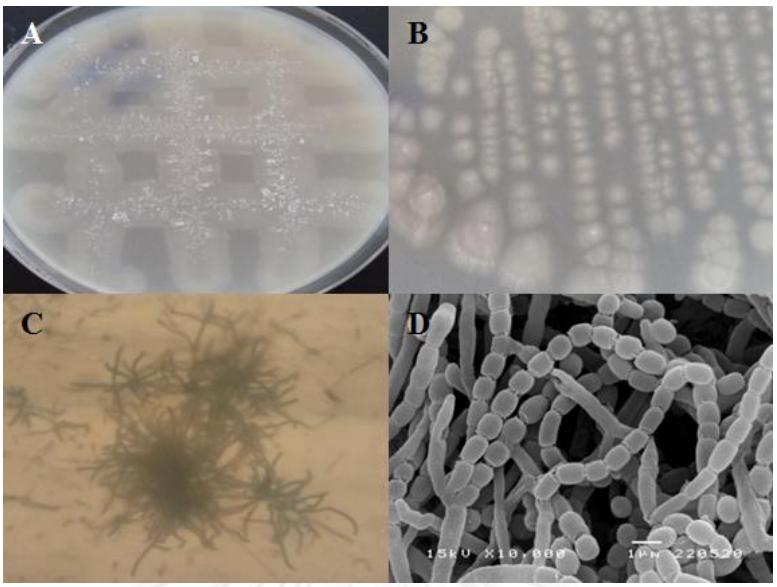


Figure 4.4 The morphological characters of strain KC-020 representing *Streptomyces* group II. A and B) the colonial appearance on ISP 3, C) light micrograph on ISP 4, and D) scanning electron micrograph on ISP4.

Group III comprised one strain, KC-072. This strain showed good growth on all agar media tested. From light and scanning electron microscopic observations, strain KC-072 had an extensively branched substrate mycelia and abundant aerial mycelia that carried hairy-surfaced spores with oval shape in spiral spore chains (Figure 4.5). The strains formed white or white to bluish gray or light greenish gray to greenish gray or medium gray aerial mycelia and grayish greenish yellow or grayish yellow to dark yellow or light olive gray or moderate yellowish brown to dark yellowish brown or moderate yellowish brown to deep yellowish brown or dark grayish yellow substrate mycelia on agar media tested. Strain KC-072 produced soluble pigments on ISP 3 and ISP 5, ISP7, YS agar and nutrient agar. The cultural characteristics of *Streptomyces* strain in group III are shown in Table 3 (Appendix C). Strain KC-072 could grow at 15-40 °C, at pH 5-12 and in the presence of 0-10% (w/v) NaCl. Starch was hydrolyzed but gelatin was not liquefied. Milk was not peptonized and coagulated. The strain could not reduce nitrate. Strain KC-072 utilized L-arabinose, D-fructose, D-glucose, D-mannitol, melibiose, *myo*-inositol, raffinose, sucrose and D-xylose as carbon sources, but not melezitose, L-rhamnose, D-sorbitol. Detailed physiological and biochemical characteristics are given in Table 4 (Appendix C).

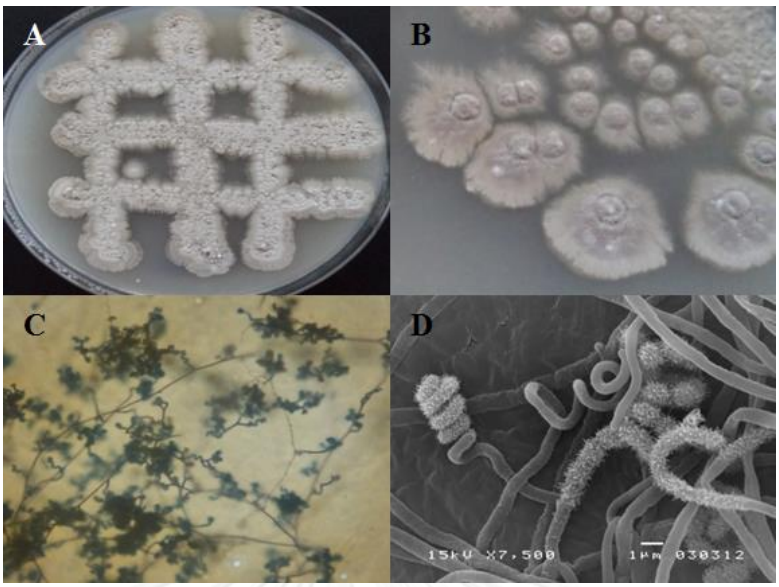


Figure 4.5 The morphological characters of strain KC-072 representing *Streptomyces* group III. A and B) the colonial appearance on ISP 3, C) light micrograph on ISP 3, and D) scanning electron micrograph on ISP2.

The almost complete 16S rRNA gene sequence of strain KC-072 was compared with sequences in the EzTaxon-e database. The result indicated the strain belonged to the genus *Streptomyces*. Strain KC-072 showed the highest 16S rRNA gene sequence similarity (99.3%) to *S. fragilis* NRRL 2424^{T} and it was clustered with *S. fragilis* NRRL 2424^{T} in phylogenetic tree (Figure 4.2). Therefore, strain KC-072 was identified as *S. fragilis*.

Group IV composed of two strains, including KC-073 and KC-155, which formed greenish white to light olive aerial mycelia on various agar media such as ISP3, ISP 4, ISP 5, ISP 7, YS agar and nutrient agar and pale yellow to yellowish brown substrate mycelia were well-developed without fragmentation. Aerial mycelia were spiral chains with hairy surface spores (Figure 4.6). They were observed to grow well on all agar media tested and produce pale orange yellow soluble pigments on ISP 4, ISP 5, ISP 7 and YS agar. The cultural characteristics of strains KC-073 and KC-155 are shown in Table 3 (Appendix C).

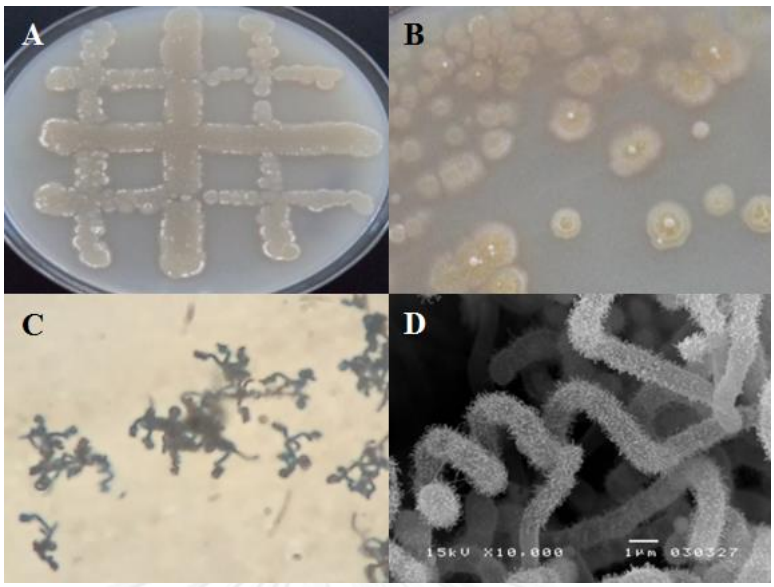


Figure 4.6 The morphological characters of strain KC-0155 representing *Streptomyces* group IV. A and B) the colonial appearance on ISP 3, C) light micrograph on ISP 4, and D) scanning electron micrograph on ISP4.

Streptomyces strains KC-073 and KC-155 were determined to be positive for nitrate reduction and starch hydrolysis and negative for gelatinization. Strain KC-155 could hydrolyze milk but not strain KC-073. Strain KC-073 was found be able to grow at pH 5-12, 10-40 °C and 0-4% (w/v) NaCl. Strain KC-155 was found be able to grow at pH 5-11, 10-40 °C and 0-5% (w/v) NaCl. They were found to use L-arabinose, D-glucose, D-mannitol, *myo*-inositol, raffinose and L-rhamnose as carbon sources, but not D-fructose, D-melibiose, melezitose, D-sorbitol, sucrose and D-xylose. Detailed physiological and biochemical characteristics of *Streptomyces* strains in group IV are presented in Table 4 (Appendix C).

Strain KC-155 was selected to be the representative strain in this group for 16S rRNA gene sequence analysis. The nearly complete 16S rRNA gene sequence of strain KC-155 was compared with sequences of known bacterial species in EzTaxon-server. The results of these comparisons revealed that strain KC-155 had the highest sequence similarity (99.5%) to *S. marokkonensis* Ap1^{T} (Table 4.2). The phylogenetic tree based on 16S rRNA gene sequence showed that strain KC-155 was from a clade with *S. marokkonensis* (Bouizgarne *et al*., 2009) (Figure 4.2).

Group V comprised four strains, including KC-055, KC-090, KC-104 and KC-105. Morphological and cultural observation of 14-day culture on various agar media, they formed extensive substrate mycelia and abundant aerial mycelia. All strains in this

group produced aerial mycelia that differentiated to long spore chains. The spore chains were spiral and cylindrical with smooth surface spores (Figure 4.7). The substrate mycelia formed colors of yellow to olive and brown and the aerial mycelia formed colors of white to gray. All strains produced soluble pigments on some agar media (Appendix C, Table 5). They were able to grow at pH 4-11, at 20-37ºC with 0-11% (w/v) NaCl. Almost strains in this group degraded starch and coagulated milk, except strain KC-105. Nitrate reduction and milk peptonization were negative. All strains utilized L-arabinose, D-fructose, D-glucose, D-mannitol, *myo*-Inositol, raffinose and L-rhamnose as carbon sources (Appendix C, Table 6).

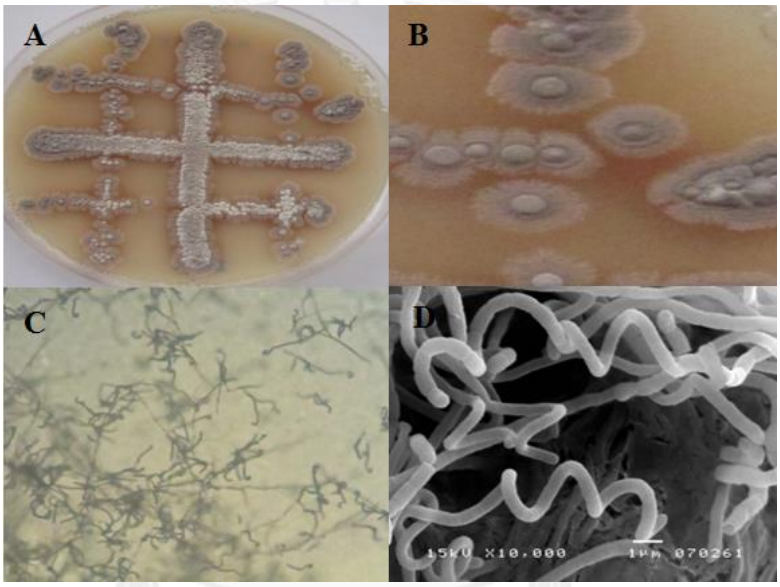


Figure 4.7 The morphological characters of strain KC-055 representing *Streptomyces* group V. A and B) the colonial appearance on ISP 3, C) light micrograph on nutrient agar, and D) scanning electron micrograph on ISP4.

Strain KC-055 was selected to be the representative strain in this group for 16S rRNA gene sequence analysis. Sequence analysis of the 16S rRNA gene sequence base on EzTaxon-e server, strain KC-055 was related the most closely to *S. parvulus* NBRC 13193^{T} (AB184326) with 100% 16S rRNA gene sequence similarity. The phylogenetic tree based on 16S rRNA gene sequence showed that strain KC-055 was from a clade with *S. parvulus* (Figure 4.2). Therefore, strain KC-055 was identified as *S. parvulus*

Group VI contained five strains, including KC-060, KC-088, KC-115, KC-118 and KC-133. These strains were observed to form extensively branched substrate mycelia and abundant aerial mycelia on most of the media, except ISP 6. The aerial mycelia consisted of long spiral spore chains with spiny surface and oval shape spores (Figure 4.8). All strains showed good growth on all agar media tested. Aerial mycelia were white to greenish gray and bluish gray and substrate mycelia were pale yellow to grayish olive. Soluble pigments were grayish yellow on ISP 7 for strain KC-060 and pale orange yellow on ISP 3 for strains KC-088 and KC-115 and on ISP 5 for strain KC-088, whereas were not produced for strains KC-118 and 133. The cultural characteristics of strains in group VI are shown in Table 7 (Appendix C).

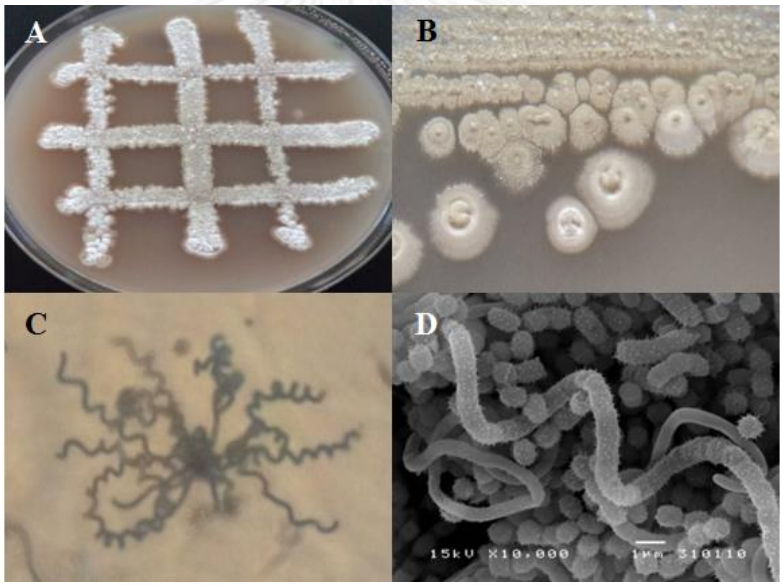


Figure 4.8 The morphological characters of strain KC-088 representing *Streptomyces* group VI. A and B) the colonial appearance on ISP 3, C) light micrograph on ISP 2, and D) scanning electron micrograph on ISP4.

They grew at 15-40°C, pH 4-12 and tolerated up to 8-10% (w/v) NaCl. All strains hydrolyzed starch but did not hydrolyze milk and gelatin. Nitrate reduction was positive for all strains. Most of strains utilized the following sugars: L-arabinose**,** D-fructose**,** D-glucose**,** D-mannitol**,** D-melibiose, *myo*-inositol**,** raffinose, L-rhamnose and D-xylose as carbon sources, but did not utilize melezitose, D-sorbitol and sucrose (Appendix C, Table 8).

Strain KC-088 was selected to be the representative strain in this group for 16S rRNA gene sequence analysis. The nearly complete 16S rRNA gene sequence of strain KC-088 was compared with sequences of known bacterial species in EzTaxon-server. The results of these comparisons exhibited that strain KC-088 had the highest sequence similarity (99.9%) to *S. malachitospinus* NBRC 101004^{T} (Table 4.2). The phylogenetic tree based on 16S rRNA gene sequence showed that strain KC-088 was from a clade with *S. malachitospinus* (Figure 4.2). Consequently, the strain KC-088 was identified as *S. malachitospinus*.

Group VII consisted of two strains, including KC-070 and KC-100 which formed an extensively branched substrate mycelium and white to medium grey and bluish gray aerial mycelia that carried smooth-surfaced spores in a spiral arrangement (Figure 4.9). Strains KC-070 and KC-100 showed good growth on all agar media tested, with pale yellow, brownish pink to reddish brown. No soluble pigment was produced into the media (Appendix C, Table 9). Temperature, pH and NaCl concentration for growth were 15-40 °C, pH 5-12 and 0-10% NaCl (w/v) for strain KC-070 and 0-9 % NaCl (w/v) for strain KC-100. Nitrate reduction and starch hydrolysis were positive, but milk peptonization and gelatinization were negative. L-Arabinose**,** D-fructose**,** D-glucose, D-mannitol**,** D-melibiose, *myo*-inositol**,** raffinose and L-rhamnose were utilized as sole carbon but melezitose**,** D-sorbitol and sucrose were not (Appendix C, Table 10).

Strain KC-070 was selected to be the representative strain in this group for 16S rRNA gene sequence analysis. Based on 16S rRNA gene sequences, strain KC-070 was related the most closely to members of the genus *Streptomyces*, in particular to *S. diastaticus* subsp. *ardesiacus* NRRL B-1773^{T} with 99.7% 16S rRNA gene sequences similarity. Therefore, strain KC-070 was identified as *S. diastaticus* subsp. *ardesiacus*.

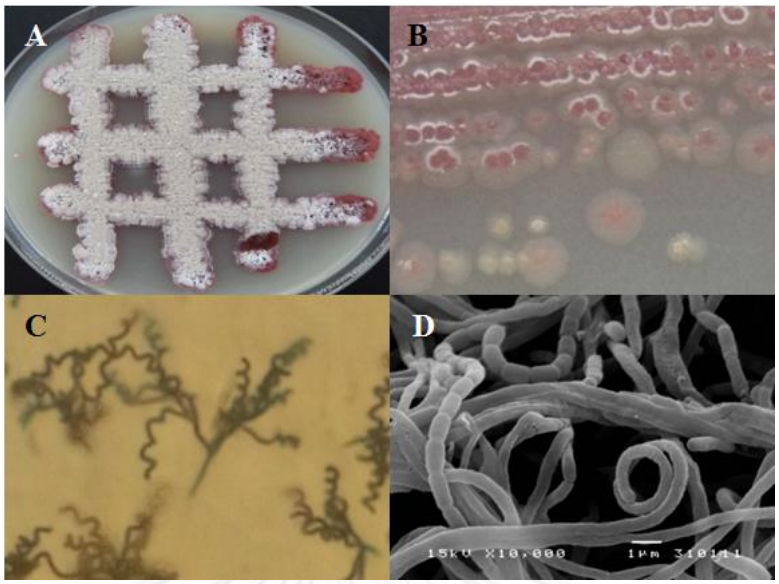


Figure 4.9 The morphological characters of strain KC-070 representing *Streptomyces* group VII. A and B) the colonial appearance on ISP 3, C) light micrograph on ISP 2, and D) scanning electron micrograph on ISP4.

Group VIII consisted of five strains, including KC-110, KC-111, KC-112, KC-135 and KC-136. These strains were observed to form extensively branched substrate mycelia and abundant aerial mycelia on all media tested. The aerial mycelia consisted of long spiral spore chains with smooth surface spores (Figure 4.10). They produced white to gray aerial mycelia on the various agar media tested. The color of substrate mycelium ranged from coloress, yellow, brown to olive. Most of strains were produced soluble pigments on ISP 2, ISP 5 and ISP 7 (Appendix C, Table 11).

They grew at 15-40°C, pH 5-12 and tolerated up to 8-9% (w/v) NaCl. All strains were determined to be negative for starch hydrolysis, milk coagulation and nitrate reduction, but positive for milk peptonization. They utilized L-arabinose, D-fructose, D-glucose, D-mannitol and D-xylose as carbon sources but did not utilize melezitose, D-melibiose, *myo*-inositol, L-rhamnose, D-sorbitol and sucrose (Appendix C, Table 12).

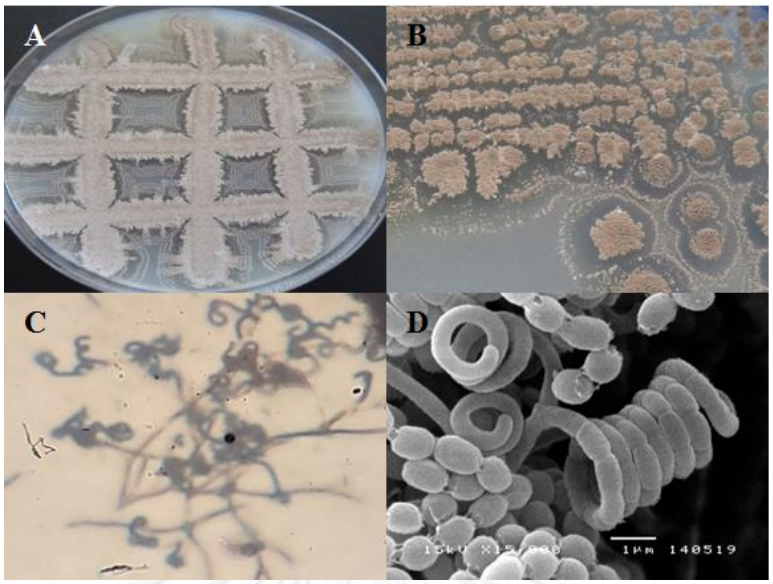


Figure 4.10 The morphological characters of strain KC-112 representing *Streptomyces* group VIII. A and B) the colonial appearance on ISP 3, C) light micrograph on YS agar, and D) scanning electron micrograph on ISP3.

Strain KC-112 was selected to be the representative strain in this group for 16S rRNA gene sequence analysis. The nearly complete 16S rRNA gene sequence of strain KC-112 was compared with sequences of known bacterial species in EzTaxon-server. The results of these comparisons revealed that strain KC-112 had the highest sequence similarity (98.8%) to *S. anandii* NRRL B-3590^T (Table 4.2). This strain was a novel species and was selected to complete for taxonomic characterization.

Strain KC-112 exhibited typical characteristics of the genus *Streptomyces*. LL-diaminopimelic acid was detected in whole-cell hydrolysates. The menaquinones detected were MK-9(H_8) (70%), MK-9(H_6) (15%) and MK-9(H_2) (15%). The *N*-acyl type of muramic acid was acetyl. Strain KC-112 contained diphosphatidylglycerol (DPG), phosphatidylethanolamine (PE), phosphatidylglycerol (PG), phosphatidylinositol (PI), phosphatidylinositol mannoside (PIMs) and unknown phospholipids as phospholipid composition. The DNA G+C content was 73 mol%.

The 16S rRNA gene sequence of strain KC-112 was most similar to *S. anandii* NRRL B-3590^T (98.8%), *S. capillispiralis* NBRC 14222^T (98.8%) and *S. fumanus* NBRC 13042^T (98.8%). The phylogenetic tree showed that strain KC-112^T formed a cluster with *S. fumanus* and *S. anandii*, with the exception of *S. capillispiralis* (Figure 4.11).

The cultural characteristics of strain KC-112 and closely related species, *S. anandii* NRRL B-3590^{T}, *S. capillispiralis* NBRC 14222^{T} and *S. fumanus* NBRC 13042^{T} are shown in Table 4.3. Strain KC-112 grew well and formed extensively branched substrate and aerial mycelia on all agar media tested. Aerial mycelia of greenish white to light olive gray or brownish gray color were produced. The aerial mycelia consisted of spiral spore chains with smooth surface and oval shaped spores (Figure 4.10). The phenotypic and differential characteristics of strain KC-112 and the closely relate type strains are listed in Tables 4.3 and 4.4. Strain KC-112 was also different from the closest related type strains in carbon utilization, strain KC-112 could not utilize *myo*-Inositol and rhamnose whereas its closely related type strains could utilize *myo*-Inositol and rhamnose. Strains KC-112 was negative for starch hydrolysis but all closely type strains were positive for starch hydrolysis.

The DNA-DNA relatedness values between strain KC-112 and the closest type strains, *S. anandii* NRRL B-3590^{T}, *S. capillispiralis* NBRC 14222^{T} and *S. fumansu* NBRC 13042^{T}, were in the range of 15-44% (Table 4.5). These values were below the 70% cutoff point recommended by Wayne *et al*. (1987) for assigning strains to the same species, and these results thus confirm that strain KC-112 is distinct from their closely related phylogenetic neighbors. Therefore, strain KC-112 is clearly a novel species within the genus *Streptomyces* and named as *Streptomyces andamanensis*.

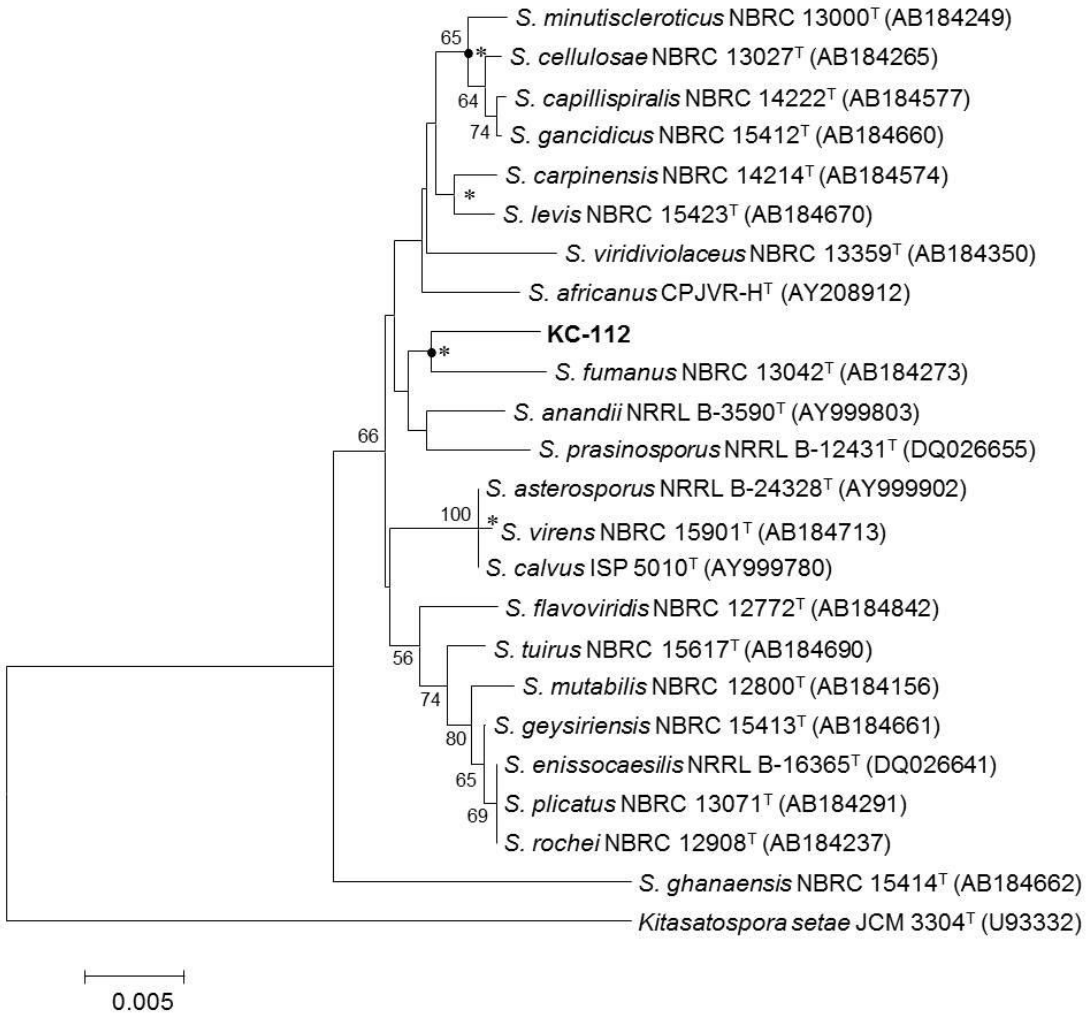


Figure 4.11 Neighbor-joining tree based on 16S rRNA gene sequences showing relationship between strain KC-112 and closely related type strains of the genus *Streptomyces*. Only bootstrap values above 50% (percentages of 1000 replications) are indicated. (•), branch also likelihood tree; Bar, 0.005 recovered in the maximum-parsimony tree; (*), branch also recovered in the maximum-likelihood tree; Bar, 0.005 nucleotide substitutions per site.

Table 4.3 Cultural characteristics of strain KC-112 and closely related type strains

Media	Strains			
	KC-112	*S. anandii* NBRC 13438^T	*S. capillispiralis* NBRC 14222^T	*S. fumansu* NBRC 13042^T
ISP 2				
Growth	Good	Good	Good	Good
	Dark yellow	Pale yellow - dark yellow	Pale yellow	Pale yellow
Reverse	Light olive brown	Pale yellow - dark yellow	Pale yellow	Light yellow - moderate yellow
Aerial mycelium	Abundant, greenish white - medium gray	Moderate, medium gray	None	Abundant, greenish white
Soluble pigment	None	None	None	None
ISP 3				
Growth	Good	Good	Good	Good
	Colorless	Greenish white - light grayish olive	Dark yellow	Pale greenish yellow
Reverse	Light olive gray	Greenish white - olive gray	Dark grayish yellow	Moderate yellow
Aerial mycelium	Abundant, light olive gray - medium gray	Moderate, greenish white - light olive gray	None	Abundant, greenish white
Soluble pigment	None	None	None	None
ISP 4				
Growth	Good	Good	Good	Good
	Colorless	Pale yellow	Dark yellow	Dark yellow
Reverse	Light grayish olive	Pale yellow	Grayish yellow - dark yellow	Grayish yellow - dark yellow
Aerial mycelium	Abundant, brownish gray	Moderate, light gray	None	Abundant, greenish white
Soluble pigment	None	None	None	None
ISP 5				
Growth	Good	Good	Good	Good
	Moderate olive brown	Greenish white	Grayish yellow - light grayish brown	Greenish white
Reverse	Light grayish olive	Greenish white	Grayish yellow - light grayish brown	Greenish white
Aerial mycelium	Abundant, light olive gray	None	None	Abundant, greenish white
Soluble pigment	Pale orange yellow	None	None	None
ISP 6				
Growth	Good	Good	Good	Good
	Colorless	Greenish gray	Pale greenish yellow – grayish greenish yellow	Pale yellowish green
Reverse	Pale yellow - moderate yellow	Dark grayish olive	Grayish greenish yellow	Pale greenish yellow - moderate yellow
Aerial mycelium	Abundant, greenish white	None	None	Abundant, greenish white
Soluble pigment	None	Dark brown	None	None
ISP 7				
Growth	Good	Good	Good	Good
	Moderate olive brown	Light greenish gray - deep greenish gray	Moderate olive brown	Grayish yellow
Reverse	Light olive gray - moderate olive brown	Olive gray	Dark greyish yellow	Grayish yellow
Aerial mycelium	Abundant, light olive gray	None	Moderate, greenish white	Abundant, greenish white
Soluble pigment	Yellowish gray	Light olive gray	Pale yellowish green	Pale yellow
YS agar				
Growth	Good	Good	Good	Good
	Colorless	Pale yellowish green - light grayish olive	Moderate yellow	Light olive brown
Reverse	Light olive gray	Pale yellowish green - grayish olive	Grayish yellow	Pale yellow - moderate olive brown
Aerial mycelium	Abundant, light olive gray	Abundant, light gray	None	Abundant, greenish white
Soluble pigment	None	None	None	Light yellowish green
Nutrient agar				
Growth	Good	Good	Good	Good
	Grayish greenish yellow	Moderate olive brown	Grayish yellow	Grayish greenish yellow
Reverse	Pale greenish yellow - grayish greenish yellow	Dark greenish yellow	Grayish yellow	Pale greenish yellow - grayish greenish yellow
Aerial mycelium	Abundant, greenish white - light greenish gray	Abundant, light greenish gray	None	Abundant, greenish white
Soluble pigment	None	Dark yellow	None	None

Table 4.4 Different characteristics of strain KC-112 and closely related type strains

Characteristics	Strains			
	KC-112	*S. anandii* NBRC 13438^T	*S. capillispiralis* NBRC 14222^T	*S. fumanus* NBRC 13042^T
Spore chain	Spiral	Spiral	Spiral	Spiral
Spore surface	Smooth	Smooth	Hairy	Smooth
Utilization of				
myo-Inositol	-	+	+	±
D-Mannitol	+	+	+	-
Melibiose	-	-	-	+
Raffinose	±	-	-	+
L-Rhamnose	-	+	+	+
Xylose	+	+	+	-
Nitrate reduction	-	+	-	+
Starch hydrolysis	-	+	+	+
Gelatin liquefaction	±	±	+	+
Milk peptonization	+	-	+	±
NaCl tolerance (%)	9	8	7	7

+ = positive, ± = weakly positive, - = negative

Table 4.5 DNA-DNA relatedness between strain KC-112 and closely related type strains

Strains	DNA-DNA relatedness with labeled strains (%)*			
	KC-112	*S. anandii* NBRC 13438^T	*S. capillispiralis* NBRC 14222^T	*S. fumansu* NBRC 13042^T
KC-112	100	32	44	21
S. anandii NBRC 13438^T	44	100	70	19
S. capillispiralis NBRC 14222^T	39	56	100	16
S. fumansu NBRC 13042^T	15	18	22	100

*Average of four independent determinations

Group IX contained of two strains, including KC-062 and KC-063. These strains showed good growth on all agar media tested. From light and scanning electron microscopic observations of these strains, they had an extensively branched substrate mycelia and aerial mycelia that carried warty surface spores (Figure 4.12). The strains formed white aerial mycelia and pale greenish yellow to strong greenish yellow and yellowish pink substrate mycelia on agar media tested. They have no soluble pigment production. The cultural characteristics of these strains in group IX are shown in Table 13 (Appendix C).

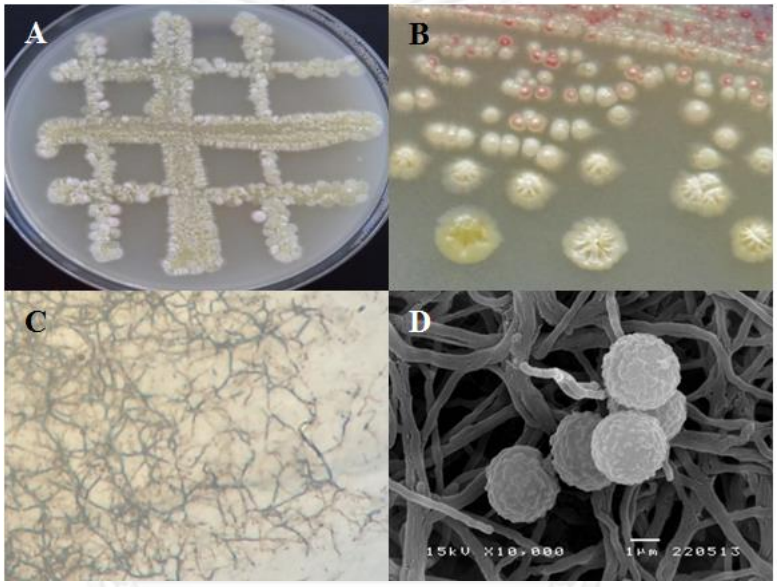


Figure 4.12 The morphological characters of strain KC-062 representing *Streptomyces* group IX. A and B) the colonial appearance on ISP 3, C) light micrograph on YS agar, and D) scanning electron micrograph on YS agar.

The strains could grow at 15-45°C and pH 5-12. They were observed to grow in the presence of 0-9% (w/v) NaCl. Starch was hydrolyzed but gelatin was not liquefied. Milk was not peptonized and coagulated. They could not reduce nitrate. In addition, they utilized L-arabinose, D-fructose, D-glucose, D-mannitol, melibiose, *myo*-inositol, raffinose, L-rhamnose and D-xylose as carbon sources, but not melezitose, D-sorbitol and sucrose. The detailed physiological and biochemical characteristics of these strains are given in Table 14 (Appendix C).

Strain KC-062 was selected to be the representative strain in this group for 16S rRNA gene sequence analysis. The almost complete 16S rRNA gene sequence of strain KC-062 was compared with sequences in the EzTaxon-e database. The result indicated the strain belonged to the genus *Streptomyces*. Strain KC-062 showed the highest 16S rRNA gene sequence similarity (99.4%) to *S. spiralis* NBRC 14215^{T} and it was clustered with *S. spiralis* in phylogenetic tree (Fig 4.2).

Group X composed of six strains, including KC-004, KC-017, KC-141, KC-142, KC-152 and KC-157 which formed extensively branched substrate mycelia and abundant aerial mycelia. These strains produced aerial mycelia, containing straight to flexous (*Rectiflexibiles*) spore chains with smooth surface spores (Figure 4.13). The colors of the substrate mycelia ranged from yellow to brown and the colors of the aerial ranged from white to gray on agar media tested. They were observed to grow well on all agar media tested and some strains could produce soluble pigments (Appendix C, Table 15).

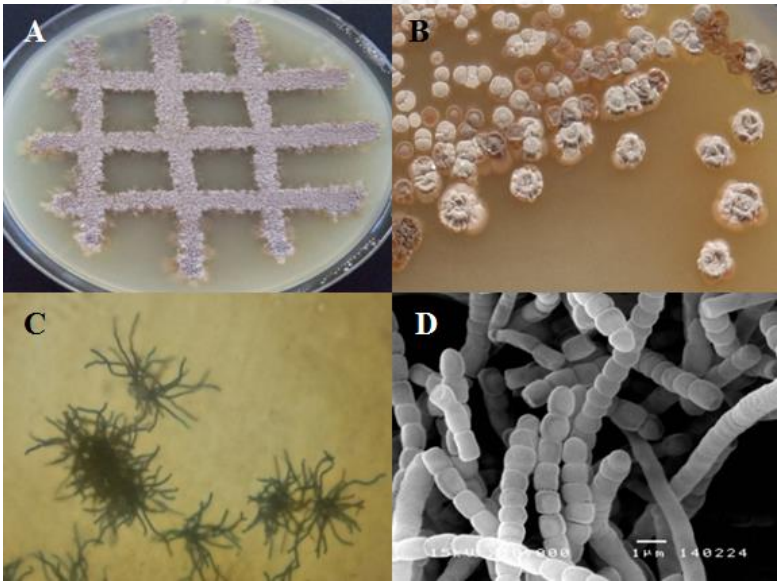


Figure 4.13 The morphological characters of strain KC-017 representing *Streptomyces* group X. A and B) the colonial appearance on ISP 3, C) light micrograph on ISP 2, and D) scanning electron micrograph on ISP2.

Most of the strains were determined to be negative for nitrate reduction, milk peptonization, milk coagulation and gelatinization and positive for starch hydrolysis. They were found be able to grow at pH 5-12, 10-37ºC. The presence of NaCl for growth ranged from 4-7% (w/v). Almost strains were found to use L-arabinose, D-fructose, D-glucose, D-mannitol, melibiose, *myo*-inositol, raffinose, L-rhamnose and D-xylose as carbon sources, but not melezitose, D-sorbitol and sucrose (Appendix C, Table 16).

Strain KC-017 was selected to be the representative strain in this group for 16S rRNA gene sequence analysis. The nearly complete 16S rRNA gene sequence of strain KC-155 was compared with sequences of known bacterial species in EzTaxon-server. Strain KC-017 had the highest sequence similarity (99.8%) to *S. aureus* NBRC 100912^{T} (Table 4.2). The phylogenetic tree based on 16S rRNA gene sequence showed that strain KC-155 was from a cluster with *S. aureus* (Figure 4.2). Consequently, strain KC-017 was identified as *S. aureus* (Manfio *et al*., 2003).

Group XI comprised of one strain, KC-035. Morphological observation of a 14-day culture of strain KC-035 grew well on almost test media, except ISP 6. This strain produced greenish white to yellowish white aerial mycelia on ISP 3, ISP 4, YS agar and nutrient agar. The aerial mycelia consisted of long spiral spore chains with smooth surface spores (Figure 4.14). The substrate mycelia colors ranged from strong yellow to light yellowish brown on the media tested. It produced pale yellow (on ISP 3, 4 and YS agar) and moderate yellow (on nutrient agar) soluble pigment on ISP 4. The cultural characteristics of *Streptomyces* strain in group XI are shown in Table 17 (Appendix C).

The strain reduced nitrate, hydrolyzed starch and gelatin but did not hydrolyze milk. It utilized D-fructose, D-glucose, D-mannitol, *myo*-inositol, raffinose, L-rhamnose and D-xylose as carbon sources but did not utilize L-arabinose, melezitose and D-sorbitol. Growth of strain KC-035 occurred at pH 5-12, at 15-37ºC with 0-6% (w/v) NaCl (Appendix C, Table 18).

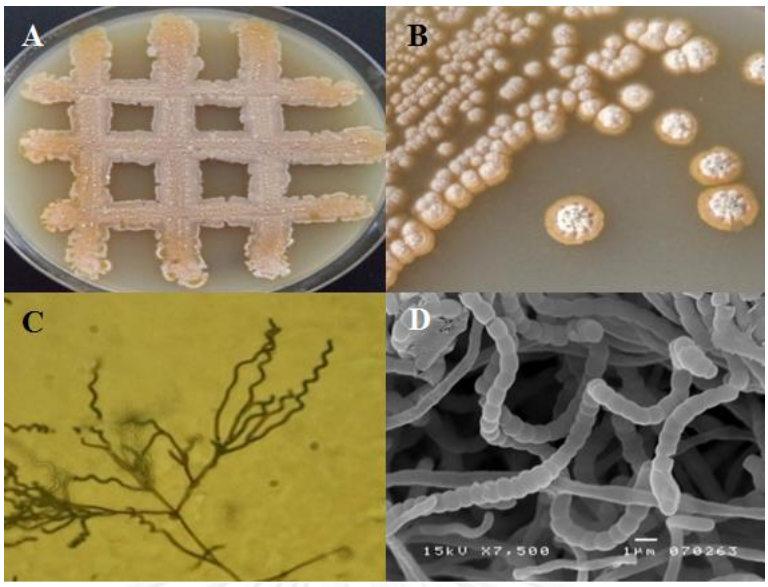


Figure 4.14 The morphological characters of strain KC-035 representing *Streptomyces* group XI. A and B) the colonial appearance on ISP 3, C) light micrograph on ISP 2, and D) scanning electron micrograph on ISP2.

The result of the 16S rRNA gene sequence analysis showed that strain KC-035 was affiliated to the genus *Streptomyces*. This strain showed the highest 16S rRNA gene sequence similarity to *S. siamensis*, KC-038^T (98.4%), *S. showdoensis* NBRC 13417^T (98.4%) and *S. hygroscopicus* subsp. *ossamyceticus* NBRC 13983^T (98.4%). Phylogenetic tree analysis using the almost complete 16S rRNA gene sequences showed that strain KC-035 was placed in a monophyletic cluster with *S. siamensis*, KC-038^T (Sripreechasak *et al*., 2013) (Figure 4.15).

Strain KC-035 exhibited typical characteristics of the genus *Streptomyces*. LL-diaminopimelic acid was detected in whole-cell hydrolysates. The menaquinones detected were MK-9(H_8) (56%), MK-9(H_6) (36%) and MK-9(H_4) (8%). The *N*-acyl type of muramic acid was acetyl. Strain KC-035 contained diphosphatidylglycerol (DPG), phosphatidylethanolamine (PE), phosphatidylglycerol (PG), phosphatidylinositol (PI), phosphatidylinositol mannoside (PIMs) and unknown phospholipids as phospholipid composition. The DNA G+C content was 72 mol%.

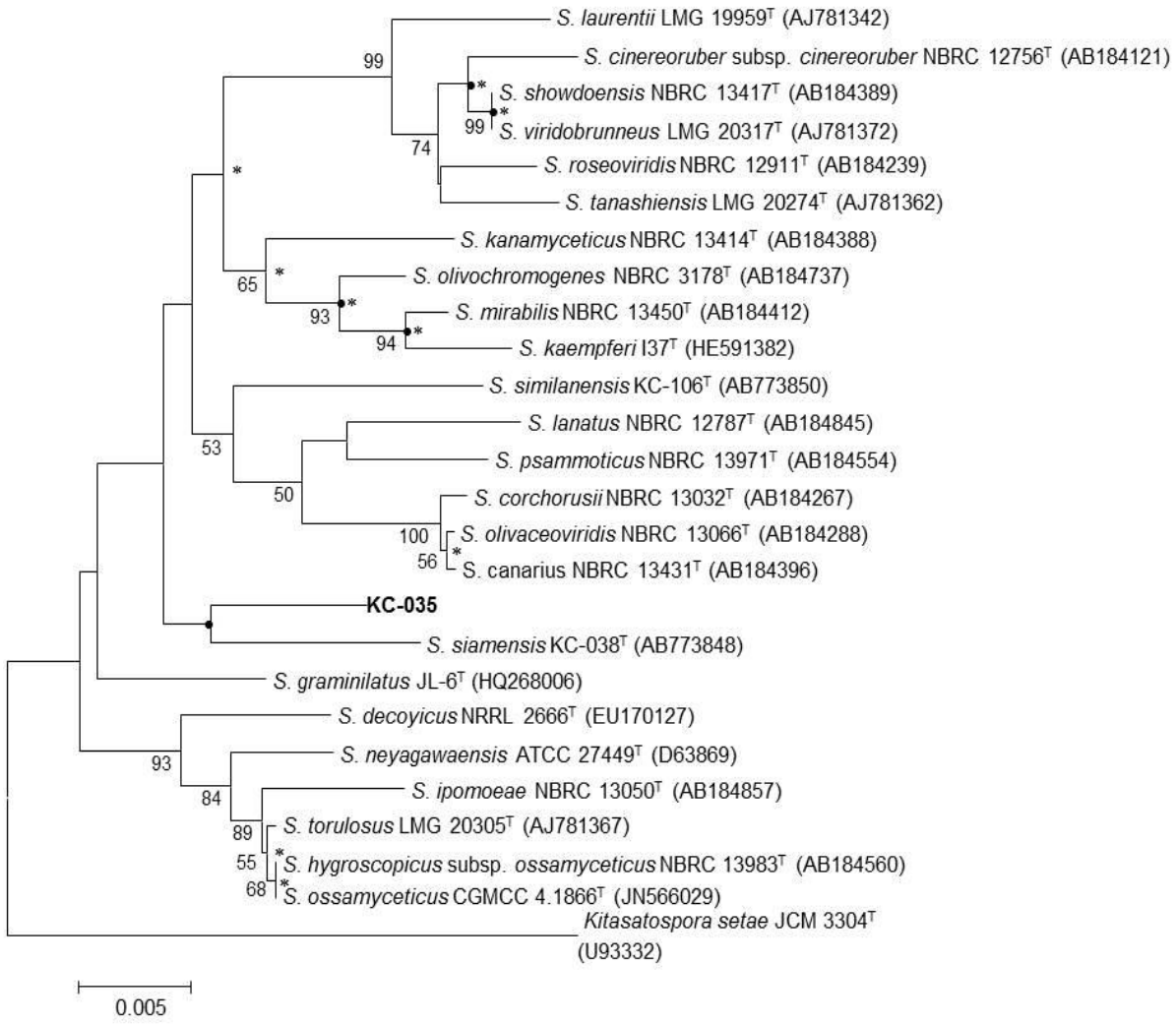


Figure 4.15 Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the relationship between strain KC-035 and closely related type strains of the genus Streptomyces. Only bootstrap values above 50% (percentages of 1000 replications) are indicated. (•), branches were also recovered in the maximum-parsimony tree; (*), branches were also recovered in the maximum-likelihood tree; Bar, 0.005 nucleotide substitutions per site.

The cultural characteristics of strain KC-035 and the type strains of the closest related species, *S. siamensis,* KC-038^{T}, *S. showdoensis* NBRC 13417^{T}, *S. psammoticus* NBRC 13971^{T} and *S. hygroscopicus* subsp. *ossamyceticus* NBRC 13983^{T} are shown in Table 4.6. Strain KC-035 could not grow on ISP 6 but the closest related species could grow well and formed extensively branched substrate mycelia. The phenotypic and differential characteristics of strain KC-035 and the closely relate type strains are listed in Table 4.7.

The DNA-DNA relatedness values between strain KC-035 and the closest type strains, *S. siamensis,* KC-038^{T}, *S. showdoensis* NBRC 13417^{T} and *S. hygroscopicus* subsp. *ossamyceticus* NBRC 13983^{T} were in the range of 9-47% (Table 4.8). These values were below the 70% cutoff point recommended by Wayne *et al*. (1987) for assigning strains to the same species, and these results thus confirm that strains KC-035 is distinct from their closely related phylogenetic neighbors. Therefore, strain KC-035 is clearly a novel species within the genus *Streptomyces* and named as *Streptomyces kungchingensis* sp. nov.

Table 4.6 Cultural characteristics of strain KC-035 and closely related type strains

Media	Strains			
	KC-035	1	2	3
ISP 2				
Growth	Good	Good	Good	Good
	Pale yellow	Pale yellow - olive gray	Dark yellow	Pale yellow
Reverse	Light yellow - moderate yellow	grayish greenish yellow	Dark yellow	Pale yellow
Aerial mycelium	None	None	Abundant, white	None
Soluble pigment	None	None	None	None
ISP 3				
Growth	Good	Good	Good	Good
	Strong yellow	Grayish yellow	Dark yellow	Greenish white
Reverse	Dark orange yellow	Grayish yellow	Dark yellow	Greenish white
Aerial mycelium	Abundant, yellowish white	Poor, white	Abundant, greenish white	Poor, white
Soluble pigment	Pale yellow	Pale yellowish green	Pale yellowish green	None
ISP 4				
Growth	Good	Good	Good	Good
	Strong yellow - deep yellow	Light grayish olive - grayish olive	Grayish greenish yellow	Dark yellowish pink - light grayish yellowish brown
Reverse	Strong yellow - deep yellow	Olive gray	Pale yellow	Light yellowish brown - dark grayish yellowish brown
Aerial mycelium	Abundant, yellowish white	None	Abundant, greenish white	Abundant, bluish gray - medium gray
Soluble pigment	Pale yellow	None	None	None
ISP 5				
Growth	Good	Good	Good	Good
	Greenish white - strong yellow	Pale yellowish green - light olive gray	Light yellow	Greenish white
Reverse	Greenish white - moderate yellow	Grayish greenish yellow - light grayish olive	Light yellow	Greenish white
Aerial mycelium	None	None	Abundant, greenish white	Poor, greenish white
Soluble pigment	None	Pale yellowish green	None	None
ISP 6				
Growth	None	Good	Good	Good
	None	Pale greenish yellow	Light grayish olive	Greenish gray
Reverse	None	Grayish greenish yellow	Light grayish olive	Dark brown
Aerial mycelium	None	None	None	Moderate, white
Soluble pigment	None	None	Grayish yellowish brown	Dark brown
ISP 7				
Growth	Good	Good	Good	Good
	Light yellowish brown - brownish black	Grayish greenish yellow - grayish olive	Light grayish olive	Grayish olive
Reverse	Light yellowish brown - brownish black	Light olive - dark olive	Light grayish olive	Dark olive brown
Aerial mycelium	None	None	Abundant, light greenish gray	None
Soluble pigment	None	Pale yellowish green	None	Light olive gray
YS agar				
Growth	Good	Good	Good	Good
	Greenish white - strong yellow	Grayish yellow - dark yellow	Dark grayish yellow	Pale yellowish green
Reverse	Pale yellow - deep yellow	Dark grayish yellow	Dark yellow	Pale yellowish green
Aerial mycelium	Poor, yellowish white	None	Abundant, greenish white	Moderate, greenish white
Soluble pigment	Pale yellow	Pale yellow	Pale yellow	Pale yellowish green
Nutrient agar				
Growth	Good	Good	Good	Good
	Light yellowish brown - moderate reddish brown	Pale yellowish green	Dark yellow	Light olive brown
Reverse	Light yellowish brown - moderate reddish brown	Pale yellowish green	Light olive brown	Dark yellow
Aerial mycelium	Abundant, greenish white	None	Abundant, greenish white - light greenish gray	Abundant, greenish white
Soluble pigment	Moderate yellow	None	Light olive brown	Light olive brown

Strains: 1, *S. siamensis*, KC-038^{T}; 2, *S. showdoensis* NBRC 13417^{T}; 3, *S. hygroscopicus* subsp. *ossamyceticus* NBRC 13983^{T}

Table 4.7 Different characteristics of strain KC-035 and closely related type strains

Characteristics	Strains			
	KC-035	1	2	3
Spore chain	Spiral	Spiral	Rectiflexibiles	Spiral
Spore surface	Smooth	Smooth	Smooth	Warty
Utilization of:				
L-Arabinose	-	+	-	+
D-Melibiose	±	-	-	-
Raffinose	+	±	-	+
Sucrose	±	±	+	+
D-Xylose	+	-	+	+
Nitrate reduction	+	+	+	+
Milk peptonization	-	+	+	-
Milk coagulation	-	-	-	-
Gelatinization	+	-	+	+
Growth at/with:	0-6	0-6	0-10	0-10
NaCl (%w/v)				
pH	5-12	5-12	5-12	5-12
Temperature (°C)	15-37	15-37	15-37	15-40

+ = positive, ± = weakly positive, - = negative

Strains: 1, *S. siamensis*, KC-038^T; 2, *S. showdoensis* NBRC 13417^T; 3, *S. hygroscopicus* subsp. *ossamyceticus* NBRC 13983^T

Table 4.8 DNA-DNA relatedness between strain KC-035 and closely related type strains

Strains	DNA-DNA relatedness with labeled strains (%)*			
	KC-035	1	4	3
KC-035	100	42	13	19
1	47	100	45	18
2	49	46	34	28
3	16	16	12	100

*Average of four independent determinations

Strains: 1, *S. siamensis*, KC-038^T; 2, S. *showdoensis* NBRC 13417^T; 3, *S. hygroscopicus* subsp. *ossamyceticus* NBRC 13983^T

Group XII consisted of two strains, including KC-106 and KC-140. These strains showed good growth on all agar media tested which formed an extensively branched substrate mycelia and abundant aerial mycelia. The aerial mycelia consisted of spiral spore chains with hairy surface spores (Figure 4.16). They formed white or brownish gray aerial mycelia and they formed pale yellowish brown to grayish yellowish brown substrate mycelia on agar media tested. They did not produce soluble pigments. (Appendix C, Table 17).

The strains KC-106 and KC-140 could grow at 15-40°C and pH 5-12 with 0-7% (w/v) NaCl. They were positive for nitrate reduction, starch hydrolysis and milk peptonization and negative for milk coagulation and gelatinization. These strains utilized L-arabinose, D-fructose, D-glucose, D-mannitol and D-xylose as carbon sources, but not melezitose, *myo*-inositol, L-rhamnose, D-sorbitol and sucrose (Appendix C, Table 18).

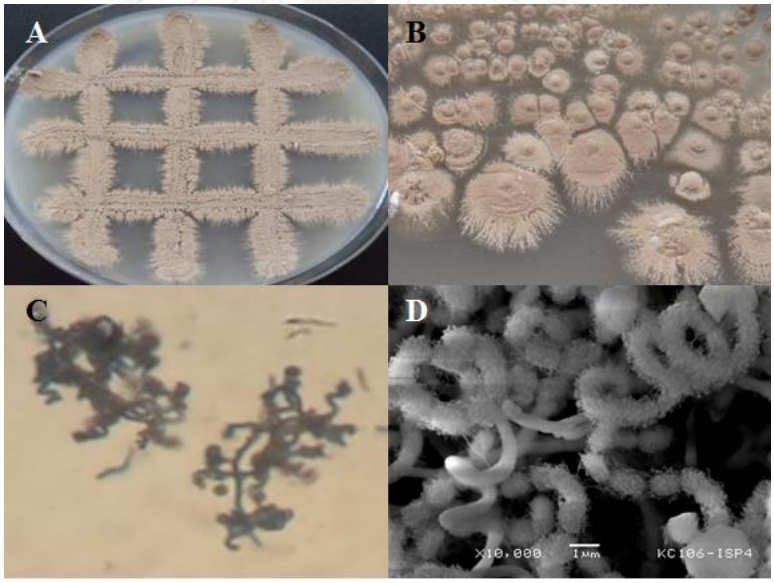


Figure 4.16 The morphological characters of strain KC-106 representing *Streptomyces* group XII. A and B) the colonial appearance on ISP 3, C) light micrograph on ISP 2, and D) scanning electron micrograph on ISP4.

The almost complete 16S rRNA gene sequence of strain 106 was compared with sequences in the EzTaxon-e database. Strain KC-106 showed the highest 16S rRNA gene sequence similarity (98.9%) to *S. seoulensis* NBRC 16668^{T} and it was

clustered with *S. seoulensis* in phylogenetic tree (Fig 4.2). Therefore, this strain was a novel species and was selected to complete for taxonomic characterization.

Strain KC-106 exhibited typical characteristics of the genus *Streptomyces*. LL-diaminopimelic acid was detected in whole-cell hydrolysates. The menaquinones detected were MK-9(H_8) (70%), MK-9(H_6) (21%) and MK-9(H_4) (10%). The *N*-acyl type of muramic acid was acetyl. Strain KC-106 contained diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositol and unknown phospholipids as phospholipid composition. Strain KC-106 contained anteiso-$C_{15:0}$ (25.0%), iso-$C_{16:0}$ (23.2%) and anteiso-$C_{17:0}$ (10.3%) as major cellular fatty acids (Table 4.9). The cellular fatty acid profiles of strain KC-106 were almost the same as those of the type strains. The DNA G+C content was 72 mol%.

The 16S rRNA gene sequence of strain KC-106 was most similar to those of *S. seoulensis* NBRC 16668^T (98.9%), *S. recifensis* NBRC 12813^T (98.9%), *S. chartreusis* NBRC 12753^T (98.7%) and *S. griseoluteus* NBRC 13375^T (98.4%). The phylogenetic tree showed that strain KC-106^T forms a cluster with three of the above species, with the exception of *S. chartreusis* NBRC 12753^T (Figure 4.17).

The cultural characteristics of strain KC-106 and the type strains of the closest related species, *S. seoulensis* NBRC 16668^T (Chun *et al*., 1997), *S. recifensis* NBRC 12813^T, *S. chartreusis* NBRC 12753^T (Calhoun *et al*., 1956), and *S. griseoluteus* NBRC 13375^T, are shown in Table 4.10. Strain KC-106 grew well and formed extensively branched substrate and aerial mycelia on all agar media tested. Aerial mycelia of white to brownish gray color were produced. The aerial mycelia consisted of long and spiral spore chains with hairy surface and oval-shaped spores (Figure 4.16), which is clearly different from the smooth surface spores produced by closely related type strains. The phenotypic and different characteristics of strain KC-106 are listed in Table 4.11. Strain KC-106 was also differentiated from its closely related type strains with respect to carbon utilization.

The DNA-DNA relatedness values between strain KC-106 and the closest type strains, *S. seoulensis* NBRC 16668^T, *S. recifensis* NBRC 12813^T, *S. chartreusis* NBRC 12753^T and *S. griseoluteus* NBRC 13375^T, were in the range of 7-46% (Table 4.12). These values were below the 70% cutoff point recommended by Wayne *et al*. (1987) for assigning strains to the same species, and these results thus confirm that strains KC-106 is distinct from their closely related phylogenetic neighbors. Therefore, strain KC-106 is clearly a novel species within the genus *Streptomyces* and named as *Streptomyces similanensis* sp. nov.

Table 4.9 Cellular fatty acid compositions (%) of strain KC-106 and closely related type strains

Fatty acids	Strains				
	KC-106	*S. seoulensis* NBRC 16668^T	*S. recifensis* NBRC 12813^T	*S. griseoluteus* NBRC 13375^T	*S. chartreusis* NBRC 12753^T
Saturated straight chain					
$C_{14:0}$	0.6	0.8	-	0.8	2.0
$C_{16:0}$	5.4	4.4	5.7	8.5	9.7
$C_{18:0}$	1.1	2.1	0.6	-	-
$C_{17:0}$ cyclo	2.0	2.4	3.3	1.8	0.4
Unsaturated straight chain					
$C_{18:1}$ $\omega 9c$	-	2.2	-	-	-
Saturated branched chain					
iso-$C_{14:0}$	5.8	10.2	6.7	4.2	7.3
iso-$C_{15:0}$	8.6	8.9	10.6	9.3	11.6
iso-$C_{16:0}$	23.2	20.1	19.3	17.9	19.8
iso-$C_{17:0}$	3.3	1.1	3.6	2.8	2.4
iso-$C_{18:0}$	1.5	1.0	1.5	1.1	-
anteiso-$C_{13:0}$	ND	-	-	0.6	1.3
anteiso-$C_{15:0}$	25.0	26.2	29.3	29.9	2.8
anteiso-$C_{17:0}$	10.3	4.2	8.0	13.6	5.8
Unsaturated branched chain					
iso-$C_{16:1}$ H	4.4	5.9	2.2	1.3	1.6
iso-$C_{17:1}$ $\omega 9c$	1.6	1.7	2.2	1.7	1.4
anteiso-$C_{17:1}$ $\omega 9c$	4.1	4.3	3.2	2.6	1.2
Summed feature[a] 3	0.9	2.0	1.4	2.2	6.9

- = the amount of fatty acid less than 0.5% was omitted, ND = not detected

[a]Summed feature 3 comprises $C_{16:1}$ $\omega 7c$ and/or $C_{16:1}$ $\omega 6c$.

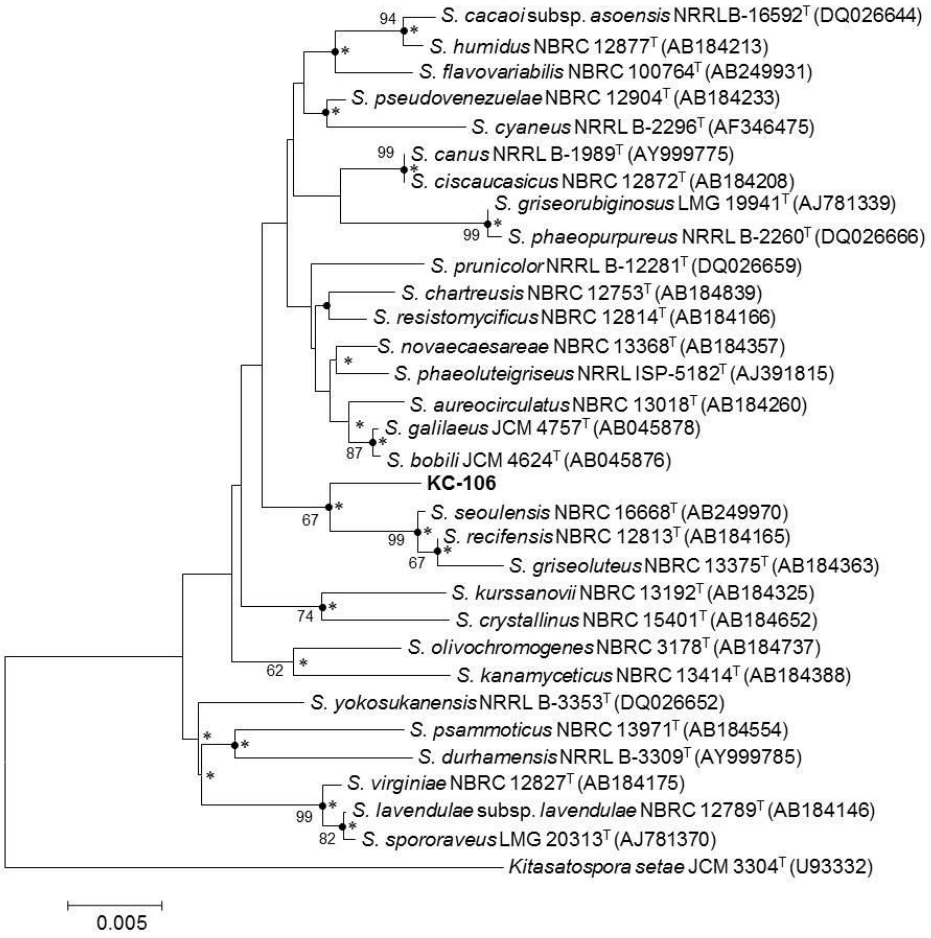


Figure 4.17 Neighbor-joining tree based on 16S rRNA gene sequences showing relationship between KC-106 and closely related type strains of the genus *Streptomyces* Only bootstrap values above 50% (percentages of 1000 replications) are indicated. (•), branches were also recovered in the maximum-parsimony tree; (*), branches were also recovered in the maximum-likelihood tree; Bar, 0.005 nucleotide substitutions per site.

Table 4.10 Cultural characteristics of strain KC-106 and closely related type strains

Media	Strains				
	KC-106^T	*S. seoulensis* NBRC 16668^T	*S. recifensis* NBRC 12813^T	*S. griseoluteus* NBRC 13375^T	*S. chartreusis* NBRC 12753^T
ISP 2					
Growth	Good	Good	Good	Good	Good
	Pale yellow olive	Colorless	Colorless	Grayish yellowish brown	Yellowish gray
Reverse	Pale yellowish brown - grayish yellowish brown	Pale yellowish brown	Pale yellowish brown - brownish gray	Pale yellowish brown - grayish yellow brown	Pale yellowish brown - light olive gray
Aerial mycelium	Abundance, white - brownish gray	Abundant, white - ashes	Abundant, white - lead gray	Abundant, white – light gray	Abundant, white - light grayish blue (19fe)
Soluble pigment	None	None	None	None	None
ISP 3					
Growth	Good	Good	Good	Good	Good
	Grayish yellow brown	Light brownish gray	Colorless	Light brownish gray	Yellowish gray -pale yellowish brown
Reverse	Light brownish gray	Pale grayish yellow - light brownish gray	Brownish white - light brownish gray	Pale grayish yellow - brownish gray	Yellowish gray - grayish yellow brown
Aerial mycelium	Abundant, brownish gray	Abundant, ashes	Abundant, white - brownish gray	Abundant, white to medium gray	Abundant, white - pale blue
Soluble pigment	None	None	None	None	None
ISP 4					
Growth	Good	Good	Good	Good	Good
	Grayish yellow brown	Greenish gray	Yellowish gray - pale grayish yellow	Light brownish gray - to grayish yellow brown	Pale grayish yellow
Reverse	Yellowish gray - grayish yellow brown	Pale grayish yellow - brownish gray	Pale grayish yellow	Light brownish gray	Pale grayish yellow
Aerial mycelium	Abundant, white - brownish gray	Abundant, white to light gray	Abundant, white	Abundant, white	Abundant, white - light gray
Soluble pigment	None	None	None	None	None
ISP 5					
Growth	Good	Good	Good	Good	Good
	Brownish white - pale yellowish brown	Brownish white	Greenish gray	Brownish white	White - grayish yellow
Reverse	Brownish white - light brownish gray	Brownish white brownish gray	Light brownish gray	Brownish white - medium gray	White - yellowish gray
Aerial mycelium	Abundant, white - brownish gray	Abundant, white	Abundant, light brownish gray - brownish gray	Abundant, white - lead gray	Moderate, white
Soluble pigment	None	None	None	None	None
ISP 6					
Growth	Good	Good	Good	Good	Good
	Yellowish gray	Pale yellow	Colorless	Pale yellow	Yellowish gray
Reverse	Pale yellow	Pale yellow	Yellowish gray	Pale yellow	Yellowish gray
Aerial mycelium	Abundant, white	Abundant, white	Abundance, white	Abundance, white	None
Soluble pigment	None	None	None	None	None
ISP 7					
Growth	Good	Good	Good	Good	Good
	Pale yellow - pale yellowish brown	Brownish white	Brownish white	Brownish white	Yellowish brown - dark yellowish brown
Reverse	Light brownish gray - pale yellowish brown	Brownish white to light brownish gray	Light brownish gray	Brownish white - brownish gray	Pale yellowish brown - brownish gray
Aerial mycelium	Abundance, white - brownish gray	Abundant, light brownish gray - brownish gray	Abundant, light brownish gray	Abundant, white - brownish gray	Abundant, light brownish gray - pale blue
Soluble pigment	None	None	None	None	None
YS agar					
Growth	Good	Good	Good	Good	Good
	Pale yellowish brown	Light brownish gray	Colorless	Pale grayish yellow	Brownish white - yellowish gray
Reverse	Grayish yellow brown	Light brownish gray - dark brown	Pale grayish yellow - dark brown	Light brownish gray - dark brown	Light brownish gray
Aerial mycelium	Abundance, white - brownish gray	Abundant, white - lead gray	Abundant, white - ashes	Abundant, white - lead gray	Abundant, white - light grayish blue
Soluble pigment	None	None	None	None	None
Nutrient agar					
Growth	Good	Good	Good	Good	Good
	Pale grayish yellow - light brownish gray	Brownish white	Brownish white	Brownish white	Pale yellow
Reverse	Pale yellowish brown - grayish yellow brown	Yellowish gray	Yellowish gray to light brownish gray	Brownish white	Pale yellow
Aerial mycelium	Abundance, white - brownish gray	Abundant, white	Abundant, white - brownish gray	Abundant, white	Poor, white
Soluble pigment	None	None	None	None	None

Table 4.11 Different characteristics of strain KC-106 and closely related type strains

Characteristics	Strains				
	KC-106^T	*S. seoulensis* NBRC 16668^T	*S. recifensis* NBRC 12813^T	*S. griseoluteus* NBRC 13375^T	*S. chartreusis* NBRC 12753^T
Spore chain	Spiral	Ractiflexibiles	Retinaculiaperti	Ractiflexibiles	Spiral
Spore surface	Hairy	Smooth	Smooth	Smooth	Spiny
Utilization of					
Raffinose	±	+	±	±	+
Melibiose	±	+	-	±	+
L-Rhamnose	-	+	-	-	+
myo-Inositol	±	-	-	-	+
Sucrose	-	-	-	-	-
Nitrate reduction	-	-	-	-	-
Gelatin liquefaction	-	+	+	±	+
NaCl tolerance (%)	7	8	6	5	6
Enzyme activity of					
Alkaline phosphatase	+	+	+	-	+
Esterase C4	+	+	+	±	+
Esterase lipase C8	-	+	±	-	±
Valine arylamidase	+	±	+	+	±
Cystine arylamidase	+	±	+	+	±
Trypsin	-	-	±	-	+
α-Chymotrypsin	-	-	-	-	+
Naphthol-AS-BI-phosphohydrase	+	+	+	±	+
ß-Galactosidase	-	-	+	-	+
α-Glucosidase	+	±	+	-	
ß-Glucosidase	+	-	-	-	+
N-acetyl-ß-glucosaminidase	+	+	+	±	+
α-Mannosidase	+	-	-	-	±

+ = positive, ± = weakly positive, - = negative

Table 4.12 DNA-DNA relatedness between KC-106 and closely related type strains

Strain	DNA-DNA relatedness with labeled strains (%)*				
	KC-106	*S. seoulensis* NBRC 16668^T	*S. recifensis* NBRC 12813^T	*S. griseoluteus* NBRC 13375^T	*S. chartreusis* NBRC 12753^T
KC-106	100	30	28	19	18
S. seoulensis NBRC 16668^T	46	100	67	61	14
S. recifensis NBRC 12813^T	39	81	100	71	21
S. griseoluteus NBRC 13375^T	38	92	96	100	21
S. chartreusis NBRC 12753^T	7	24	20	24	100

*Average of four independent determinations

Group XIII consisted of four strains, including KC-119, KC-124, KC-125 and KC-134. They showed good growth on all agar media tested. From light and scanning electron microscopic observations of these strain, they had an extensively branched substrate mycelia and abundant mycelia that carried spiny-surfaced spores with oval shape in spiral spore chains (Figure 4.18). The results of morphological characteristics indicated that the strains belong to the genus *Streptomyces*. The strains formed yellowish white aerial mycelia and pale yellow, pale yellowish pink to yellowish brown substrate mycelia on agar media tested. They could produce soluble pigments. The cultural characteristics of these strains in group XIII are shown in Table 19 (Appendix C).

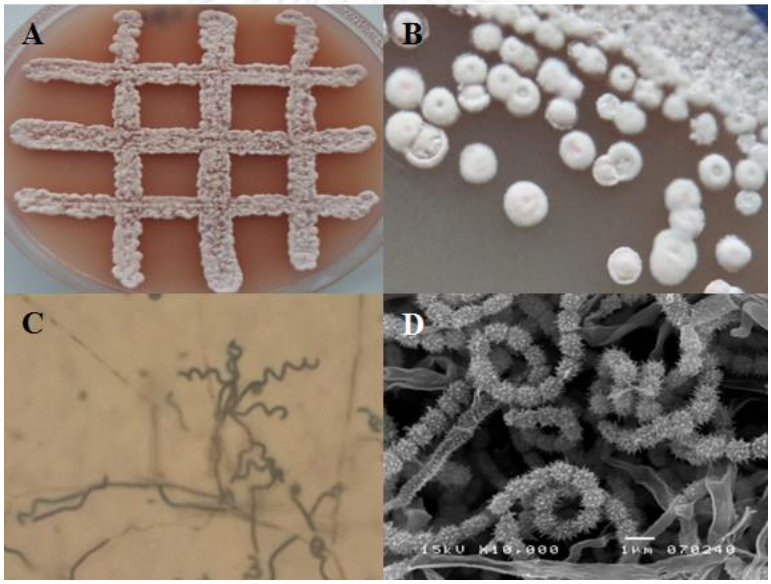


Figure 4.18 The morphological characters of strain KC-134 representing *Streptomyces* group XIII. A and B) the colonial appearance on ISP 3, C) light micrograph on ISP 2, and D) scanning electron micrograph on ISP2.

These strains could grow at 15-37ºC and pH 5-12. They were observed to grow in the presence of 0-8% (w/v) NaCl. Starch hydrolysis, milk peptonization and milk coagulation were positive, but nitrate reduction and gelatinization were negative. In addition, they utilized L-arabinose, D-fructose, D-glucose, D-mannitol, melibiose, *myo*-inositol, raffinose, L-rhamnose, D-sorbitol, sucrose and D-xylose as carbon sources, but not melezitose. The detailed physiological and biochemical characteristics of these strain are given in Table 20 (Appendix C).

Strain KC-134 was selected to be the representative strain in this group for 16S rRNA gene sequence analysis. The almost complete 16S rRNA gene sequence of strain KC-134 was compared with sequences in the EzTaxon-e database. Strain KC-134 showed the highest 16S rRNA gene sequence similarity (99.6%) to *S. violarus* NBRC 13104^{T} and it was clustered with *S. violarus* in phylogenetic tree (Figure 4.2). Therefore, strain KC-134 was identified as *S. violarus*.

Group XIV consisted of six strains, including strains KC-031, KC-032, KC-033, KC-038, KC-150 and KC-156. These strains grew well and produced white to gray aerial mycelia on various agar media tested. The aerial mycelia consisted of long spiral spore chains with smooth surface spores (Figure 4.19). The cultural characteristics of *Streptomyces* strains in group XIV are shown in Table 21 (Appendix C). They grew at 15-40°C and pH 5-12 and tolerated up to 6-8% (w/v) NaCl. All strains were positive for nitrate reduction, starch hydrolysis and milk peptonization but negative for milk coagulation and gelatinization. They utilized L-arabinose, D-fructose, D-glucose, D-mannitol, melibiose, *myo*-Inositol, L-rhamnose and D-xylose as carbon source but did not utilize melezitose, D-sorbitol and sucrose (Appendix C, Table 22).

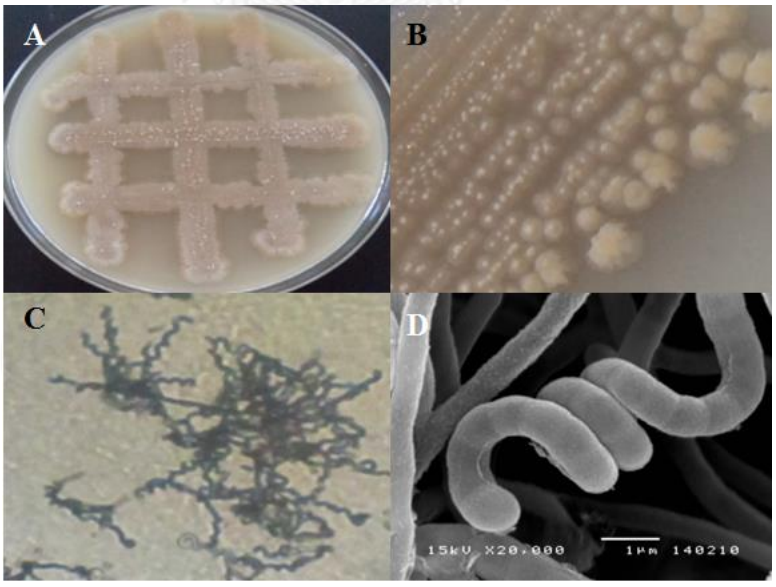


Figure 4.19 The morphological characters of strain KC-038 representing *Streptomyces* group XIV. A and B) the colonial appearance on ISP 3, C) light micrograph on ISP 4, and D) scanning electron micrograph on ISP4.

Strains KC-031 and KC-038 were selected to be the representative strains in this group for 16S rRNA gene sequence analysis. The nearly complete 16S rRNA gene sequences of strains KC-038 and KC-031 were compared with sequences of known bacterial species in EzTaxon-server. Strains KC-031 and KC-038 showed the highest sequence similarity (98.1%) to *S. olivochromogenes* NBRC 3178^T (AB184737) (Table 4.2). Therefore, theses strains were selected to complete for taxonomic characterization.

Strains KC-038 and KC-031 exhibited typical characteristics of the genus *Streptomyces*. LL-diaminopimelic acid was detected in whole-cell hydrolysates. The menaquinones detected were as follows: MK-9(H_6) (62%), MK-9(H_4) (23%) and MK-9(H_8) (15%) for KC-038^T. The *N*-acyl type of muramic acid was acetyl. Strains KC-038 and KC-031 contained diphosphatidylglycerol (DPG), phosphatidylethanolamine (PE), phosphatidylglycerol (PG), phosphatidylinositol (PI), phosphatidylmethylinosides (PIMs), and unknown phospholipids as phospholipid composition Strains KC-038 and KC-031 contained $C_{16:0}$ (23.5, 19.7%), iso-$C_{16:0}$ (18.4, 22.3%) and anteiso-$C_{15:0}$ (17.7, 16.6%) as major cellular fatty acids. The cellular fatty acid profiles of strains KC-038 and KC-031 were almost the same as those of the type strains, but the amount of some fatty acids was different, as shown in Tables 4.13. The DNA G+C content was 72 mol% for strains KC-038 and KC-031.

The 16S rRNA gene sequence similarity value between strains KC-038 and KC-031 was 99.9%, and they showed the highest sequence similarities to *S. olivochromogenes* NBRC 3178^T (98.1%) and *S. psammoticus* NBRC 13971^T (98.1%) and clustered with them (Figure 4.20).

The cultural characteristics of strains KC-038 and KC-031, along with those of the type strains of the closest related species, *S. olivochromogenes* NBRC 3178^T and *S. psammoticus* NBRC 13971^T are shown in Table 4.14. Strains KC-038 and KC-031 grew well and formed extensively branched substrate mycelia on the various agar media tested. Aerial mycelia of white to gray color were produced on ISP 2-5 and 7, YS agar and nutrient agar, while the related type strains produced white aerial mycelia. The aerial mycelia consisted of long spiral chains with smooth surface spores and the spores were rod shaped (Figures 4.19). Soluble pigment was produced on ISP 6. The phenotypic and differential characteristics of strains KC-038 and KC-031 are listed in Table 4.15. Strains KC-038 and KC-031 were highly similar to each other but were differentiated from the closest related type strains with respect to carbon utilization.

The DNA-DNA relatedness value between strains KC-038 and KC-031 was 100%; therefore, these two strains were classified as the same species. The DNA-DNA relatedness values between strain KC-038 and the closest related type strains, *S. olivochromogenes* NBRC 3178^T and *S. psammoticus* NBRC 13971^T, were in the range of 4–36% (Table 4.16). These values were below the 70% cutoff point recommended by Wayne *et al*. (1987) for assigning strains to the same species, and these results thus confirm that strains KC-038 is distinct from their closely related phylogenetic neighbors. Therefore, strains KC-038 is clearly a novel species within the genus *Streptomyces* and named *Streptomyces siamensis* sp. nov.

Table 4.13 Cellular fatty acid compositions (%) of strains KC-038, KC-031 and closely related type strains

Fatty acid	KC-038	KC-031	*S. olivochromogenes* NBRC 3178^T	*S. psammoticus* NBRC 13971^T
Saturated straight chain				
$C_{14:0}$	4.0	3.0	0.7	3.2
$C_{16:0}$	23.5	19.7	7.6	23.9
$C_{17:0}$	1.2	1.0	0.5	1.0
$C_{18:0}$	-	-	-	0.5
$C_{17:0}$ cyclo	0.5	0.7	2.9	3.7
Unsaturated straight chain				
$C_{17:1}$ $\omega 8c$	0.6	0.6	-	-
Saturated branched chain				
iso-$C_{14:0}$	8.6	8.5	8.0	2.0
iso-$C_{15:0}$	7.7	8.9	13.0	10.8
iso-$C_{16:0}$	18.4	22.3	22.1	10.5
iso-$C_{17:0}$	1.7	2.7	4.9	2.9
iso-$C_{18:0}$	-	-	0.9	0.6
anteiso-$C_{13:0}$	0.5	-	-	-
anteiso-$C_{15:0}$	17.7	16.6	25.2	22.6
anteiso-$C_{17:0}$	4.2	4.6	6.7	9.8
Unsaturated branched chain				
iso-$C_{16:1}$ H	0.6	0.9	1.0	-
iso-$C_{17:1}$ $\omega 9c$	0.6	1.0	1.9	0.5
anteiso-$C_{17:1}$ $\omega 9c$	0.5	0.6	1.2	0.7
Summed feature[a] 3	6.0	5.5	1.2	4.4

Abbreviation: -, the amount of fatty acid less than 0.5% was omitted. ND, not detected.

[a]Summed feature 3 comprises $C_{16:1}$ $\omega 7c$ and/or $C_{16:1}$ $\omega 6c$.

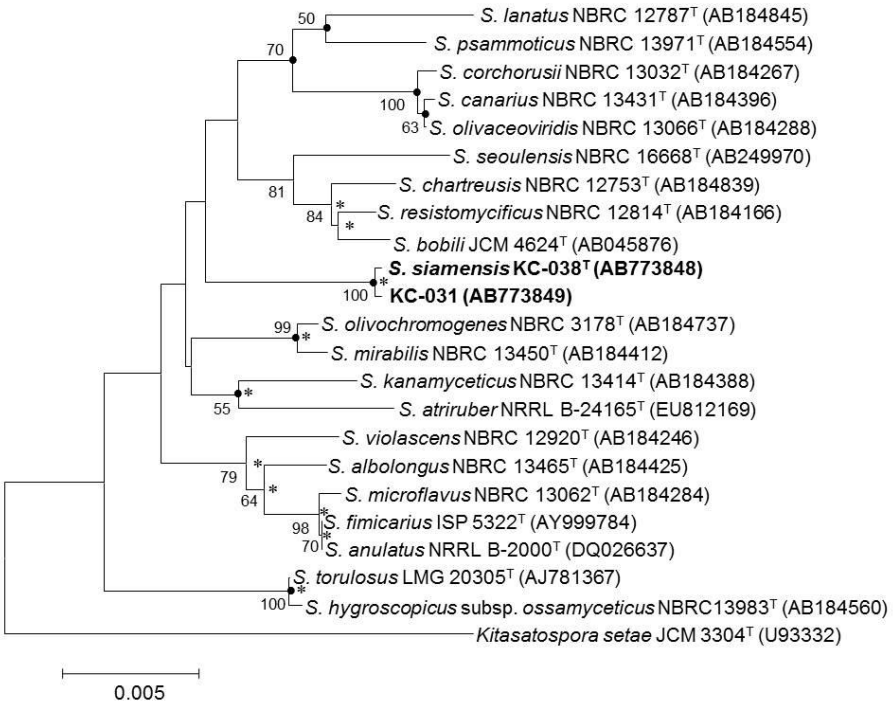


Figure 4.20 Neighbor-joining tree based on 16S rRNA gene sequences showing relationship between KC-038, KC-031 and closely related type strains of the genus Streptomyces. Only bootstrap values above 50% (percentages of 1000 replications) are indicated. (•), branch also recovered in the maximum-parsimony tree; (*), branch also recovered in the maximum-likelihood tree; Bar, 0.005 nucleotide substitutions per site.

Table 4.14 Cultural characteristics of strain KC-038, KC-031 and closely related type strains

Media	Strains			
	KC-038	KC-031	*S. olivochromogenes* NBRC 3178^T	*S. psammoticus* NBRC 13971^T
ISP 2				
Growth	Good	Good	Good	Good
	Pale yellowish brown	Pale yellowish brown	Pale yellowish brown	Pale yellowish brown
Reverse	Grayish olive - brownish gray	Grayish yellow brown	Pale yellowish brown	Pale yellowish brown
Aerial mycelium	Abundant, white -brownish gray	Moderate, white	Poor, white	Moderate, white (a)
Soluble pigment	None	None	None	None
ISP 3				
Growth	Good	Good	Good	Good
	Light yellow - light olive	Grayish yellowish brown	Yellowish gray - pale yellowish brown	Yellowish brown
Reverse	Pale greenish yellow - grayish yellowish brown	Yellowish gray - pale yellowish brown	Yellowish gray - pale yellowish brown	Grayish yellowish brown
Aerial mycelium	Abundant, brownish gray	Moderate, white	Moderate, white	Abundant, white - brownish white
Soluble pigment	None	None	None	None
ISP 4				
Growth	Good	Good	Good	Good
	light olive	Grayish yellowish brown - grayish yellowish brown	Pale yellowish brown	Grayish yellowish brown
Reverse	Grayish yellowish brown - yellowish brown	Dark yellowish brown	Pale yellowish brown	Pale yellowish brown
Aerial mycelium	Abundant, light brownish gray brownish gray	Abundant, brownish gray	None	Moderate, white
Soluble pigment	None	None	none	None
ISP 5				
Growth	Good	Good	Good	Good
	Light brownish gray - light olive	Brownish white	White	Brownish white
Reverse	Light brownish gray - grayish yellowish brown	Light brownish gray	White	Brownish white
Aerial mycelium	Abundant, light brownish gray	Abundant, brownish white	Poor, white	Abundant, white
Soluble pigment	None	None	None	None
ISP 6				
Growth	Good	Good	Good	Good
	Dark brownish gray	Grayish yellowish brown	Pale yellowish brown	Pale yellowish brown
Reverse	Dark brownish gray	Grayish yellowish brown	Pale yellowish brown	Pale yellowish brown
Aerial mycelium	None	None	None	None
Soluble pigment	Dark brown	Yellowish brown	None	Reddish yellow
ISP 7				
Growth	Good	Good	Good	Good
	Light brownish gray - pale yellow olive	Light yellowish brown	white	Brownish white
Reverse	Light brownish gray - light olive	Light brownish gray	white	Brownish white
Aerial mycelium	Abundant, light brownish gray	Abundant, light brownish white	Moderate, white	Abundance, white
Soluble pigment	None	None	None	None
YS agar				
Growth	Good	Good	Good	Good
	Light brownish gray - pale olive	Pale yellowish brown	Pale yellow	Grayish yellowish brown
Reverse	Pale yellowish brown - grayish yellowish brown	Pale yellowish brown	Pale yellow	Pale yellowish brown -grayish yellowish brown
Aerial mycelium	Abundance, white -brownish gray	Moderate, brownish gray	None	Abundant, white
Soluble pigment	None	None	None	None
Nutrient agar				
Growth	Good	Good	Good	Good
	Pale yellow	Pale yellow	Pale yellow - Pale yellowish brown	Pale yellow
Reverse	Pale yellow - yellowish gray	Pale yellow	Pale yellow - Pale yellowish brown	Pale yellow
Aerial mycelium	Poor, white	None	None	Poor, white
Soluble pigment	None	None	None	None

Table 4.15 Different characteristics of strain KC-038T, KC-031 and closely related type strains

Characteristics	KC-038^T	KC-031	*S. olivochromogenes* NBRC 3178^T	*S. psammoticus* NBRC 13971^T
Spore chain	Spiral	Spiral	Spiral	Ractiflexibiles
Spore surface	Smooth	Smooth	Smooth	Smooth
Utilization of				
L-Arabinose	+	+	+	-
D-Xylose	+	+	+	-
Raffinose	+	+	-	-
Melibiose	+	+	+	-
D-Mannitol	+	+	+	-
L-Rhamnose	+	+	+	-
myo-Inositol	+	+	+	-
Sucrose	-	-	+	+
Gelatin liquefaction	-	-	±	-
NaCl tolerance (%)	6	6	6	3
Enzyme activity of				
Esterase lipase C8	-	±	±	±
Cystine arylamidase	±	±	±	-
Trypsin	±	+	-	-
α-Galactosidase	-	-	±	-
β-Galactosidase	±	+	+	+
α-Glucosidase	-	±	+	+
β-Glucosidase	+	+	-	-
N-Acetyl-β-glucosaminidase	+	+	±	-
α-Mannosidase	±	±	-	+

+ = positive, ± = weakly positive, - = negative

Table 4.16 DNA-DNA relatedness between strains KC-038, KC-031 and closely related type strains

Strains	DNA-DNA relatedness with labeled strains (%)*			
	KC-038^T	KC-031	*S. olivochromogenes* NBRC 3178^T	*S. psammoticus* NBRC 13971^T
KC-038^T	100	100	36	13
KC-031	100	100	11	3
S. olivochromogenes NBRC 3178^T	17	21	100	4
S. psammoticus NBRC 13971^T	4	9	2	100

*Average of four independent determinations

Group XV consisted of four strains, including KC-085, KC-98, KC-101 and KC-103. These strains were observed to form extensively branched substrate mycelia and abundant aerial mycelia on all media tested. The aerial mycelia consisted of long straight to flexuous (*Rectiflexibiles*) spore chains with smooth surface spores (Figure 4.21). They produced white, light greenish gray to bluish gray aerial mycelia on various agar media tested. The colors of substrate mycelia ranged from pale yellow, pale yellowish green to dark olive brown. All strains were produced soluble pigment on ISP 2, ISP 4, YS agar and nutrient agar. The cultural characteristics of these strains are shown in Table 23 (Appendix C).

They grew at 15-37ºC, pH 5-12 and tolerated up to 7-8% (w/v) NaCl. All strains were determined to be negative for gelatinization and positive for nitrate reduction, starch hydrolysis and milk peptonization. Almost strains utilized L-arabinose, D-glucose, D-melibiose, raffinose, L-rhamnose and xylose as carbon sources but did not utilize D-mannitol, melezitose, D-sorbitol and sucrose. The biochemical and physiological characteristics of strains in group XV are presented in the Table 24 (Appendix C).

Strain KC-101 was selected to be the representative strain in this group for 16S rRNA gene sequence analysis. The almost complete 16S rRNA gene sequence of strain KC-101 was compared with sequences of known bacterial species in EzTaxon-server. The results of these comparisons revealed that this strain had the highest

sequence similarity to *S. drozdowiczii* NBRC 101007^{T} (99.7%) (Table 4.2). Therefore, strain KC-101 was identified as *S. drozdowiczii* (Semedo *et al*. 2004).

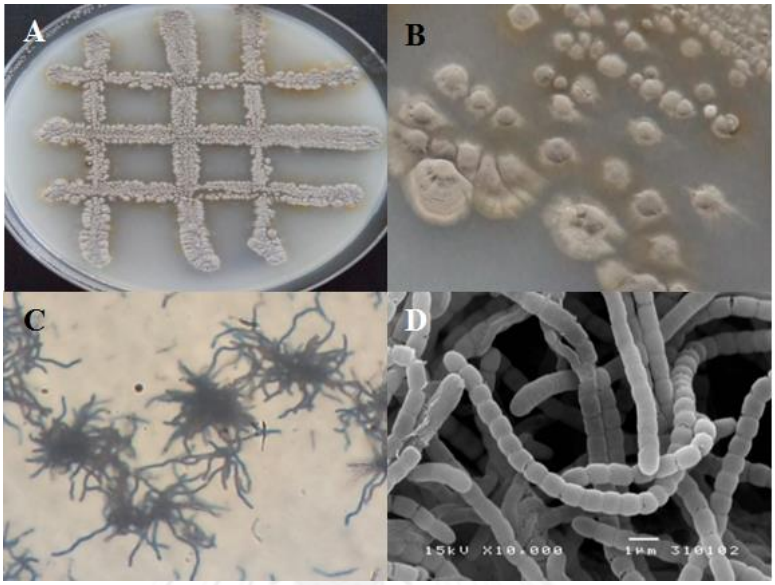


Figure 4.21 The morphological characters of strain KC-101 representing *Streptomyces* group XV. A and B) the colonial appearance on ISP 3, C) light micrograph on ISP 2, and D) scanning electron micrograph on ISP 2.

Group XVI consisted of two strains, KC-034 and KC-036 which formed an extensively branched substrate mycelium and white, bluish gray to medium gray aerial mycelia. The aerial meclia carried smooth-surfaced spores in a straight to flexuous (*Rectiflexibiles*) arrangement (Figure 4.22). They showed good growth on all agar media tested, with dark grayish yellow, grayish greenish yellow to grayish olive substrate mycelium. They produce grayish greenish yellow soluble pigments on ISP 2 and nutrient agar (Appendix C, Table 25).

Temperature, pH and NaCl concentration for growth were 15-37ºC, pH 5-12 and 0-4% (w/v) NaCl, respectively. Nitrate reduction and starch hydrolysis were positive and milk peptonization was weakly positive, but milk coagulation and gelatinization were negative. They utilized only D-glucose as carbon sources but did not utilize L-arabinose, D-fructose, D-mannitol, melezitose, D-melibiose, *myo*-inositol, raffinose, L-rhamnose, D-sorbitol, sucrose and D-xylose. The physiological and biochemical characteristics are listed in Table 26 (Appendix C).

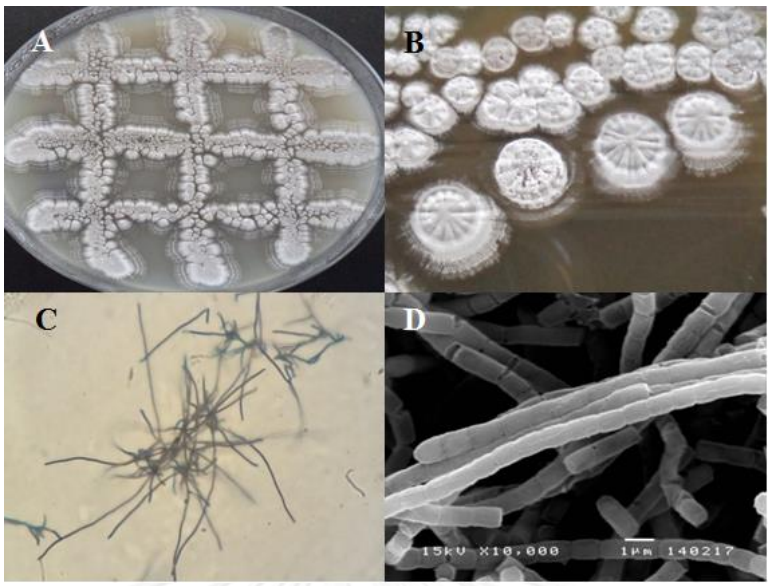


Figure 4.22 The morphological characters of strain KC-036 representing *Streptomyces* group XVI. A and B) the colonial appearance on ISP 3, C) light micrograph on ISP 3, and D) scanning electron micrograph on YS agar.

Strain KC-036 was selected to be the representative strain in this group for the 16S rRNA gene sequence analysis. Phylogenetic tree based on 16S rRNA gene sequences, strain KC-036 was related the most closely to members of the genus *Streptomyces*, in particular to *S. exfoliatus* NBRC 13191^{T} (99.9% similarity). Therefore, the morphological and 16S rRNA gene sequence data were considered that strain KC-036 was identified as *S. exfoliatus*.

Group XVII contained two strains, including KC-117 and KC-128. These strains were observed to form extensively branched substrate mycelia and abundant aerial mycelia on some agar media tested. The aerial mycelia consisted of long straight to flexuous (*Rectiflexibiles*) spore chains with smooth surface and oval shape spores (Figure 4.23). The results of morphological characteristics of these strain indicated that they belong to the genus *Streptomyces*. All strains showed good growth on all agar media tested. Aerial mycelia were white to light greenish gray and substrate mycelia were colorless, pale yellowish green to brilliant greenish yellow. Soluble pigments were vivid greenish yellow on ISP 2, light yellow to greenish yellow on ISP 3, light orange yellow on ISP 5 and brilliant greenish yellow on YS agar. The cultural characteristics of strains in group XVII are shown in Table 25 (Appendix C).

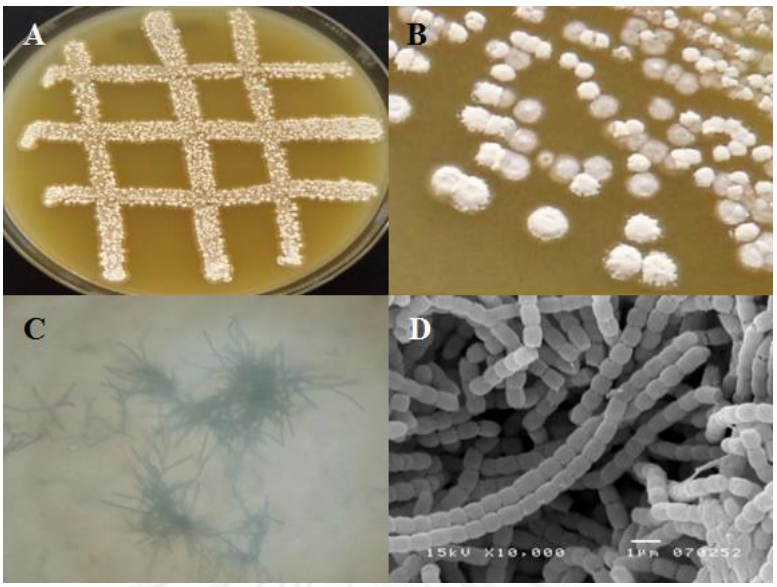


Figure 4.23 The morphological characters of strain KC-117 representing *Streptomyces* group XVII. A and B) the colonial appearance on ISP 3, C) light micrograph on ISP 4, and D) scanning electron micrograph on ISP 4.

They grew at 15-37°C, pH 5-12 and tolerated up to 7% (w/v) NaCl. They hydrolyzed starch and milk but did not hydrolyze gelatin. Nitrate reduction was positive for these strains. Milk coagulation was positive for strain KC-117. They utilized the following sugars: D-fructose, D-glucose and D-mannitol as carbon sources, but did not utilize L-arabinose, melezitose, D-melibiose, *myo*-inositol, raffinose, L-rhamnose, D-sorbitol and sucrose (Appendix C, Table 26).

Strain KC-117 was selected to be the representative strain in this group for 16S rRNA gene sequence analysis. The nearly complete 16S rRNA gene sequence of strain KC-117 was compared with sequences of known bacterial species in EzTaxon-server. The results of these comparisons exhibited that strain KC-117 showed the highest sequence similarity to *S. sindenensis* NBRC 3399^{T} (100%) (Table 4.2). The phylogenetic tree based on 16S rRNA gene sequence showed that strain KC-117 was from a clade with *S. sindenensis* (Figure 4.2). Consequently, strain KC-117 was identified as *S. sindenensis*.

Group XVIII comprised of four strains, namely KC-120, KC-121, KC-122 and KC-130**.** Morphological and cultural observation of 14-day culture on various agar media revealed that they formed extensive substrate mycelia and abundant aerial mycelia. All strain in this group produced aerial mycelia that differentiated to long spore chains. The spore chains were straight to flexuous (*Rectiflexibiles*) with oval shaped and smooth surface spores (Figure 4.24). The substrate mycelia formed colors of colorless, grayish greenish yellow to moderate olive brown and the aerial mycelium formed colors of greenish white to light greenish gray. All strains produced soluble pigments on some agar media. The cultural characteristics of these strains are shown in Table 27 (Appendix C).

They were able to grow at between pH 5 and pH 12. The temperature for growth was 15-37°C. Almost strains grew in presence of 0-8% (w/v) NaCl. They degraded starch and milk. Nitrate reduction was positive but milk coagulation and gelatinization were negative. All strains utilized D-fructose, D-glucose, D-mannitol and xylose as carbon sources, but did not utilize L-arabinose, D-melibiose, melezitose, *myo*-Inositol, raffinose, L-rhamnose, D-sorbitol and sucrose. In addition, the physiological and biochemical characteristics are detailed in Table 28 (Appendix C).

Figure 4.24 The morphological characters of strain KC-121 representing *Streptomyces* group XVIII. A and B) the colonial appearance on ISP 3, C) light micrograph on ISP 4, and D) scanning electron micrograph on ISP 4.

Strain KC-121 was selected to be the representative strain in this group for 16S rRNA gene sequence analysis. Strain KC-121 was related the most closely to *S. cavourensis* NBRC 13026^{T} with 99.9% similarity. The phylogenetic tree based on 16S rRNA gene sequence showed that strain KC-121 was from a clade with *S. cavourensis* (Figure 4.2). On the basis of morphological characteristics and 16S rRNA gene sequence analysis, strain KC-121 was identified *S. cavourensis* (Yuan *et al*., 2010).

Group XIX contained one strain, KC-087. This strain grew well and formed a highly branched substrate mycelia on any test media. The strain produced white to medium gray aerial mycelia on the various agar media tested, except ISP 6 and ISP 7. The aerial mycelia consisted of spiral spore chains with rugose surface spores (Figure 4.25). The substrate mycelium colors ranged from colorless on nutrient agar, pale yellow on ISP 2, ISP 3, ISP 5 and YS agar, grayish greenish yellow on ISP 4 to light olive brown on ISP 7. It produced grayish yellow soluble pigment on ISP 2 and ISP 3 (Appendix C, Table 29).

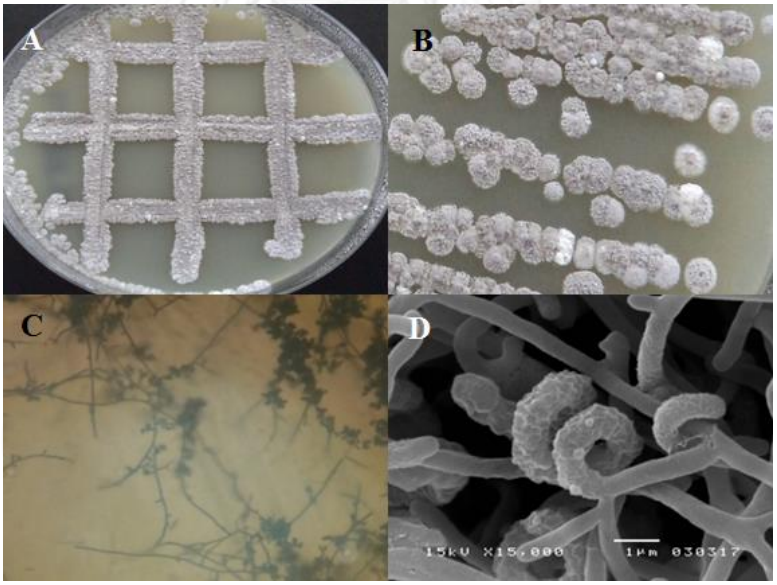


Figure 4.25 The morphological characters of strain KC-087 representing *Streptomyces* group XIX. A and B) the colonial appearance on ISP 3, C) light micrograph on ISP 4, and D) scanning electron micrograph on ISP 4.

The strain was positive for nitrate reduction and starch hydrolysis, weakly positive for milk peptonization and negative for milk coagulation and gelatinization. It utilized L-arabinose, D-fructose, D-glucose, D-mannitol, melibiose, *myo*-inositol, raffinose, L-rhamnose, sucrose and D-xylose as carbon sources but did not utilize melezitose and D-sorbitol. Growth of strain KC-087 occurred at pH 5-12 and with 0-7% NaCl (w/v). The temperature range for growth was 15-37°C. Detailed physiological and biochemical characteristics are presented in Table 30 (Appendix C)

Phylogenetic tree analysis based on the 16S rRNA gene sequence showed that strain KC-087 was affiliated to the genus *Streptomyces* (Figure 4.2). Based on EzTaxon-e analysis, strain KC-087 was related the most closely to *S. iranensis* HM 35^{T} with 99.8%. The 16S rRNA gene sequence similarity that is shown in the Table 4.2. On the basis of phenotypic and 16S rRNA gene sequence analyses, strain KC-087 was identified as *S. iranensis* (Hamedi *et al*., 2010).

Group XX composed of two strains, including KC-037 and KC-058, which formed greenish white to moderate greenish gray aerial mycelia and pale yellow to strong greenish yellow substrate mycelia on various agar media tested. These strains produced aerial mycelia that differentiated to spiral spore chains and the spore surfaces appeared to be a rugose (Figure 4.26). They were observed to grow well on all agar media tested. Strain KC-037 produce strong greenish yellow soluble pigment on ISP 2 and strain KC-058 produced pale yellow on ISP 7. The cultural characteristics of these strains are shown in Table 29 (Appendix C).

Streptomyces strains KC-037 and KC-058 were determined to be positive for nitrate reduction and starch hydrolysis and negative for gelatinization, milk peptonization and milk coagulation. The pH range for growth was pH 5-12 for strain KC-037 and pH 5-11 for strain KC-058. They were found be able to grow at 10-40°C and 0-7% (w/v) NaCl. The carbon utilization were positive f D-fructose, D-glucose, D-mannitol, D-melibiose, *myo*-inositol, raffinose, L-rhamnose and D-xylose and weakly positive for L-arabinose and negative for melezitose, D-sorbitol and sucrose. Detailed physiological and biochemical characteristics of *Streptomyces* strains in group XX are presented in Table 29 (Appendix C).

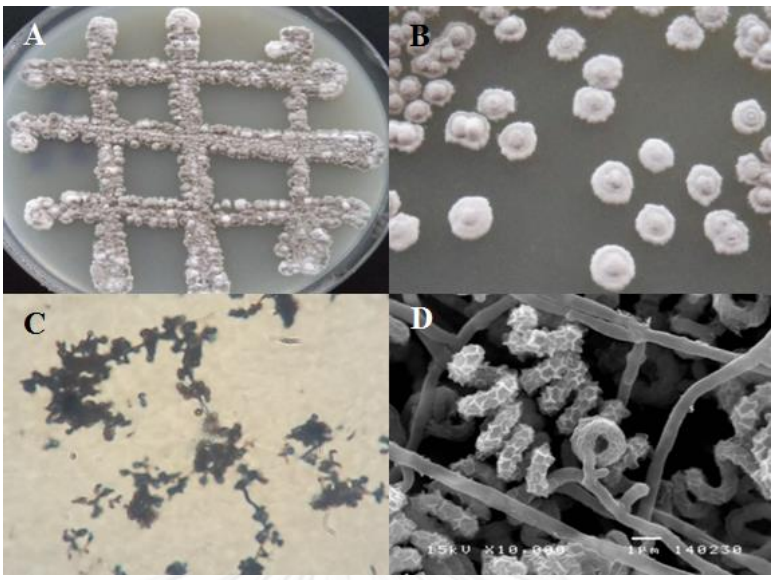


Figure 4.26 The morphological characters of strain KC-058 representing *Streptomyces* group XX. A and B) the colonial appearance on ISP 3, C) light micrograph on YS agar, and D) scanning electron micrograph on ISP 4.

Strain KC-058 was selected to be the representative strain in this group for 16S rRNA gene sequence analysis. The almost complete 16S rRNA gene sequence of strain KC-058 was compared with sequences of known bacterial species in EzTaxon-server. The results of these comparisons revealed that strain KC-058 had the highest sequence similarity (99.3%) to *S. rapamycinicus* NRRL B-5491^{T} (Appendix C, Table 30). The phylogenetic tree based on 16S rRNA gene sequence showed that strain KC-058 was from a clade with *S. rapamycinicus* (Figure 4.2). Therefore, strain KC-058 was identified as *S. rapamycinicus* (Kumar & Goodfellow, 2008)

Group XXI contained one strain, KC-079. This strain showed good growth on all agar media tested. From light and scanning electron microscopic observations of strain KC-079, it produced spiral spore chains with rugose surface spores (Figure 4.27). The results of morphological characteristics of strain KC-079 indicated that the strain belong to the genus *Streptomyces*. Strain KC-079 formed greenish white to olive gray aerial mycelia and pale yellow, grayish greenish yellow to moderate olive brown substrate mycelia on agar media tested. It produced pale greenish yellow (on ISP 3 and YS agar) and grayish greenish yellow (ISP 2) soluble pigments. The cultural characteristics of *Streptomyces* strain in group XXI are shown in Table 29 (Appendix C).

Strain KC-079 could grow at 15-37ºC and pH 5-12. It was observed to grow in the presence of 0-4% (w/v) NaCl. Starch and gelatin were hydrolyzed but not gelatin. Milk coagulation and nitrate reduction were negative. Strain KC-079 utilized L-arabinose, D-fructose, D-glucose, D-mannitol, melibiose, *myo*-inositol, raffinose, L-rhamnose and D-xylose as carbon sources, but not melezitose, D-sorbitol and sucrose. Detailed physiological and biochemical characteristics are given in Table 30 (Appendix C).

Figure 4.27 The morphological characters of strain KC-079 representing *Streptomyces* group XXI. A and B) the colonial appearance on ISP 3, C) light micrograph on ISP 7, and D) scanning electron micrograph on ISP 4.

The almost complete 16S rRNA gene sequence of strain KC-079 was compared with sequences in the EzTaxon-e database. Strain KC-079 showed the highest 16S rRNA gene sequence similarity to *S. yatensis* NBRC 101000^{T} (99.4%) and it was clustered with *S. yatensis* NBRC 101000^{T} in phylogenetic tree (Figure 4.2). Therefore, strain KC-079 was identified as *S. yatensis* (Saintpierre *et al*., 2003).

Group XXII comprised of five strains, including KC-080, KC-093, KC-094, KC-138 and KC-151. These strain showed good growth on all agar media tested. From light and scanning electron microscopic observations, they had an extensively branched substrate mycelia and aerial mycelia that carried rugose-surfaced spores in spiral spore chains (Figure 4.28). They formed white to gray series aerial mycelia and colorless, light grayish olive to moderate olive brown substrate mycelia on the agar media tested. Strains KC-080, KC-093 and KC-151 could not produce soluble pigments on all agar media tested. Strain KC-093 produced soluble deep yellow to dark yellow soluble pigments on ISP 2, ISP 3, ISP4, ISP 6, YS agar and nutrient agar and strain KC-138 produced only grayish greenish yellow on ISP 7. The cultural characteristics of *Streptomyces* strains in group XXII are shown in Table 31 (Appendix C).

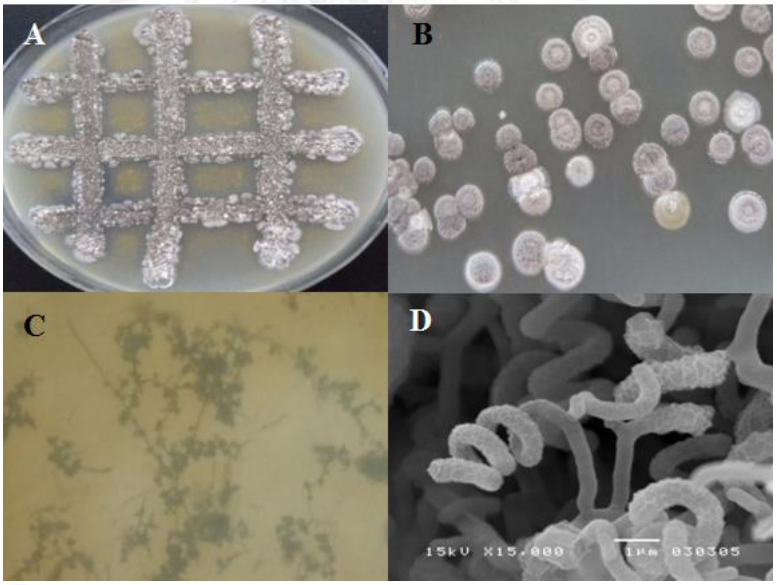


Figure 4.28 The morphological characters of strain KC-138 representing *Streptomyces* group XXII. A and B) the colonial appearance on ISP 3, C) light micrograph on ISP 4, and D) scanning electron micrograph on ISP 4.

The temperature for growth of these strain were 15-37ºC and pH 5-12. They tolerated up to 7% (w/v) NaCl for growth. They were positive for nitrate reduction, starch hydrolysis and milk peptonization and negative for milk coagulation and gelatinization. They utilized L-arabinose, D-fructose, D-glucose, D-mannitol, melibiose, *myo*-inositol, L-rhamnose and D-xylose as carbon sources, but not melezitose,

raffinose, D-sorbitol and sucrose. Detailed physiological and biochemical characteristics are given in Table 32 (Appendix C).

Strain KC-138 was selected to be the representative strain in this group for the 16S rRNA gene sequence analysis. The nearly complete 16S rRNA gene sequence of strain KC-138 was compared with sequences of known bacterial species in EzTaxon-server. The results of these comparisons revealed that strain KC-138 had the highest sequence similarity to *S. samsunensis* M1463^{T} (99.8%) (Table 4.2). The phylogenetic tree based on 16S rRNA gene sequence showed that strain KC-138 was from a cluster with *S. samsunensis* (Figure 4.2). Consequently, strain KC-138 was identified as *S. samsunensis* (Sazak *et al*., 2011).

Group XXIII consisted of one strain, KC-145. The strain was observed to form extensively branched substrate mycelia and abundant aerial mycelia on all agar media tested. The aerial mycelia contained long straight to flexuous spore chains (*Rectiflexibiles*) with smooth surface spores (Figure 4.29). Strain KC-145 produced white to greenish gray aerial mycelia. The colors of substrate mycelium were light yellowish brown on ISP2, moderate pink to dark pink on ISP 3, moderate yellowish brown on ISP 4, pale yellowish green on ISP 5, grayish greenish on ISP 6, dark grayish yellow on ISP 7, dark pink to strong yellowish brown on YS agar and moderate olive brown on nutrient agar. It produced soluble pigments all agar media tested (Appendix C, Table 33).

It grew at 15-40°C, pH 5-12 and tolerated up to 7% (w/v) NaCl. The strain hydrolyzed starch and milk but did not hydrolyze gelatin. It could not reduce nitrate. It utilized only D-glucose as carbon sources but did not utilize L-arabinose, D-fructose, D-mannitol, melezitose, melibiose, *myo*-inositol, L-rhamnose, raffinose, D-sorbitol, sucrose and D-xylose. The biochemical and physiological characteristics of all strains in group XXIII was presented in the Table 34 (Appendix C).

Phylogenetic tree analysis based on the 16S rRNA gene sequences (Figure 4.2) indicted that strain KC-145 was closely related to *S. cinereoruber* subsp. *cinereoruber* NBRC 12756^{T} (AB184121) with 100% sequence similarity . Consequently, strain KC-145 was identified as *S. cinereoruber* subsp. *cinereoruber*.

Figure 4.29 The morphological characters of strain KC-145 representing *Streptomyces* group XXIII. A and B) the colonial appearance on ISP 3, C) light micrograph on ISP 2, and D) scanning electron micrograph on ISP 2.

Group XXIV contained one strain, KC-003. It grew well and formed a highly branched substrate mycelium on any agar media tesed. This strain produced white to greenish gray and bluish gray aerial mycelia on various agar media tested. The aerial mycelia consisted of long straight to flexuous spore chains (*Rectiflexibiles*) with smooth surface spores (Figure 4.30). The substrate mycelium colors ranged from grayish greenish yellow to moderate olive brown on media tested. It produced soluble pigments with strong yellowish brown on ISP 2, brownish pink on ISP 5 and 7, deep yellowish brown on ISP 6 and dark grayish yellow on nutrient agar (Appendix C, Table 33).

Strain KC-003 was positive for gelatinization and negative for nitrate reduction, starch hydrolysis, milk peptonization and milk coagulation. It utilized D-glucose and sucrose as carbon sources but did not utilize L-arabinose, D-fructose, D-mannitol, melezitose, melibiose, *myo*-inositol, raffinose, L-rhamnose, D-sorbitol and D-xylose. Growth of strain KC-003 occurred at pH 5-12 and 15-37°C with 0-2% (w/v) NaCl. Detailed physiological and biochemical characteristics are presented in the Table 34 (Appendix C).

Phylogenetic tree analysis based on the 16S rRNA gene sequence showed that strain KC-003 was affiliated to the genus *Streptomyces* (Figure 4.2.). Based on

EzTaxon-e analysis, strain KC-003 was related the most closely to *S. misakiensis* NBRC 12891^{T} (AB184223) with 99.8%. The 16S rRNA gene sequence similarity that is shown in the Table 4.2. On the basis of phenotypic and 16S rRNA gene sequence analyses, strain KC-003 was identified as *S. misakiensis*.

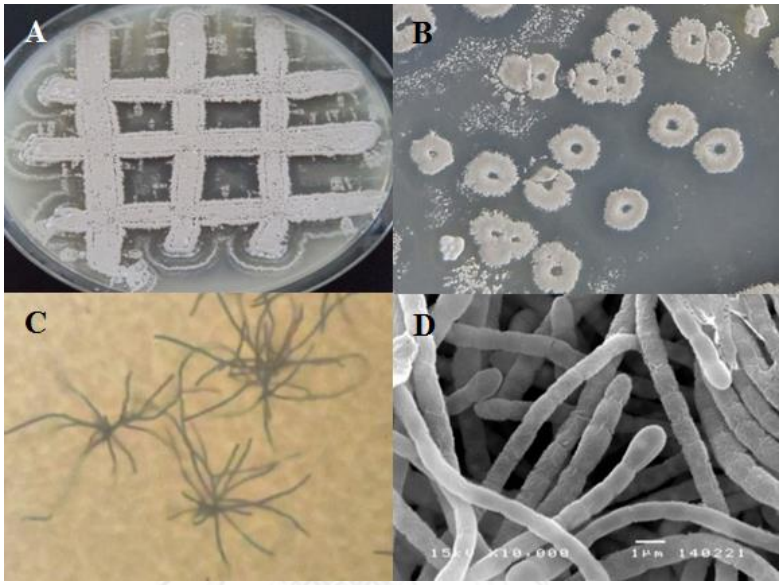


Figure 4.30 The morphological characters of strain KC-003 representing *Streptomyces* group XXIV. A and B) the colonial appearance on ISP 3, C) light micrograph on ISP 2, and D) scanning electron micrograph on ISP 2.

4.2.2 Characteristics of *Amycolatopsis* strains

Strain KC-132 contained *meso*-DAP, arabinose and galactose in cell wall peptidoglycan (wall chemotype IV). The polar lipids were found to include phosphatidylglycerol (DPG), phosphatidylethanolamine (PE), hydroxyphosphatidyl-ethylethanolamine (OH-PE), phosphatidylglycerol (PG), phosphatidylinositolmanno-sides (PIMs), Phosphatidylinositol (PI), two unknown glycolipids and polar lipids. The results of chemical analysis indicated that strain KC-132 has chemotaxonomic marker typical of the genus *Amycolatopsis*. Strain KC-132 was observed to form extensively branched substrate mycelia and abundant aerial mycelia on all agar media tested. The aerial mycelia contained long straight to flexuous spore chains with rod shape spore. The surface of spore-like structures was smooth (Figure 4.31). Strain KC-132 produced white aerial mycelia and pale yellowish pink to strong brown substrate on different agar media. It produced light orange (on ISP 2), pale yellowish pink (on ISP 3 and ISP 4), light orange yellow (ISP 5), light yellowish pink (on ISP 6 and YS agar) and deep reddish purple (on ISP 7) soluble pigments (Appendix C, Table 35).

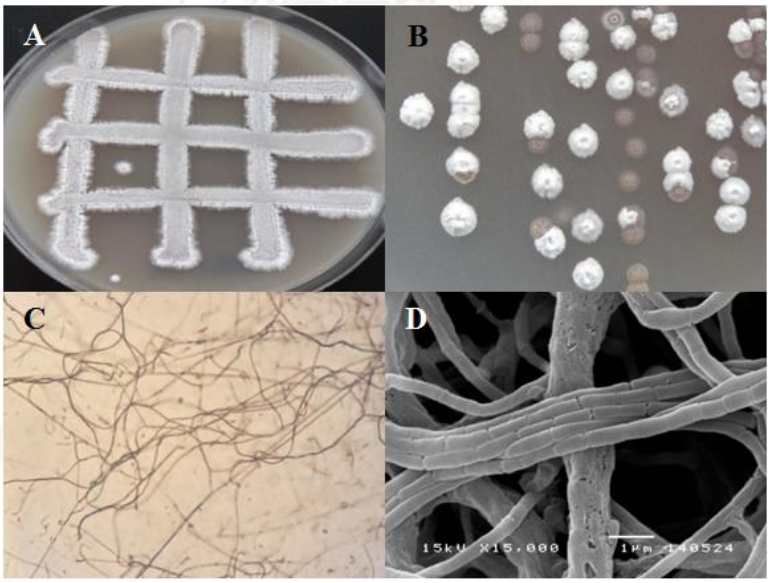


Figure 4.31 The morphological characters of strain KC-132. A and B) the colonial appearance on ISP 3, C) light micrograph on ISP 2, and D) scanning electron micrograph on ISP 2.

Strain KC-132 was positive Nitrate reduction, starch hydrolysis, milk peptonization, milk coagulation and gelatinization. It utilized L-arabinose, D-fructose, D-glucose, D-mannitol, melibiose, *myo*-inositol, raffinose and xylose as carbon sources but did not utilize L-rhamnose, D-sorbitol and sucrose. Growth of strain KC-132 occurred at pH 5-12, 15-40ºC and with 0-7% (w/v) NaCl. Detailed physiological and biochemical characteristics are presented in the Table 36 (Appendix C).

The nearly complete 16S rRNA gene sequence of strain KC-132 was compared with sequences of known bacterial species in EzTaxon-server. The results of these comparisons revealed that strain KC-132 had the highest sequence similarity to *A. keratiniphila* subsp. *keratiniphila* DSM 44409^{T} (99.3%) (Table 4.2). Phylogenetic analysis of 16S rRNA gene sequences showed that strain KC-132 was a member of the genus *Amycolatopsis* (Figure 32).

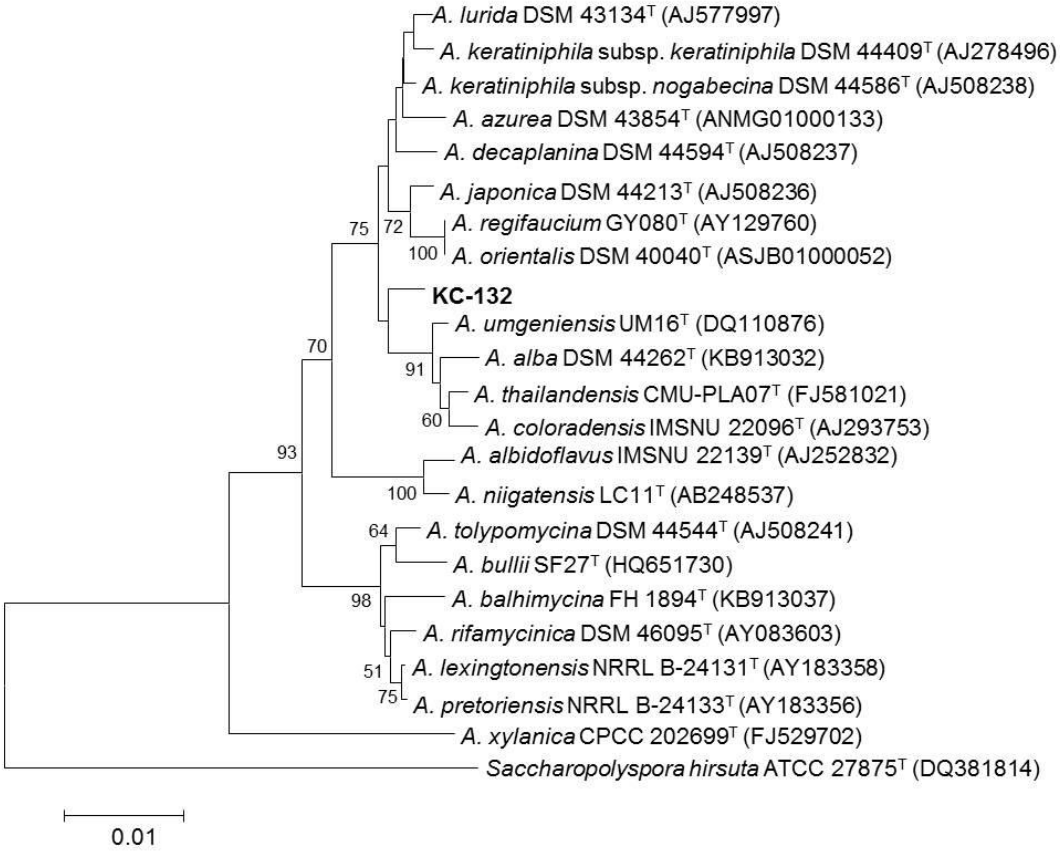


Figure 4.32 Neighbor-joining tree based on the almost complete 16 rRNA gene sequences of strain KC-132 and the members of the genus *Amycolatopsis*

4.2.3 Characteristics of *Kitasatospora* strains

Group 1 consisted of two strains, including KC-001 and KC-005. These strain contained LL-DAP and *meso*-DAP, galactose, manose and ribose in cell wall peptidoglycan (wall chemotype I/III). The polar lipids were found to include phosphatidylglycerol (DPG), phosphatidylethanolamine (PE), phosphatidylglycerol (PG), phosphatidylinositolmannosides (PIMs), Phosphatidylinositol (PI), unknown glycolipid and unknown lipid. The results of chemical analysis indicated that they had chemotaxonomic marker typical of genus *Kitasatospora*.

They formed an extensively branched substrate and aerial mycelium with smooth surfaced spores (Figure 4.33). The colors of the substrate mycelia ranged from yellowish white, greenish white to pale yellow and the colors of aerial mycelia ranged from greenish white to light greenish gray or bluish gray on different media (Table 35). Strain KC-001 produce dark yellow soluble pigment and strain KC-005 produced dark grayish yellow soluble pigment on ISP2.

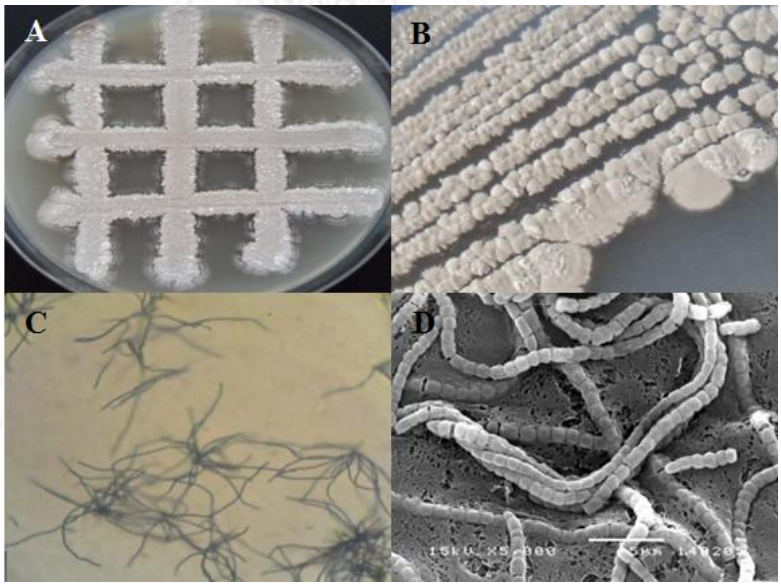


Figure 4.33 The morphological characters of strain KC-005 representing *Kitasatospora* group 1. A and B) the colonial appearance on ISP 3, C) light micrograph on ISP 2, and D) scanning electron micrograph on ISP 2.

Strains KC-001 and KC-005 were positive for starch hydrolysis and milk peptonization and negative for Nitrate reduction, milk coagulation and gelatinization. It utilized D-glucose and xylose as carbon sources but did not utilize, L-arabinose, D-fructose, D-mannitol, melezitose, melibiose, *myo*-inositol, raffinose and L-rhamnose, D-sorbitol and sucrose. Growth of these strains occurred at pH 5-12, 15-37°C and with 0-3% (w/v) NaCl. Detailed physiological and biochemical characteristics are presented in the Table 36 (Appendix C).

The nearly complete 16S rRNA gene sequence of strain KC-001 and KC-005 were compared with sequences of known bacterial species in EzTaxon-server. The results of these comparisons showed that these strains had the highest sequence similarity to *K. saccharophila* SK15^{T} (100%) (Table 4.2). Phylogenetic analysis of 16S rRNA gene sequences showed that they formed a cluster along with was *K. saccharophila* SK15^{T} (Figure 35). Therefore, strain KC-001 and KC-005 were identified as *K. saccharophila* (Li *et al*., 2008).

Group 2 consisted of one strain, KC-143. This strain contained LL-DAP and *meso*-DAP, galactose, manose and ribose in cell wall peptidoglycan (wall chemotype I/III). The polar lipids were found to include phosphatidylethanolamine (PE), Phosphatidylinositol (PI), phosphatidylinositolmannosides (PIMs), phosphatidylglycerol (DPG), two unknown glycolipids and unknown lipid. The results of chemical analysis indicated that they had chemotaxonomic marker typical of genus *Kitasatospora*.

Strain KC-143 formed an extensively branched substrate and aerial mycelia with smooth surface spores (Figure 4.34). The colors of the substrate mycelia ranged from grayish greenish yellow to green black and the colors of aerial mycelia ranged from white to light greenish gray or bluish gray on different media. It produced soluble pigments on ISP2, ISP3, ISP7 and nutrient agar (Table 35).

Strain KC-143 could hydrolyze milk but not hydrolyze starch and gelatin. It was negative for Nitrate reduction and milk coagulation. It utilized D-glucose and xylose as carbon sources but did not utilize, L-arabinose, D-fructose, D-mannitol, melezitose, melibiose, *myo*-inositol, raffinose and L-rhamnose, D-sorbitol and sucrose. Growth of strain KC-143 occurred at pH 5-12, 15-37°C with 0-4% (w/v) NaCl. Detailed physiological and biochemical characteristics are given in the Table 36 (Appendix C).

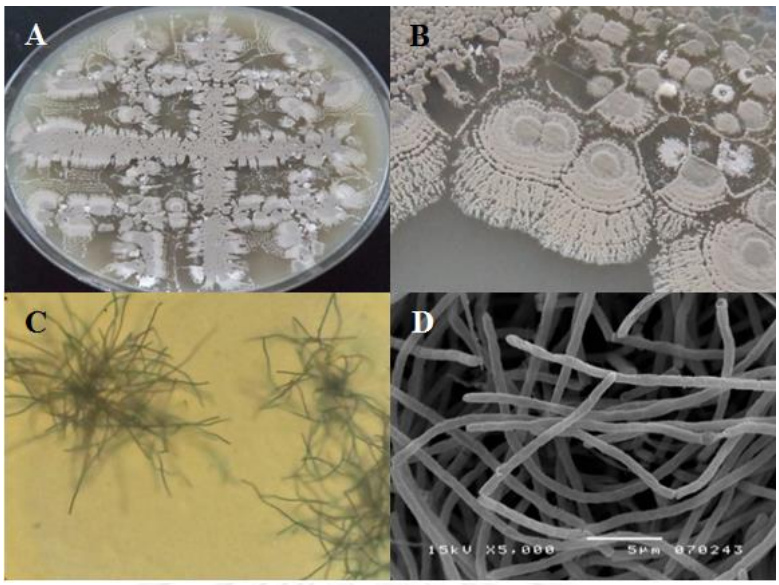


Figure 4.34 The morphological characters of strain KC-143 representing *Kitasatospora* group 2. A and B) the colonial appearance on ISP 3, C) light micrograph on ISP 2, and D) scanning electron micrograph on ISP 4.

The almost complete 16S rRNA gene sequence of strain KC-143 was compared with sequences of known bacterial species in EzTaxon-server. The results revealed that strain KC-143 had the highest sequence similarity to *K. putterlickiae* F18-98^{T} (99.3%) (Table 4.2). Phylogenetic analysis of 16S rRNA gene sequences showed that strain KC-143 was a member of the genus *Kitasatospora* (Figure 34).

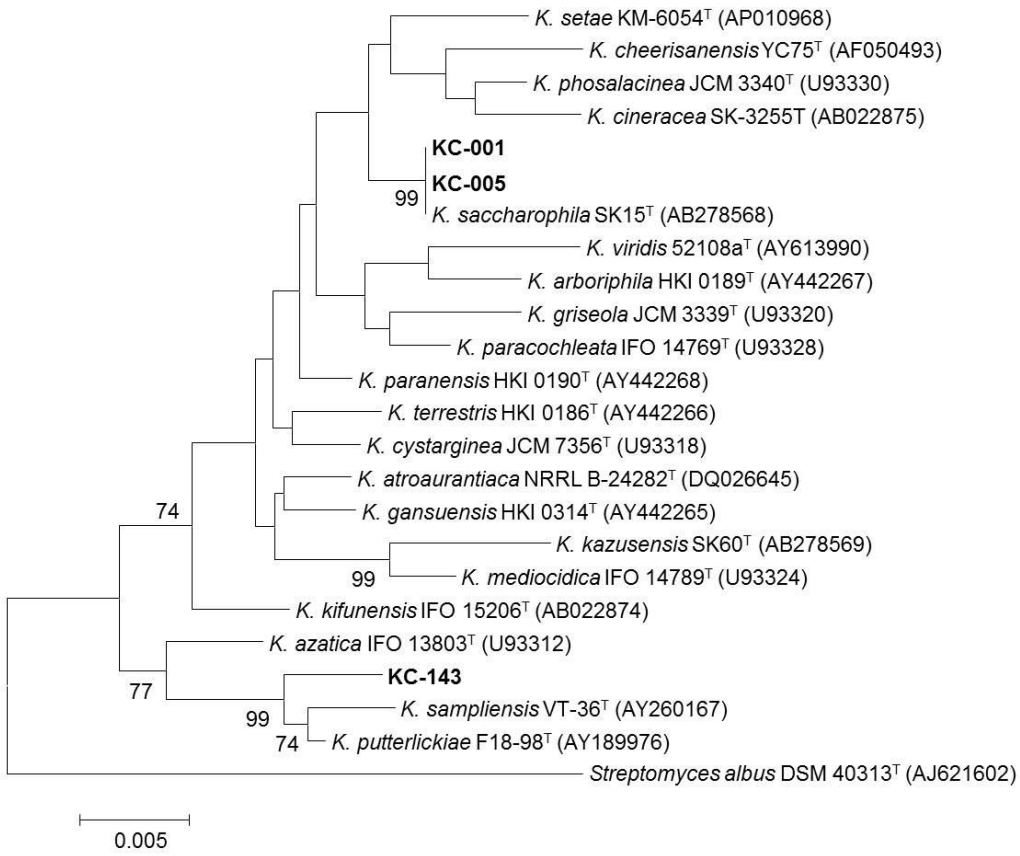


Figure 4.35 Neighbor-joining tree based on the almost complete 16 rRNA gene sequences of strains KC-001, KC-005, KC-143 and the members of the genus *Kitasatospora*

4.2.4 Characteristics of *Nonomuraea* strain

Strain KC-061 grew well on ISP 2, 3, 4, 5 and 7 media, YS agar and nutrient agar. The aerial mycelia appeared white on ISP 2, whitish purple on ISP and pale orange on ISP 5 and 7. No aerial mycelia were formed when the strain cultivated on ISP 4 and 6 media, YS agar and nutrient agar. The substrate mycelium branched extensively and the colors on various media were reddish brown to yellowish brown. Brown soluble pigments on ISP 2 and 3 media, YS agar and nutrient agar were produced (Table 4.17). The aerial hyphae produced long and spiral spore chains, which had spiral bearing >10 spores (Figure 4.36). The strain grew at 14-38 °C and at pH 6.0-11.0 and tolerated up to 4% (w/v) NaCl. Nitrate was reduced to nitrite. Casein and starch were hydrolyzed but gelatin was not liquefied. Milk was peptonized weakly and was not coagulated.

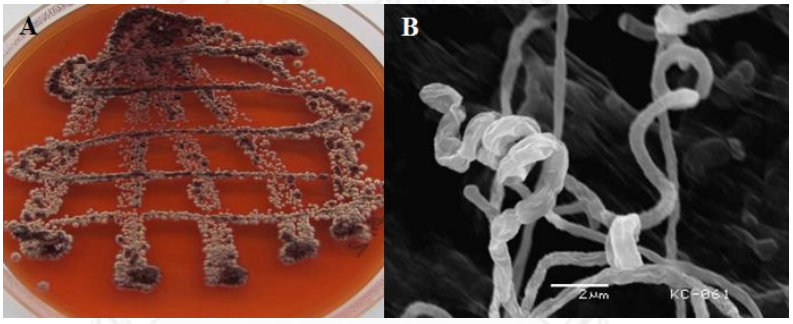


Figure 4.36 The morphological characters of strain KC-061. A) the colonial appearance on ISP 2 and B) scanning electron micrograph on ISP 3.

Strain KC-061 utilized D-glucose, *myo*-inositol, D-mannitol, melibiose, raffinose, L-rhamnose and D-xylose as carbon sources but did not utilize sucrose. Enzyme activities of the API ZYM system were positive for alkaline phosphatase, leucine arylamidase, α-chymotrypsin, acid phosphatase, naphthol-AS-BI-phospho-hydrase, a-glucosidase, β-glucosidase and N-acetyl-*β*-glucosaminidase and weakly positive for esterase (C4), esterase lipase (C8), valine arylamidase and α-mannosidase but negative for cystine arylamidase, trypsin, α-galactosidase and β-galactosidase (Table 4.18).

The 16S rRNA gene sequence analysis showed that strain KC-061 belongs to the genus *Nonomuraea*. The 16S rRNA gene sequence similarity values between the strain KC-061 and the type strains in the genus *Nonomuraea* ranged from 95.4 to

99.3%. The phylogenetic trees based on 16S rRNA gene sequences of strain KC-061 and the type strains of members in the genus *Nonomuraea* revealed that strain KC-061 constructed a cluster with *N. monospora* PT708^T, *N. rhizophila* YIM 67092^T and *N. rosea* GW 12687^T (Figure 4.37). The strain KC-061 showed high 16S rRNA gene sequence similarity values to *N. monospora* PT708^T (99.3%), *N. rhizophila* DSM45382T (98.6%), N. *dietziae* NBRC 14039^T (98.5%) and *N. rosea* DSM 45177^T (98.3%). These results indicate that strain KC-061 is the closest with *N. monospora* PT708^T. However, *N. monospora* PT708^T, producing single spore is clearly distinguished from the strain KC-061, which produced spiral spore chains.

Strain KC-061 contained *meso*-DAP isomer as diagnostic diamino acid and galactose, mannose, madurose and ribose as diagnostic sugars in whole cells. The *N*-acyl type of muramic acid was acetyl. The predominant menaquinone was MK-9(H_4) (80%) and the minor menaquinones were MK-9(H_6) (10%), MK-9(H_2) (7%), MK-9(H_0) (2%) and MK-10(H_4) (1%). Diphosphatidylglycerol (DPG), phospha tidylethanolamine (PE), phosphatidylmonomethylethanolamine (PME), hydroxyphosphatidylmonomethylethanolamine (OH-PME), hydroxyphosphatidylethanolamine (OH-PE), phosphatidylglycerol (PG), ninhydrin positive phosphoglycolipid and unknown phospholipid (PL1) were detected as phospholipids. The major fatty acids were iso-$_{C16:0}$ (25.3%), 10-methyl-$_{C17:0}$ (13.4%), $C_{17:1}$ *ω8c* (7.7%), C16:1 *ω7c*/$C_{16:1}$ *ω6c* (7.0%), $C_{16:0}$ (6.9%), iso-$C_{16:1}$ G (6.4%), iso-$C_{15:0}$ (4.8%), 10-methyl-$C_{16:0}$ (4.0%) and $C_{16:0}$ 2-OH (3.8%). Strain KC-061 contained the same cellular fatty acid profiles as *Nonomuraea* species but it showed different in the amount of fatty acids (Table 4.19). The G+C content of genomic DNA was 72.4 mol%. Chemotaxonomic analyses confirmed that strain KC-061 exhibited typically chemical characteristics of members of the genus *Nonomuraea*.

DNA-DNA relatedness values between strain KC-061 and strains *N. monospora* PT708^T, *N. rhizophila* DSM 45382^T, *N. dietziae* NBRC 14309^T and *N. rosea* DSM 45177^T were 47%, 55%, 48% and 54%, respectively and reciprocally, strain *N. monospora* PT708^T showed 54 %, 50%, 39% and 46% DNA-DNA relatedness to KC-061, *N. rhizophila* DSM 45382^T, *N. dietziae* NBRC 14309^T and *N. rosea* DSM 45177^T, respectively. These values are obtained from three independent determinations and are below the 70% cutoff point recommended by Wayne *et al*. for assigning strains to the same species and confirms the separation of strain KC-061 from its closely related phylogenetic neighbors. Therefore, strain KC-061 clearly represents a novel species of the genus *Nonomuraea* and named as *Nonomuraea thalandensis* KC-061.

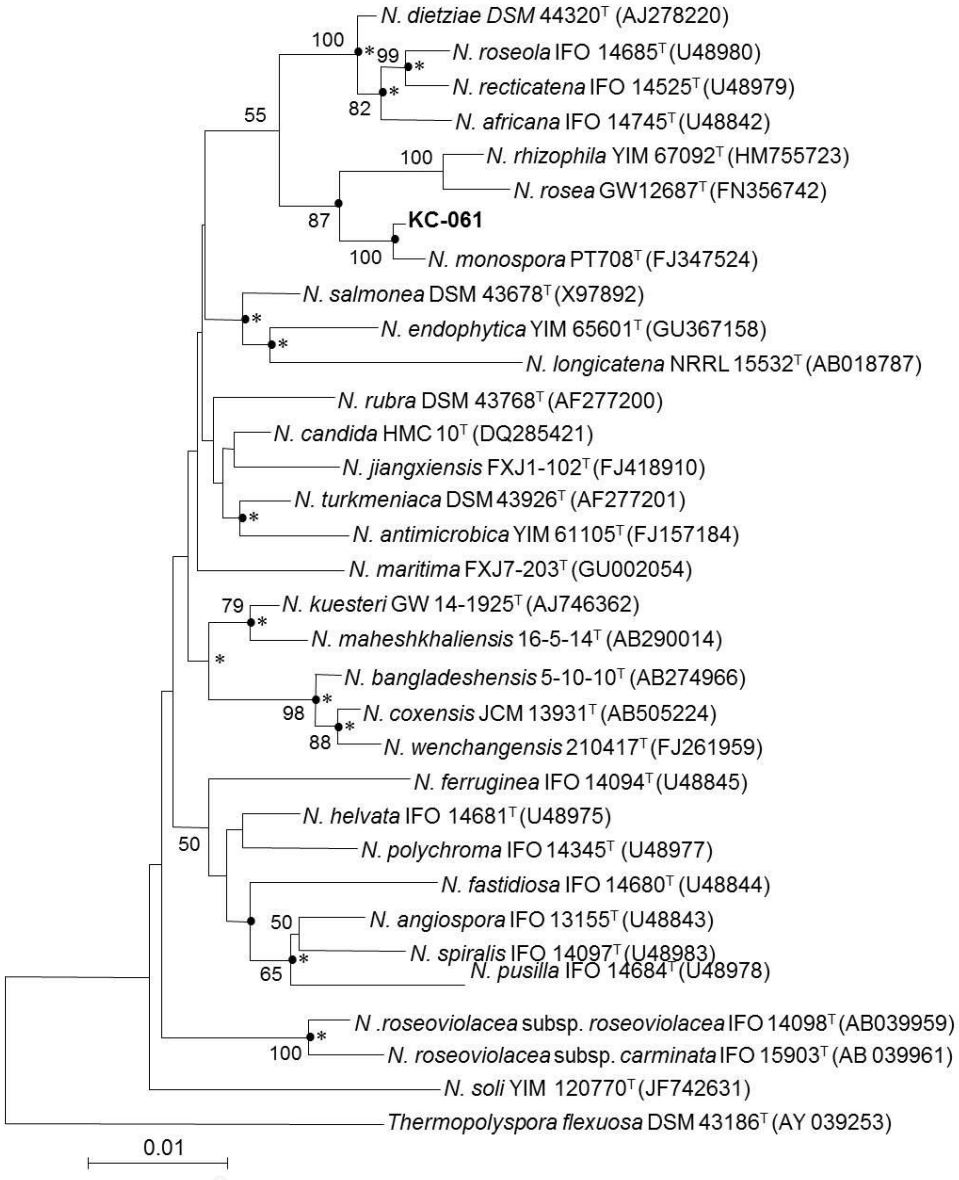


Figure 4.37 Neighbor-joining tree based on 16S rRNA gene sequences showing relationship between KC-061 and members of the genus *Nonomuraea*. Only bootstrap values above 50% (percentages of 1000 replications) are indicated. (•), branch also recovered in the maximum-parsimony tree; (*), branch also recovered in the maximum-likelihood tree; Bar, 0.01 nucleotide substitutions per site.

Table 4.17 Differential characteristics of strain KC-061 and closely related type strains

Media	Strains				
	KC-061	*N. monospora*	*N. rhizophila*	*N. dietziae*	*N. rosea*
ISP 2					
Growth	Good	Good	Good	Good	Good
	Deep reddish brown	Reddish brown	Grayish yellow brown	Pale yellowish brown - brownish orange	Pale yellowish brown and light rose brown
Reverse	Deep reddish brown	Reddish brown	Pale yellowish brown	Pale yellowish brown - light brown	Pale yellowish brown and reddish gray
Aerial mycelium	Poor, white	Abundant, yellowish white	Abundant, white	Moderate, pale yellowish brown	None
Soluble pigment	Deep orange	Moderate orange	None	None	None
ISP 3					
Growth	Good	Good	Good	Good	Good
	Brownish orange - reddish brown	Reddish orange	Brownish white - pale yellowish brown	Yellowish brown - light brown	Dark grayish purple
Reverse	Brownish orange	Reddish orange	Yellowish gray	Yellowish brown	Dark reddish purple - dark grayish purple
Aerial mycelium	Moderate, whitish purple	Moderate, pale pink	Abundance, brownish white	Abundant, whitish purple	Poor, medium gray
Soluble pigment	Light brown	Light brown	None	Grayish yellowish brown	Medium gray
ISP 4					
Growth	Good	Good	Good	Good	Good
	Yellowish brown	Moderate yellow	Pale yellowish brown	pale yellowish brown	Pale yellowish brown - light olive brown
Reverse	Grayish yellow	Pale yellow	Brownish white	Grayish yellow	Grayish yellow
Aerial mycelium	None	None	None	None	None
Soluble pigment	None	None	None	None	None
ISP medium 5					
Growth	Good		Good	Good	Good
	Pale orange - pale yellowish brown	Pale yellow - grayish greenish yellow	Yellowish gray - pale yellowish brown	Yellowish gray pale yellowish brown	Yellowish gray - pale yellowish brown
Reverse	Pale orange - pale yellowish brown	Grayish greenish yellow	Yellowish gray - pale yellowish brown	Yellowish gray - pale yellowish brown	Yellowish gray - pale yellowish brown
Aerial mycelium	Poor, pale orange	Poor, white	Abundant, brownish white	Abundant, white	None
Soluble pigment	None		None	None	None
ISP 6					
Growth	Moderate	Moderate	Good	Moderate	Moderate
	Light olive brown	Moderate brown	Yellowish brown	Yellowish brown	Light olive brown
Reverse	Light olive brown	Moderate brown	Yellowish brown	Yellowish brown	Light olive brown
Aerial mycelium	None	None	None	None	None
Soluble pigment	None	None	None	None	None
ISP 7					
Growth	Good	Good	Good	Good	Good
	Pale orange - pale yellowish brown	Pale yellow - grayish greenish yellow	Yellowish gray pale yellowish brown	Yellowish gray - pale yellowish brown	Yellowish gray - pale yellowish brown
Reverse	Pale orange - pale yellowish brown	Grayish greenish yellow	Yellowish gray pale yellowish brown	Yellowish gray - pale yellowish brown	Yellowish gray - pale yellowish brown
Aerial mycelium	Poor, pale orange	Poor, white	Abundant, brownish white	Abundant, white	None
Soluble pigment	None	None	None	None	None
YS agar					
Growth	Good	Good	Good	Good	Good
	Dark reddish brown dark grayish reddish brown	Moderate reddish	Pale yellowish brown	Light olive brown - grayish reddish brown	Yellowish gray - grayish reddish purple
Reverse	Deep reddish brown	Moderate reddish	Pale yellowish brown	Light olive brown	Pale yellow - grayish reddish purple
Aerial mycelium	None	None	Moderate, brownish white	Abundance, white	None
Soluble pigment	Deep orange	Deep yellow	None	Pale yellowish brown	None
Nutrient agar					
Growth	Good	Good	Good	Good	Good
	Dark reddish brown	Light red	Pale yellowish brown	Pale yellowish brown	Yellowish brown - dark reddish brown
Reverse	Deep reddish brown	Light red	Pale yellowish brown	Pale yellowish brown	Grayish brown - dark reddish brown
Aerial mycelium	None	None	Poor, pale yellowish brown	Poor, pale yellowish brown	None
Soluble pigment	Vivid orange	Light reddish orange	None	None	None

Table 4.18 Different characteristics of strain KC-061 and closely related type strains

Characteristics	Strains				
	KC-061	*N. monospora*	*N. rhizophila*	*N. dietziae*	*N. rosea*
Utilization of					
L-Arabinose	±	-	-	+	+
D-Fructose	+	-	+	+	+
D-Mannitol	+	-	+	-	+
Melibiose	+	-	+	-	-
Raffinose	+	-	+	-	+
Sucrose	-	-	-	-	+
D-Xylose	+	+	-	+	+
Nitrate reduction	+	+	-	-	+
Degradation of casein	±	+	-	-	-
Gelatin liquefaction	-	+	-	-	-
Starch hydrolysis	+	+	-	±	+
NaCl tolerance (%)	4	3	3	3	2
Enzyme activity of					
Cystine arylamidase	-	-	+	+	+
Esterase C4	±	±	+	+	+
Esterase lipase C8	±	-	±	+	±
α-Galactosidase	-	±	±	-	±
β-Galactosidase	-	+	+	+	+
β-Glucosidase	+	±	±	±	±
α-Mannosidase	±	+	±	±	±
Trypsin	-	+	+	+	+
Valine arylamidase	±	-	+	+	+

+ = positive, ± = weakly positive, - = negative.

Table 4.19 Cellular fatty acid compositions (%) of KC-061 and closely related type strains

Fatty acid	Strains				
	KC-061	*N. monospora*	*N. rhizophila*	*N. dietziae*	*N. rosea*
Saturated straight-chain					
$C_{13:0}$	0.6	0.2	1.2	0.6	1.1
$C_{14:0}$	3.3	3.3	4.2	5.9	3.6
$C_{16:0}$	6.9	11.0	8.2	23.4	9.5
$C_{17:0}$	1.7	1.2	3.4	8.7	4.0
Unsaturated straight-chain					
$C_{15:1}$ $\omega 6c$	0.2	ND	0.3	ND	ND
$C_{17:1}$ $\omega 8c$	7.7	6.7	8.4	9.9	8.3
$C_{18:1}$ $\omega 9c$	2.8	3.5	0.6	5.3	0.9
Saturated branched-chain					
iso-$C_{14:0}$	1.3	0.7	3.1	1.3	2.5
iso-$C_{15:0}$	4.8	5.1	3.2	3.5	5.1
iso-$C_{16:0}$	25.3	23.5	27.2	9.6	18.9
iso-$C_{17:0}$	1.0	1.4	0.4	0.7	1.0
iso-$C_{18:0}$	0.6	0.4	ND	0.2	ND
anteiso-$C_{15:0}$	0.3	0.5	0.8	1.6	2.1
anteiso-$C_{17:0}$	0.7	1.1	0.4	0.7	1.4
10-methyl $C_{16:0}$	4.0	6.8	4.7	1.8	4.6
10-methyl $C_{17:0}$	13.4	13.2	13.1	6.2	14.6
10-methyl $C_{18:0}$ (TBSA)	2.4	3.2	1.0	2.6	1.6
Unsaturated branched-chain					
iso-$C_{15:1}$ G	0.3	ND	0.2	0.2	0.4
iso-$C_{15:1}$ G	6.4	4.4	4.2	1.0	4.7
Hydroxy					
$C_{13:0}$ 2-OH	ND	0.2	ND	0.1	ND
$C_{15:0}$ 2-OH	0.7	0.2	2.9	1.1	3.0
$C_{16:0}$ 2-OH	3.8	1.7	3.6	3.5	3.2
$C_{17:0}$ 2-OH	0.5	0.2	0.5	0.6	0.5
Summed feature[a] 3	7.0	7.1	4.1	0.5	3.1

ND = not detected.

[a]Summed feature 3 comprises $C_{16:1}$ $\omega 7c$ and/or $C_{16:1}$ $\omega 6c$.

4.3 Screening of antimicrobial activity

On the basis of primary screening, a total of forty five *Streptomyces* isolates cultured in production media no. 51 and 53 showed antimicrobial activity whereas *Amycolatopsis* and *Kitasatospora* strains had no antimicrobial activity. The detailed antimicrobial activity based on cultivation of the isolated strains in production media no. 51 and 53 is given in Table 4.20. The potency of antimicrobial activity based on inhibition zone was divided into 3 levels which were strong or potent (>20 mm), moderate (14-20 mm) and weakly (<14 mm).

The *Streptomyces* strains in group I which were identified as *S. tendae* comprising strains KC-066, KC-075, KC-095, KC-097 and KC-102 showed antimicrobial activity. They showed antimicrobial activity against *B. subtilis* and *K. rhizophila*, except strain KC-097 showing antimicrobial activity against only *B. subtilis*. Therefore, these strains in group I revealed anti-Gram positive bacterial activity. Among strains in group I, strain KC-102 exhibited strong anti-Gram positive bacteria activity against *B. subtilis*.

A *Streptomyces* strain in group II, KC-020 identified as *S. enissocaesilis* presented antimicrobial activity against *B. subtilis*, *E. coli*, *K. rhizophila* and *X. campestris* pv. *oryzae*. Consequently, strain KC-020 exhibited anti-Gram positive and anti-Gram negative bacterial activities and also anti-rice pathogen activity.

The *Streptomyces* strains in groups III (KC-072), V (KC-070 and KC-100), and XIV (KC-031, KC-032, KC-033, KC-038, KC-150 and KC-156) had no antimicrobial activity.

The *Streptomyces* strains in group IV, including KC-073 and KC-155 which were identified as *S. marokkonensis* inhibited anti-Gram positive bacterial activity against *B. subtilis* and *K. rhizophila*.

The *Streptomyces* strains in group V, including KC-055, KC-090, KC-104 and KC-105 which were identified as *S. parvulus* demonstrated antimicrobial activity against *B. subtilis*, *K. rhizophila* and *X. campestris* pv. *oryzae*. From this result, it indicated that these strains showed anti-Gram positive bacterial and also anti-rice pathogen activity.

The *Streptomyces* strains in group VI, including KC-060, KC-088, KC-115, KC-118 and KC-133 which were identified as *S. malachitospinus* showed antimicrobial activity such as strains KC-060 inhibited *B. subtilis* and *X. campestris* pv. *oryzae* in weakly activity when it was cultured in production medium no. 53, strain KC-115 inhibited *B. subtilis* and *K. rhizophila* when it was cultured in production medium no.

51, strain KC-118 inhibited *K. rhizophila* when it was cultured in production medium no. 51 and KC-133 inhibited *B. subtilis* when it was also cultured in production medium no. 51. Strain KC-088 exhibited no antimicrobial activity in both of production media.

The *Streptomyces* strains in group VIII, including KC-110, KC-111, KC-112, KC-135 and KC-136 which were identified as a novel species in the genus *Streptomyces* had no antimicrobial activity against all test microorganisms, except strain KC-112 showing weakly antimicrobial activity against *B. subtilis.*

The *Streptomyces* strains in group IX, including KC-062 and KC-063 which were identified as *S. spiralis* demonstrated weakly antimicrobial activity against *B. subtilis* and *X. campestris* pv. *oryzae* when they were cultured in 53 production medium.

The *Streptomyces* strains in group X which were identified as *S. aureus* comprising strains KC-004, KC-017, KC-141, KC-142, KC-152 and KC-157 exhibited no antimicrobial activity against all test microorganisms, except strain KC-141 showing weakly antimicrobial activity against *B. subtilis* and *K. rhizophila*.

The *Streptomyces* strains in group XI, KC-035 identified as a novel *Streptomyces krungchingensis* in the genus *Streptomyces* exhibited weakly antimicrobial activity against *B. subtilis* and *K. rhizophila* when it was cultured in the production media. Consequently, strain KC-035 showed anti-Gram positive bacterial activity.

The *Streptomyces* strains in group XII, including KC-106 and KC-104 which were identified as a novel species in the genus *Streptomyces* namely *S. similanensis*. They inhibited *B. subtilis* and *K. rhizophila*. Hence, they showed anti anti-Gram positive bacterial activity.

The *Streptomyces* strains in group XIII which were identified as *S. violarus* comprising strains KC-119, KC-124, KC-125 and KC-134. The almost strains showed antimicrobial activity, except strain KC-134. Strains KC-119 and KC-124 showed anti-Gram positive bacteria against *B. subtilis*, *K. rhizophila* and *X. campestris* pv. *oryzae*. Furthermore, strain KC-124 showed anti-Gram negative bacterial activity against *E. coli*. Strain KC-125 showed anti- *B. subtilis* and anti-*K. rhizophila* activities.

The *Streptomyces* strains in group XV, including KC-085, KC-098, KC-101 and KC-103 which were identified as *S. drozdowiczii.* Tthey showed weakly antimicrobial activity, except strain KC-085. They exhibited anti-Gram positive bacteria against *B.*

subtilis. Furthermore, strains KC-098 and KC-103 exhibited also anti-Gram positive bacterial activity against anti-*K. rhizophila*.

The *Streptomyces* strains in group XVII, including KC-117 and KC-128 identified as *S. sindenensis*. Strain KC-117 showed potent antimicrobial activity against *B. subtilis*, *K. rhizophila* and *X. campestris* pv. *oryzae* whereas strain KC-128 exhibited only weakly antimicrobial activity against *E. coli*.

The *Streptomyces* strains in group XVIII, including KC-120, KC-121, KC-122 and KC-130 which were identified as *S. cavourensis* showed antifungal activity against *M. racemosus*. Strain KC-112 exhibited potent antifungal activity and exhibited awealkly ntimicrobial activity against *B. subtilis* and *K. rhizophila* and strain KC-122 and 130 exhibited also anti-*K. rhizophila* activity.

A *Streptomyces* strain in group XIX, KC-087 identified as *S. iranensis* inhibited *B. subtilis*, *E. coli*, *K. rhizophila* and *M. racemosus* when it was cultured in production medium no. 53, but not in production medium no. 51. Consequently, it showed anti antibacterial and antifungal activity.

The *Streptomyces* strains in group XX, KC-037 and KC-058 which identified as *S. rapamycinicus* showed potent antimicrobial activity against the almost test microorganisms such as *B. subtilis*, *Candida albicans*, *K. rhizophila*, *M. racemosus* and *X. campestris* pv. *oryzae*.

A *Streptomyces* strain in group XXI, KC-079 identified as *S. yatensis* demonstrated antimicrobial activity against *B. subtilis*, *Candida albicans*, *K. rhizophila*, *M. racemosus* and *X. campestris* pv. *oryzae*. From this result, it indicated that these strains showed anti-Gram positive bacterial, anti-rice pathogen and antifungal activity.

The *Streptomyces* strains in group XXII, including KC-080, KC-093, KC-094, KC-138 and KC-151 which were identified as *S. samsunensis* showed antimicrobial activity against *B. subtilis*, *Candida albicans*, *K. rhizophila* and *M. racemosus.* Furthermore, strain KC-138 exhibited potent antimicrobial activity against *X. campestris* pv. *oryzae*.

A *Streptomyces* strain in group XXIII, KC-145 identified as *S. cinereoruber* subsp. *cinereoruber* exhibited antimicrobial activity against *B. subtilis*, *E. coli*, *K. rhizophila* and *X. campestris* pv. *oryzae* when it was cultured in production medium no. 51 whereas the strain exhibited only antimicrobial activity against *B. subtilis* and *K. rhizophila*.

Based on antimicrobial activity, some *Streptomyces* strains showed antimicrobial activity when they were cultured in production media no. 51 or 53 whereas some strains showed antimicrobial activity when they were cultured in the both of media. Therefore, types of production media affect to produce secondary metabolites of the strains that showed different antimicrobial activity. From this research, it suggested that the primary screening of antimicrobial activity should be use various production media to obtain suitable the production medium for bioactive secondary metabolite production.

Table 4.20 Antimicrobial activity of *Streptomyces*, *Amycolatopsis* and *Kitasatospra* strains

	Strains	Inhibition zone (mm) of the strain											
		Cultured in production medium no. 51						Cultured in production medium no. 53					
		B	*C*	*E*	*K*	*M*	*X*	*B*	*C*	*E*	*K*	*M*	*X*
Streptomyces strains													
Group I	KC-047	-	-	-	-	-	-	-	-	-	-	-	-
	KC-054	-	-	-	-	-	-	-	-	-	-	-	-
	KC-066	14	-	-	14	-	-	9	-	-	-	-	-
	KC-074	-	-	-	-	-	-	-	-	-	-	-	-
	KC-075	13	-	-	13	-	-	10	-	-	10	-	-
	KC-076	-	-	-	-	-	-	-	-	-	-	-	-
	KC-095	18	-	-	16	-	-	15	-	-	12	-	-
	KC-096	-	-	-	-	-	-	-	-	-	-	-	-
	KC-097	11	-	-	-	-	-	11	-	-	-	-	-
	KC-102	23	-	-	19	-	-	15	-	-	13	-	-
Group II	KC-020	16	-	22	24	-	22	10	-	10	10	-	-
Group III	KC-072	-	-	-	-	-	-	-	-	-	-	-	-
Group IV	KC-073	13	-	-	9	-	-	11	-	-	11	-	-
	KC-155	15	-	-	14	-	-	9	-	-	-	-	-
Group V	KC-055	17	-	-	22	-	13	18	-	-	27	-	15
	KC-090	25	-	-	27	-	22	20	-	-	18	-	11
	KC-104	25	-	-	27	-	20	21	-	-	28	-	15
	KC-105	25	-	-	30	-	22	21	-	-	22	-	14
Group VI	KC-060	-	-	-	-	-	-	10	-	-	-	-	11
	KC-088	-	-	-	-	-	-	-	-	-	-	-	-
	KC-115	14	-	-	9	-	-	-	-	-	-	-	-
	KC-118	-	-	-	13	-	-	-	-	-	-	-	-
	KC-133	10	-	-	-	-	-	-	-	-	-	-	-
Group VII	KC-070	-	-	-	-	-	-	-	-	-	-	-	-
	KC-100	-	-	-	-	-	-	-	-	-	-	-	-
Group VIII	KC-110	-	-	-	-	-	-	-	-	-	-	-	-
	KC-111	-	-	-	-	-	-	-	-	-	-	-	-
	KC-112	10	-	-	-	-	-	-	-	-	-	-	-
	KC-135	-	-	-	-	-	-	-	-	-	-	-	-
	KC-136	-	-	-	-	-	-	-	-	-	-	-	-
Group IX	KC-062	-	-	-	-	-	-	10	-	-	-	-	11
	KC-063	-	-	-	-	-	-	10	-	-	-	-	14
Group X	KC-004	-	-	-	-	-	-	-	-	-	-	-	-
	KC-017	-	-	-	-	-	-	-	-	-	-	-	-
	KC-141	13	-	-	13	-	-	-	-	-	-	-	-
	KC-142	-	-	-	-	-	-	-	-	-	-	-	-
	KC-152	-	-	-	-	-	-	-	-	-	-	-	-
	KC-157	-	-	-	-	-	-	-	-	-	-	-	-
Group XI	KC-035	10	-	-	-	-	-	10	-	-	-	-	-
Group XII	KC-106	11	-	-	13	-	-	9	-	-	9	-	-
	KC-140	12	-	-	12	-	-	-	-	-	-	-	-
Group XIII	KC-119	10	-	-	12	-	-	17	-	-	16	-	13
	KC-124	11	-	-	12	-	-	16	-	14	20	-	20
	KC-125	10	-	-	-	-	-	-	-	11	-	-	-
	KC-134	-	-	-	-	-	-	-	-	-	-	-	-

Table 4.20 (continued)

	Strains	Inhibition zone (mm)											
		Cultured in production medium no. 51						Cultured in production medium no. 53					
		B	*C*	*E*	*K*	*M*	*X*	*B*	*C*	*E*	*K*	*M*	*X*
Streptomyces strains													
Group XIV	KC-031	-	-	-	-	-	-	-	-	-	-	-	-
	KC-032	-	-	-	-	-	-	-	-	-	-	-	-
	KC-033	-	-	-	-	-	-	-	-	-	-	-	-
	KC-038	-	-	-	-	-	-	-	-	-	-	-	-
	KC-150	-	-	-	-	-	-	-	-	-	-	-	-
	KC-156	-	-	-	-	-	-	-	-	-	-	-	-
Group XV	KC-085	-	-	-	-	-	-	-	-	-	-	-	-
	KC-098	10	-	-	10	-	-	-	-	-	-	-	-
	KC-101	11	-	-	-	-	-	-	-	-	-	-	-
	KC-103	10	-	-	10	-	-	-	-	-	-	-	-
Group XVI	KC-034	-	-	-	-	-	-	-	-	-	-	-	-
	KC-036	-	-	-	-	-	-	-	-	-	-	-	-
Group XVII	KC-117	26	-	-	30	-	21	22	-	-	26	-	19
	KC-128	-	-	-	-	-	-	-	-	12	-	-	-
Group XVIII	KC-120	-	-	-	-	20	-	-	-	-	-	21	-
	KC-121	-	-	-	10	21	-	9	-	-	12	21	-
	KC-122	-	-	-	9	20	-	-	-	-	-	16	-
	KC-130	-	-	-	11	20	-	-	-	-	12	18	-
Group XIX	KC-087	-	-	-	-	-	-	12	12	-	15	14	-
Group XX	KC-037	18	-	-	28	13	13	12	11	-	30	12	-
	KC-058	24	17	-	27	21	17	20	16	-	22	22	14
Group XXI	KC-079	17	16	-	11	19	15	12	12	-	15	14	-
Group XXII	KC-080	14	9	-	12	10	-	14	17	-	11	21	-
	KC-093	15	13	-	12	19	-	10	13	-	10	18	-
	KC-094	18	15	-	15	18	-	10	15	-	11	15	-
	KC-138	25	16	-	22	18	21	10	19	-	13	13	-
	KC-151	10	11	-	9	11	-	-	-	-	-	-	-
Group XXIII	KC-145	14	-	11	15	-	14	13	-	-	13	-	-
Group XXIV	KC-003	-	-	-	-	-	-	-	-	-	-	-	-
Kitastospora strains													
Group 1	KC-143	-	-	-	-	-	-	-	-	-	-	-	-
Group 2	KC-001	-	-	-	-	-	-	-	-	-	-	-	-
	KC-005	-	-	-	-	-	-	-	-	-	-	-	-
Amycolatopsis strain													
	KC-132	-	-	-	-	-	-	-	-	-	-	-	-

- = no inhibition zone, *B* = *Bacillus subtilis* ATCC 6633, *C* = *Candida albicans* KF1, *E* = *Escherichia coli* NIHJ KB213, *K* = *Kocuria rhizophila* ATCC 9341, *M* = *Mucor racemosus* IFO 4581, and *X* = *Xanthomonas campestris* pv. *oryzae* KB88

4.4 Secondary metabolites of the selected *Streptomyces* strains

After screening antimicrobial activity of all actinomycete strains, twenty four *Streptomyces* strains showing antimicrobial activity were selected for cultivation in production medium no. 54. The secondary metabolite profiles of the crude EtOAc extracts from these *Streptomyces* strains were analyzed by HPLC. Based on the HPLC chemical profiles compared with the BIOTEC database, two *Streptomyces* strains including *Streptomyces tendae* strain KC-097 and *Streptomyces cavourensis* strain KC-121 were selected for further 20-L large scale fermentation processes.

4.4.1 Isolation and structure elucidation of secondary metabolites from the *Streptomyces tendae* strain KC-097

The HPLC profile pattern of the crude EtOAc extract from *S. tendae* strain KC-097 showed the major peaks at RT 5.39 (peak A) and 5.73 (peak B) and the minor peaks at RT 6.233 (peak C) and 6.908 (peak D) (Figure 4.38). The peaks C and D exhibited the interesting UV spectra when compared with the BIOTEC database. This crude extract was subjected to purification by using TLC and column chromatographic techniques to achieve six pure compounds that were represented by the HPLC peaks at RT 5.425 (KC097-A), RT 5.783 (KC097-B), RT 6.25 (KC097-C), RT 6.26 (KC097-D), RT 6.975 (KC097-E), RT 5.77 (KC097-F) (Appendix E, Figure 1). The crude extract of these compounds showed no biological activities such as antibacterial, antifungal, antimalarial, antitubercular, anticancer and cytotoxic activities.

The chemical structures of these compounds were elucidated by analyses of mass and NMR spectra and were identified as phenethylacetamide (KC097-A), germicidin B (KC097-B), germicidin C or phomapyrone C (KC097-C), germicidin A (KC097-D), isogermicidin A (KC097-E) and acetyltryptamine (KC097-F). The weights of compounds KC097-A, KC097-B, KC097-C, KC097-D, KC097-E, and KC-097F were 175.4 mg, 2.3 mg, 8.4 mg, 28.0 mg, 1.8 mg and 32.9 mg, respectively. These pure compounds also exhibited no biological activities, except *N*-acetyltryptamine exhibited cytotoxicity against cancer cell NCI-H187 (IC_{50} 46.87 µg/ml).

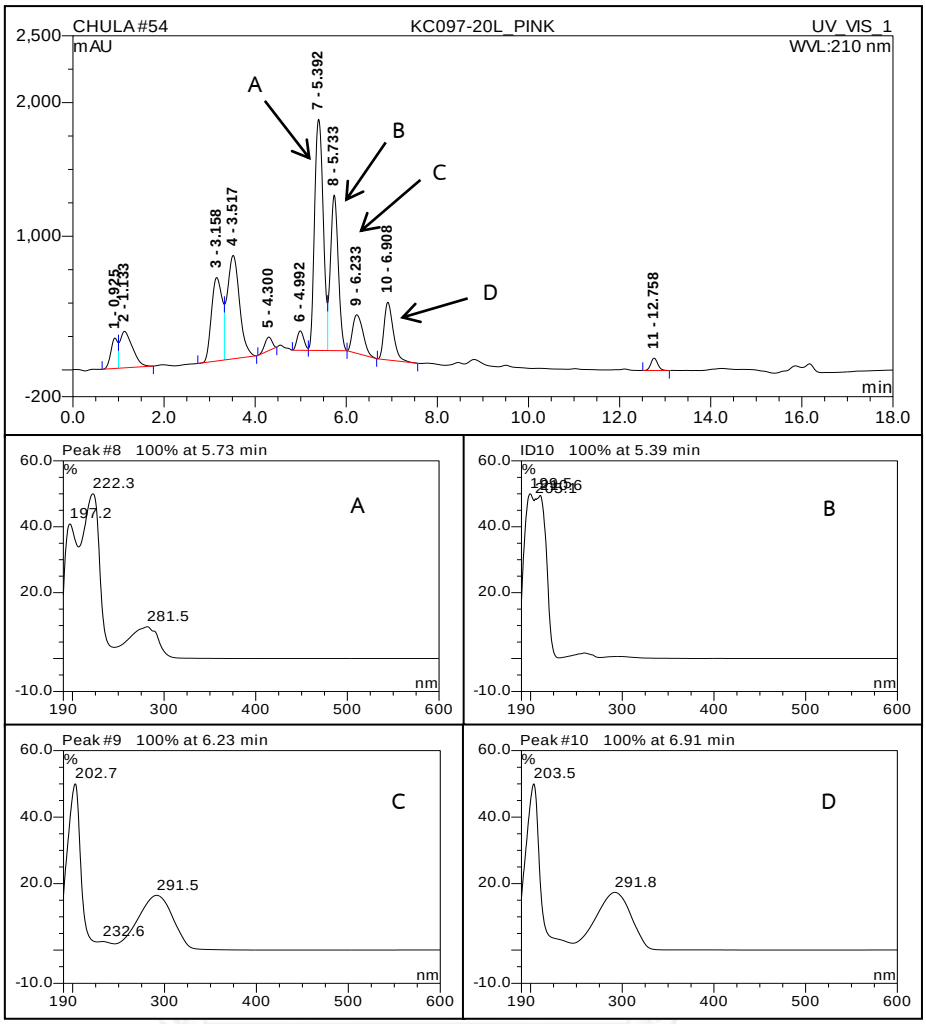


Figure 4.38 The HPLC profile of the EtOAc crude extract from *Streptomyces tendae* strain KC-097. HPLC conditions (column: C-18 column (5 µm), 2.1x50 mm; solvent: 0-100% CH_3CN in H_2O + 0.05% formic acid; flow rate: 0.5 ml/min for 18 min), A) The UV chromatogram of the peak at RT 5.39 min, B) The UV chromatogram of the peak at RT 5.73 min, C) The UV chromatogram of the peak at RT 6.23 min and D) The UV chromatogram of the peak at RT 6.90 min.

Compound KC097-A (Figure 4.39) was obtained as a yellow oil (175.4 mg) with the molecular formula of $C_{10}H_{13}NO$, determined by its HRESI mass spectrum showing a pseudomolecular ion at *m/z* 186.0888 $[M+Na]^+$ (Appendix E, Figure 2) and NMR data (Table 4.18).

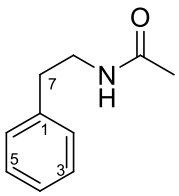


Figure 4.39 Chemical structure of KC097-A (phenethylacetamide)

The ^{1}H NMR spectrum of KC-097A (Appendix E, Figure 3) showed seven proton signals which were a singlet methyl at δ_H 1.91, two methylenes including one triplet at δ_H 2.81 and one quartet at δ_H 3.50, three methines including one doublet at δ_H 7.19 and two triplets at δ_H 7.23 and 7.31. In addition, the ^{1}H NMR spectrum showed a broad singlet amide proton at δ_H 5.79.

The ^{13}C NMR spectrum of KC-097A (Appendix E, Figure 4) revealed eight carbon signals which comprised one methyl carbon at δ_C 23.4 (C-11), two methylene carbons at δ_C 35.8 (C-7), 40.9 (C-8), three methine carbons at δ_C 128.9 (C-2 and C-6), 129.5 (C-3 and C-9), 126.7 (C-4), one quaternary carbon at δ_C 139.1 (C-1) and one amide carbonyl carbon at δ_C 170.5 (C-10). The ^{13}C and ^{1}H NMR spectral data are shown in Table 4.21.

Therefore, the ^{1}H NMR and ^{13}C NMR spectral data and molecular mass indicated the presence of one amide carbonyl and two methylenes, one methyl and one phenyl group. Based on the spectroscopic information, the structure of KC097-A was assigned as phenethylacetamide.

The previous study reported that phenethylacetamide was isolated from the culture of *Bacillus* sp. by Maskey *et al*. (2002). This compound had no antimicrobial and antimicroalgal activities.

Table 4.21 The ^{13}C and ^{1}H NMR spectral data (in $CDCl_3$) of KC097-A (phenethylacetamide)

Position	δ_C (ppm)	δ_H (ppm), multiplicity, (*J* in Hz)
1	139.1	-
2, 6	128.9	7.19, 2H, d (7.29)
3, 5	129.0	7.23, 2H, t (7.24)
4	126.7	7.31, 1H, t (7.38)
7	35.8	2.81, 2H, t (7.01)
8	40.9	3.50, 2H, q (6.63)
8-NH-CO-CH_3	-	5.79, 1H, br s
8-NH-CO-CH_3	170.5	-
8-NH-CO-CH_3	23.4	1.91, 3H, s

Compound KC097-B (Figure 4.40) was isolated as a colorless solid (2.3 mg). Its molecular formula was determined to be $C_{10}H_{14}O_3$ by a pseudomolecular ion $[M+Na]^+$ at *m/z* 205.0833 in the HRESI mass spectrum (Appendix E, Figure 5).

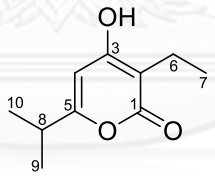


Figure 4.40 Chemical structure of KC097-B (germicidin B)

The ^{1}H NMR spectrum of KC097-B (Appendix E, Figure 6) revealed six proton signals containing, two methyls (δ_H 1.10 and 1.21), one methylene (δ_H 2.46), one methine (δ_H 2.70), one aromatic (δ_H 6.09) and one hydroxyl proton (δ_H 3.54).

The ^{13}C NMR and DEPT 135 spectra of KC097-B (Appendix E, Figures 7 and 8) exhibited eight carbon signals, which comprised three methyls at δ_C 12.5 (7-CH_3), 20.0 (9 and 10-CH_3), one methylene at δ_C 16.4 (6-CH_2), two methines at δ_C 98.1 (4-CH), 32.5 (8-CH) and three quaternary carbons at δ_C 167.0 (C-1), 104.7 (C-2) and 168.4 (C-3 and C-5). Inspection of 2D NMR spectra (HBQC, HMBC, COSY) (Appendix E, Figures 9-11)

readily revealed a germicidin-type structure of KC097-B. Consequently, the NMR spectral data (Table 4.22) indicated that KC097-B is germicidin B.

Aoki *et al*. (2011) reported that germicidin B was isolated from a liquid culture of *Streptomyces coelicolor* A3(2) and also extracted from *S. coelicolor* A3(2) spores. The compound inhibited germination of its own spores.

Table 4.22 The ^{13}C, ^{1}H, COSY and HMBC NMR spectral data (in $CDCl_3$) of KC097-B (germicidin B)

Position	δ_C (ppm)	δ_H (ppm), multiplicity (*J* in Hz)	COSY	HMBC (H to C)
1	167.0	-	-	-
2	104.7	-	-	-
3	168.4	-	-	-
4	98.1	6.09, 1H, s	-	2, 3, 5, 8
5	168.4	-	-	-
6	16.4	2.46, 2H, q (7.46)	7	1, 2, 7
7	12.5	1.10, 3H, t (7.46)	6	2, 6
8	32.5	2.70, 1H, septet (6.88)	9, 10	5, 9, 10
9, 10	20.0	1.21, 2 x 3H, d (6.88)	8	5, 8
3-OH	-	3.54, br s	-	-

Compound KC097-C was obtained as a white solid (8.4 mg). The molecular formula was established as $C_{10}H_{14}O_3$, an isomer of KC097-B, by giving a pseudomolecular mass peak at m/z 205.0849 $[M+Na]^+$ in the HRESI mass spectrum (Appendix E, Figure 12)

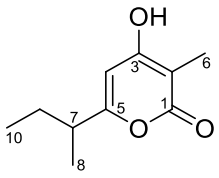


Figure 4.41 Chemical structure of KC097-C (germicidin C)

The ^{1}H NMR spectrum of KC097-C (Appendix E, Figure 14) showed seven proton signals which comprised one singlet aromatic at δ_H 6.09 (H -4), three methyls at δ_H 1.96 (H-6), δ_H 1.19 (H-8) and δ_H 0.87 (H-10), two methylenes at δ_H 1.52 and 1.68 (H-9) and one methine at δ_H 2.46 (H-7).

The ^{13}C NMR and DEPT 135 spectra of KC097-C (Appendix E, Figures 14 and 15) exhibited ten carbon signals, which comprised three methyls at δ_C 8.1 (C-6), 17.8 (C-8) and 11.5 (C-10), one methylene at δ_C 27.4 (C-9), two methines at δ_C 99.2 (C-4) and 39.7 (C-7) and four quaternary carbons at δ_C 167.5 (C-1), 98.6 (C-2), 165.2 (C-3) and 167.2 (C-5).

The 2D NMR spectra of KC097-C (HBQC, HMBC, COSY) (Appendix E, Figures 16-19) were similar to those of KC097-B. It indicated the same core structure, but they were different in the substituted groups at C-2 and C-5. KC097-C had a methyl group substituted at C-2 and an isobutyl group at C-5 whereas KC097-B had an ethyl group at C-2 and an isopropyl group at C-5. Therefore, the NMR spectral data (Table 4.23) suggested that KC097-C was identified as germicidin C or phomapyrone C as shown in Figure 4.41.

Germicindin C or phomapyrone C have been previously isolated from the blackleg fungus *Phoma lingam* (Pedras *et al*., 1994) and the sponge-derived fungus *Paecilomyces lilacinus* (Elbandy *et al*., 2009).

Table 4.23 ^{13}C, ^{1}H, COSY and HMBC NMR spectral data (in $CDCl_3$) of KC097-C (germicidin C)

Position	δ_C (ppm)	δ_H (ppm), multiplicity (*J* in Hz)	COSY	HMBC (H to C)
1	167.5	-	-	-
2	98.6	-	-	-
3	165.2	-	-	-
4	99.2	6.09, 1H, s	-	2, 5, 7
5	167.2	-	-	-
6	8.1	1.96, 3 H, s	-	1, 2, 3
7	39.7	2.46, 1H, sextet (6.80)	8, 9	4, 5, 8, 9, 10
8	17.8	1.19, 3 H, d (7.36)	7	5, 7, 9
9	27.4	1.52, 1H, m	7, 10	5, 7, 8, 10
		1.68, 1H, m	7, 10	5, 7, 8, 10
10	11.5	0.87, 3 H, t (7.36)	9	7, 9

Compound KC097-D (Figure 4.42) was isolated as a pale yellow solid (28.0 mg). The molecular of this compound was determined to be $C_{11}H_{16}O_3$ by its HRESI mass spectrum showing a pseudomolecular ion at *m/z* 195.1022 $[M-H]^-$ (Appendix E, Figure 19). Analyses of its ^{1}H, ^{13}C NMR, and 2D NMR spectra (Appendix E, Figures 19-25) suggested that KC097-D was similar to germicidin A (Table 4.24).

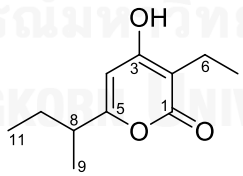


Figure 4.42 Chemical structure of KC097-D (germicidin A)

Germicidin A was first isolated from the submerge culture of *Streptomyces viridochromogenes* NRRL B-1551.T It had inhibitory effect on germination of its own arthrospores at a concentration as low as 200 pM (40 pg/ml) but did not inhibit the growth of various other Gram-positive and Gram-negative bacteria and various fungi (Petersen *et al*., 1993).

Moreover, germicidin A was also isolated from the endophytic *Streptomyces* sp. A00122 of Camptotheca acuminate (Li *et al*., 2013).

Aoki *et al*. (2011) reported that germicidins A, B, C and D were isolated from a liquid culture of *Streptomyces coelicolor* A3(2) and germicidins B, C and D were also extracted from *S. coelicolor* A3(2) spores which inhibited germination of *S. coelicolor* A3(2) spores. Furthermore, germicidin A inhibited not only spore germination but also hyphal elongation.

Table 4.24 ^{13}C, ^{1}H, COSY and HMBC spectral data of KC097-D and germicidin A

Position	KC097-D (in $CDCl_3$)				Germicidin A (in $CDCl_3$) (Petersen and Zähner, 1993)	
	δ_C (ppm)	δ_H (ppm), multiplicity, (*J* in Hz)	COSY	HMBC (H to C)	δ_C (ppm)	δ_H (ppm), multiplicity, (*J* in Hz)
1	168.6	-	-	-	176.5	-
2	104.9	-	-	-	104.7	-
3	167.3[a]	-	-	-	167.2	-
4	100.3	6.19, 1H, s	-	2, 3, 5, 6, 8	99.6	6.08, s
5	167.4[a]	-	-	-	165.9	-
6	16.6	2.49, 2H, q (7.40)	7	1, 2, 7	16.5	2.46, q (7.4)
7	12.7	1.10, 3H, t (7.40)	6	2, 6	12.5	1.08, t (7.4)
8	39.9	2.45, 1H, sextet (6.80)	9, 10	5, 9, 10, 11	39.7	2.41, m
9	18.0	1.18, 3H, d (6.92)	8	5, 8, 10	17.7	1.16, d (7.0)
10	27.6	1.50, 1H, m	8, 11	5, 8, 9, 11	27.4	1.49, m
		1.67, 1H, m	8, 11	5, 8, 9, 11		1.65, m
11	11.8	0.86, 3H, t (7.40)	10	8, 10	11.5	0.84, t (7.4)
3-OH	-	10.42, 1H, br s	-	-	-	ND

[a] = exchangeable, ND = not determined

Compound KC097-E (Figure 4.41) was obtained as a pale yellow solid (1.8 mg) with the molecular formula of $C_{11}H_{16}O_3$, an isomer of KC097-D, determined by its HRESIM mass spectrum showing a pseudomolecular ion at *m/z* 219.0999 $[M+Na]^+$ (Appendix E, figure 26).

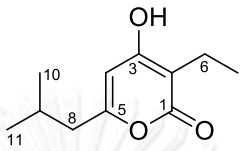


Figure 4.43 Chemical structure of KC097-E (isogermicidin A)

The ^{1}H NMR spectral data of KC097-E (Appendix E, Figure 27) showed six proton signals which comprised two methyls at δ_H 1.1 (C-7) and δ_H 0.94 (C-8), two methylenes at δ_H 2.46 (C-6) and 2.29 (C-8) and two methines at δ_H 5.93 (C-4) and 2.07 (C-9).

The ^{13}C NMR and DEPT 135 spectra of KC097-E (Appendix E, Figures 28-29) presented ten carbon signals, which comprised two methyls at δ_C 12.7 (C-7), 22.4 (C-8 and C-10), two methylenes at δ_C 16.7 (C-6) and 43.0 (C-8), two methines δ_C 101.0 (C-4) and 27.0 (C-9) and four quaternary carbons at δ_C 166.7 (C-1), 104.8 (C-2), 164.4 (C-3) and 163.2 (C-5).

The 2D NMR spectra of KC097-E (HMQC, HMBC, COSY) (Appendix E, Figures 30-32) were similar to those of KC097-A. It indicated that the same core structure, but they were different in a substituted group at C-5. KC097-E had a *gem*-dimethylethyl group substituted at C-5 whereas KC097-D had an isobutyl group at C-5. Therefore, the NMR spectral data (Table 4.25) suggested that KC097-E was identified as isogermicidin A as shown in Figure 4.43.

Isogermicidin A has been previously isolated from the culture supernatant of *Streptomyces coeilicolor* M145 by Song *et al*. (2006).

Table 4.25 ^{13}C, ^{1}H, COSY and HMBC NMR spectral data (in $CDCl_3$) of KC097-E (isogermicidin A)

Position	δ_C (ppm)	δ_H (ppm), multiplicity, (*J* in Hz)	COSY	HMBC (H to C)
1	166.7	-	-	-
2	104.8	-	-	-
3	164.4	-	-	-
4	101.0	5.93, 1H, s	-	2, 3
5	163.2	-	-	-
6	16.7	2.46, 2H, q (7.42)	7	1, 2, 3, 7
7	12.7	1.11, 3H, t (7.42)	6	2, 6
8	43.0	2.29, 2H, d (7.12)	9	4, 5, 9, 10, 11
9	27.0	2.07, 1H, m	8, 10, 11	-
10, 11	22.4	0.94, 2 x 3H, d (6.60)	9	8, 9

Compound KC097-F (Figure 4.44) was isolated as a white brown gum (32.9 mg). The molecular formula was established as $C_{12}H_{14}N_2O$ by giving a pseudomolecular mass peak at *m/z* 225.0995 $[M+Na]^+$ in the HRESI mass spectrum (Appendix E, Figure 33).

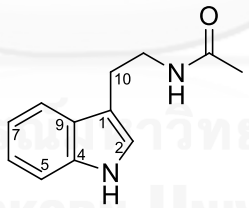


Figure 4.44 Chemical structure of KC097-F (*N*-acetyltryptamine)

The ^{1} H-NMR spectrum of KC097-F (Appendix E, Figure 34) revealed the presence of four aromatic protons at δ_H 7.59 (H-5), 7.09 (H-6), 7.01 (H-7) and 7.37 (H-8), one indole proton at δ_H 7.16, one methyl (H-2) at δ_H 1.86 (H-14) and two methylenes at δ_H 2.95 (H-10) and 3.48 (H-11).

The ^{13}C-NMR spectrum of KC097-F (Appendix E, Figure 35) exhibited 12 carbon signals, which comprised one methyl at δ_C 22.3 (C-14), two methylenes at δ_C 25.7 (C-10) and 40.1 (C-11), five methines at δ_C 118.7 (C-5), 111.5 (C-6), 111.4 (C-7), 111.6 (C-8)

and four quaternary carbons at δ_C 121.4 (C-1), 122.6 (C-2), 115.3 (C-9), 169.3 (C-13). The NMR spectral data of KC097-F (Table 4.26) were identified as *N*-acetyltryptamine.

KC097-F (*N*-acetyltryptamine) exhibited cytotoxicity against cancer cell NCI-H187 (IC_{50} 46.87 µg/ml)

This compound was known as a secondary metabolite which was isolated from different species of plants (*Prosopis nigra*; Liguminosae) (Maeda *et al*., 1993) as well as from bacteria such as myxobacterium *Archangium gephyra* strain Ar T205 (Shaaban *et al*., 2007) and an actinobacterium *Streptoverticillium* sp. IPV-2793 (Brambilla *et al*., 1995). Recently, Mehdi *et al*. (2009) isolated *N*-acetyltryptamine from *Streptomyces* sp. strain TN58. This compound showed antifungal activity against filamentous fungi but not against the unicellular fungus *Candida tropicalis* R2 CIP203 and also showed antibacterial activity against Gram-positive bacterium *Micrococcus luteus*. Furthermore, *N*-acetyltryptamine was isolated from the fermentation broth of *Streptomyces djakartensis* NW35. It exhibited significant antibacterial activity against *Bacillus subtilis*, *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas syringae* pv. actinidiae and MRSA (Zhange *et al*, 2013).

Table 4.26 ^{13}C, ^{1}H, COSY and HMBC NMR spectral data of compound KC097-F and *N*-acetyltryptamine

Position	KC097-F (in acetone-d_6)		*N*-acetyltryptamine (in $CDCL_3$) (Zhang *et al*., 2013)	
	δ_C (ppm)	δ_H (ppm), multiplicity, (*J* in Hz)	δ_C (ppm)	δ_H (ppm), multiplicity, (*J* in Hz)
1	121.4	-	128.8	-
2	122.4	7.16, 1H, s	123.3	7.05, 1H, s
3	NH	10.04, 1H, br s	NH	-
4	122.6	-	138.2	-
5	118.7	7.59, 1H, d (7.88)	122.3	7.54, 1H, d (9.0)
6	111.5	7.09, 1H, t (7.50)	119.2	7.06, 1H, t (8.0)
7	111.4	7.01, 1H, t (7.42)	112.2	6.99, 1H, t (8.0)
8	111.6	7.37, 1H, d (8.08)	119.6	7.31, 1H, d (7.5)
9	115.3	-	122.3	-
10	25.7	2.95, 2H, t (7.34)	22.6	2.92, 2H, t (7.0)
11	40.1	3.48, 2H, q (6.68)	41.6	3.45, 2H, t (7.0)
11-NH-CO-CH_3	-	-	-	-
11-NH-CO-CH_3	169.3	-	173.2	-
11-NH-CO-CH_3	22.3	1.86, 3H, s	22.6	1.89, 1H, s

4.4.2 Isolation and structure elucidation of secondary metabolites from the *Streptomyces cavourensis* strain KC-121

The crude EtOAc extract of *Streptomyces cavourensis* strain KC-121 comprised two interesting peaks at RT 11.39 min. (peak A) and RT 13.62 min. (peak B) in the determined HPLC chromatogram (Figure 4.43). These peaks were revealed the interesting UV spectra profiles compared with the BIOTEC database (Figure 4.45). The crude extract showed various biological activities including antitubercular activity against *Mycobacterium tuberculosis* strain H37Ra with MIC at 25.00 µg/ml, antimalarial activity against *Plasmodium falciparum* K1 with IC_{50} at 0.19 µg/ml, cytotoxicity against Vero cells with IC_{50} at 0.34 µg/ml, cytotoxicity against cancer cells including KB, MFC-7 and NCI-H187 cells with IC_{50} at 28.03, 3.83 and 0.30 µg/ml, respectively. The crude extract was purified by using column chromatographic techniques to obtain two pure compounds which were KC121-A (bafiomycin D) and KC121-B (21-*O*-methyl-bafilomycin A_1)

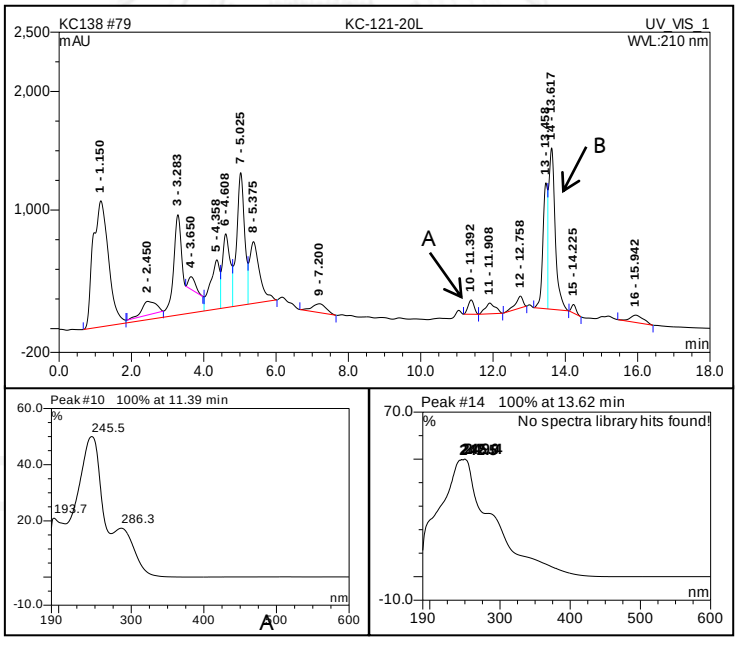


Figure 4.45 The HPLC profile of the crude EtOAC extract from *Streptomyces cavourensis* strain KC-121. HPLC conditions (column: C-18 column (5 µm), 2.1x50 mm; solvent: 0-100% CH_3CN in H_2O + 0.05% formic acid; flow rate: 0.5 ml/min for 18 min), A) The UV chromatogram of the peak at RT 11.39 min. and B) The UV chromatogram of the peak at RT 13.62 min.

Compound KC121-A was obtained in the form of a yellow solid (3.6 mg). Its molecular formula was determined as $C_{35}H_{56}O_8$ based on the HRESI mass data with a pseudomolecular ion peak of $[M+Na]^+$ at *m/z* 627.3865 (Appendix E, Figure 36).

The structure of KC121-A was elucidated by extensive analyses of ^1H, ^{13}C NMR and 2D NMR spectroscopic information (Appendix E, Figures 37-43).

The ^{13}C NMR and DEPT 135 spectra of KC121-A (Appendix E, Figures 38-39) showed 35 carbon signals corresponding to two methoxy carbons (2-OCH_3 and 14-OCH_3), nine methyl carbons (C-25, C-26, C-27, C-28, C-29, C-30, C-31, C-32, and C-33), one methylene carbons (C-9), six methine carbons (C-6, C-8, C-16, C-18, C-22, and C-24), seven olefinic carbons (C-3, C-5, C-11, C-12, C-13, C-20, and C-21), five oxymethine carbons (C-7, C-14, C-15, C-17, and C-23), three olefinic quaternary carbons (C-2, C-4, and C-10) and two carbonyl carbons (C-1 and C-19) as presented in Table 4.27.

The ^1H and ^{13}C NMR spectral data of KC121-A (Table 4.24) showed similar assignments to those of bafilomycin D (Yu *et al*., 2011**).** Therefore, KC121-A was elucidated as bafilomycin D as shown in Figure 4.46.

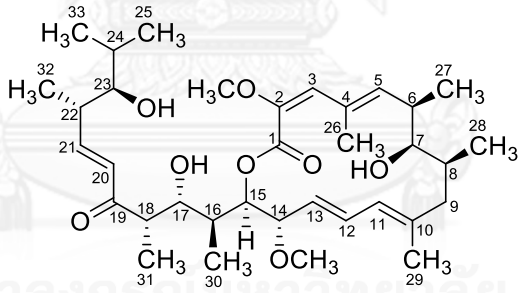


Figure 4.46 Chemical structure of KC121-A (bafiomycin D)

Bafilomycin D was previously isolated from the culture of *Streptomyces griseus* Tü 2559 and showed antibacterial activity against Gram-positive bacteria including *Enterococcus* sp. IBC 27101, *Enterobacter* sp. ACTT 9790 and *Staphylococcus aureus* FK 422, fungicidal activity against *Phythium ultimum*, *Mycosphaerella musae* and *Fusarium nivale* and insecticidal activity against *Plutella maculipensis* (lepidoptera), *Phaedon cochleariae* (coleoptera), *Dysdercus intermedius* (heteroptera) and *Ceratiits capitata* (diptera) (Kretschmer *et al*., 1985).

Recently, Yu *et al*. (2011) reported that the compound was also produced by the endophytic *Streptomyces* sp. YIM56209. It exhibited potent cytotoxicity against A-549 human lung adenocarcinoma and HT-29 human colorectral adenocarcinoma cancer cell lines.

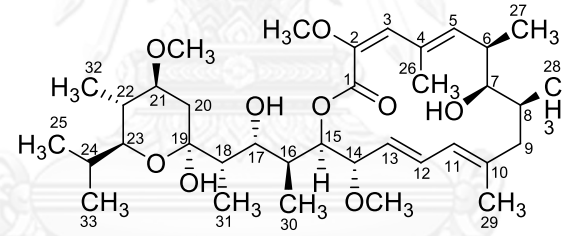
Table 4.27 The ^{13}C, ^{1}H, COSY and HMBC NMR spectral data of KC121-A and bafilomycin D

Position	KC121-A (in acetone-d_6)				Bafilomycin D (in $CDCl_3$) Yu *et al*., 2011	
	δ_C (ppm)	δ_H (ppm), multiplicity, (*J* in Hz)	COSY	HMBC (H to C)	δ_C (ppm)	δ_H (ppm), multiplicity, (*J* in Hz)
1	166.3	-	-	-	166.7	-
2	141.5	-	-	-	141.7	-
3	132.2	6.66, s	-	1, 2, 5, 27	133.2	6.64, s
4	131.7	-	-	-	133.0	-
5	143.7	5.94, d (8.9)	6	3, 27	142.6	5.76, d (9.0)
6	37.3	2.54, m	5	5, 28	37.1	2.52, m
7	79.6	3.32, m	-	-	81.5	3.30, d (7.0)
8	40.2	1.89, m	7, 9	-	40.1	1.91, m
9	41.5	2.05, 2H, m	8	10, 11	41.6	2.12, m and 1.99, m
10	143.2	-	-	-	143.1	-
11	124.5	5.81, d (12.9)	12	9, 30	125.5	5.81, d (11.0)
12	132.9	6.64, dd (15.4, 12.9)	11, 13	11, 14	133.1	6.48, dd (15.0, 10.5)
13	126.2	5.19, dd (15.3, 8.1)	12, 14	11, 14	127.3	5.17, dd (15.0, 9.0)
14	83.3	4.01, t (8.1)	13, 15	12, 16, 31	83.6	3.82, dd (8.5, 8.0)
15	76.0	5.16, d (8.1)	14	1, 14, 32	76.6	5.06, d (8.5)
16	38.8	2.05, m	15, 17	-	38.8	2.06, m
17	72.0	3.81, m	16, 18	-	72.9	3.76, m
18	45.9	3.01, dd (6.9, 3.3)	17	-	46.5	2.98, m
19	201.5	-	-	-	203.4	-
20	128.5	6.29, d (15.8)	21	19, 22	129.6	6.28, d (15.5)
21	148.7	6.93, dd (15.8, 8.7)	20, 22	19	148.8	6.91, dd (16.0, 8.5)
22	40.1	2.52, m	23, 34	21	40.3	2.52, m
23	79.2	3.14, t (5.6)	20, 24	-	80.0	3.18, t (6.0)
24	31.4	1.62, sext (6.7)	23, 25, 35	-	31.2	1.72, m
25	19.0	0.89, 3H, d (6.7)	24	23, 24, 35	17.1	0.92, d (7.0)
26	13.2	1.97, 3H, s	-	3, 4, 5	14.2	1.98, s
27	17.2	1.05, 3H, d (8.6)	6	5, 6, 7	17.7	1.08, d (6.5)
28	21.8	0.93, 3H, d (6.0)	8	7, 8, 9	22.2	0.94, d (7.5)
29	19.0	1.89, 3H, s	-	9, 10, 11	20.2	1.91, s
30	10.2	0.98, 3H, d (7.0)	16	15, 16,17	11.0	0.94, d (6.5)
31	8.6	1.09, 3H, d (6.9)	18	17, 18, 19	10.5	1.21, d (7.0)
32	16.9	1.05, 3H, d (7.2)	22	21, 22, 23	16.9	1.08, d (7.0)
33	17.1	0.91, 3H, d (6.4)	24	23, 24, 25	19.9	0.94, d (6.5)
2-O$\underline{CH_3}$	59.2	3.64, 3H, s	-	2	60.4	3.68, s
14-O$\underline{CH_3}$	54.9	3.22, 3H, s	-	14	55.9	3.22, s

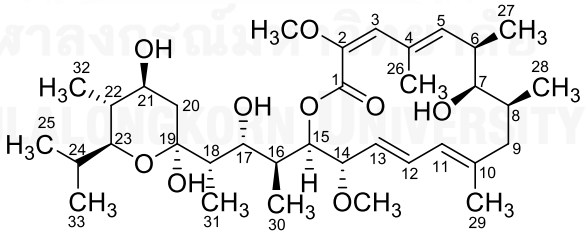
Compound KC121-B was isolated as a white solid (1.2 mg) and was determined to have the molecular formula $C_{36}H_{60}O_9$ based on the HRESI mass data with a pseudomolecular ion peak of $[M+Na]^+$ at m/z 659.4133 and the NMR data (Appendix E, Figures 44-51).

The ^{13}C NMR and DEPT 135 spectra of KC121-B revealed 36 carbon signals for two methoxyls (2-OCH_3, 14-2-OCH_3), nine methyls (C-25, C-26, C-27, C-28, C-29, 30, 31, 32 and 33), two methylenes (C-9 and C-20), seventeen methines (C-3, C-5, C-6, C-7, C-8, C-11, C-12, C-13, C-14, C-15, C-16, C-17, C-18, C-21, C-22, C-23 and C-24) and five quaternary carbons (C-1, C-2, C-4, C-10, C-19). Inspection of the NMR data revealed a bafilomycin-type 16-membered macrolide (Table 4.28).

The ^{1}H and ^{13}C spectra of KC121-B showed similar patterns to those of bafilomycin-A_1 (Michael *et al*., 1997) except one methoxyl at δ_H 3.27 and δ_C 55.4 connected to C-21 according to the HMBC data. Thus, the structure of KC121-B was determined to be 21-(*O*-methyl)-bafilomycin A_1 (Michael *et al*., 1997). The chemical structures of KC121-B and bafilomycin-A_1 are shown in Figure 4.47



(A)



(B)

Figure 4.47 Chemical structures of KC121-B (A) and bafilomycin A_1 (B)

Table 4.28 ^{13}C, 1H, COSY and HMBC spectral data of KC121-B (21-*O*-methyl-bafilomycin A_1) and Bafilomycin A_1

Position	KC121-B (in acetone-d_6)				Bafilomycin A_1 (in $CDCl_3$) Werner & Hagenmaier, 1984	
	δ_C (ppm)	δ_H (ppm), multiplicity, (*J* in Hz)	COSY	HMBC (H to C)	δ_C (ppm)	δ_H (ppm), multiplicity, (*J* in Hz)
1	166.2	-	-	-	167.3	-
2	141.3	-	-	-	141.3	-
3	132.7	6.70, s	-	1, 2, 5, 27	141.3	6.71, d (0.7)
4	131.7	-	-	-	143.0	-
5	144.3	5.96, d (9.0)	6	3, 27	125.1	5.79, d (9.2)
6	37.2	2.53, m	5, 28	-	36.8	2.55, m
7	79.4	3.31, m	-	-	81.0	3.29, m
8	40.5	1.88, m	7	-	40.1	1.91, dq (11.5, 6.5)
9	41.5	2.05, m	-	7, 8, 10, 11	41.3	2.15, dm (14.0) 1.94, dd (14.0, 11.5)
10	143.5	-	-	-	132.8	-
11	124.5	5.81, d (10.5)	12	-	142.7	5.82, dm (10.5, 1.2)
12	133.1	6.66, dd (15.1, 10.5)	11, 13	-	133.0	6.53, dd (15.0, 10.5)
13	126.4	5.17, dd (15.1, 9.1)	12	11	127.0	5.15, dd (15.0, 9.5)
14	83.1	4.03, d (9.1)	15	15, 31	82.3	3.89, dd (9.5, 8.7)
15	76.7	5.11, dd (7.9, 1.3)	14	1, 14	76.8	4.96, dd (8.7, 1.2)
16	39.1	2.05, m	17	-	37.2	2.12, dqd (11.0, 6.7, 1.2)
17	69.5	3.53, m	16	-	70.6	4.13, ddd (11.0, 4.1, 2.0)
18	38.5	2.12, m	-	19, 33	42.1	1.76, qm (7.2, 1-2)
19	103.1	-	-	-	98.9	-
20	34.5	2.42, dd (13.4, 4.6)	21	-	43.5	2.30, dd (11.9, 4.7)
21	79.6	3.31, m	20	-	70.8	3.69, ddd (11.0, 9.9, 4.7)
22	45.8	3.08, m	-	-	41.0	1.33, ddq (10.2, 9.9, 6.5)
23	77.3	3.08, m	-	19	75.9	3.48, dd (10.2, 2.2)
24	28.2	1.31, m	-	-	27.9	1.89, sept.d (6.7, 2.2)
25	13.6	0.88, d (6.6)	-	23, 24, 36	12.2	0.90, d (6.7)
26	13.2	1.98, d (1.0)	-	3, 4, 5	14.0	1.98, d (1.2)
27	16.9	1.05, d (7.0)	6	5, 6, 7	17.2	1.06, d (7.0)
28	21.6	0.92, d (6.8)	-	7, 8, 9	14.5	0.93, d (6.5)
29	19.2	1.93, s	-	9, 10, 11	20.1	1.93, s
30	10.2	0.96, d (7.0)	-	15, 16, 17	9.8	0.83, d (6.7)
31	7.0	0.97, d (7.0)	-	17, 18, 19	7.0	1.04, d (7.2)
32	11.6	0.87, d (6.2)	-	21, 23	21.7	0.94, d (6.5)
33	20.1	1.03, d (6.8)	-	23, 24, 25	21.2	0.76, d (6.7)
C2-O$\underline{CH_3}$	59.3	3.66, m	-	2	59.9	3.63, s
C14-O$\underline{CH_3}$	54.8	3.22, s	-	14	55.5	3.24, s
C21-O$\underline{CH_3}$	55.4	3.27, s	-	21	-	-

Since the limited amount of KC121-A and KC121-B, the compounds were not determined biological activity. However, the original fraction KC121F2F3 containing KC121-A and KC121-B exhibited various biological activities including, antimalarial activity against *Plasmodium falciparum* K1 with IC_{50} value at 0.21 µg/ml, cytotoxicity against Vero cells with IC50 values at 0.37 µg/ml, cytotoxicity against cancer cells including KB, MFC-7 and NCI-H187 with IC_{50} values at 18.39, 4.18 and 0.23 µg/ml, respectively. This fraction had no antitubercular activity against *Mycobacterium tuberculosis* strain H37Ra when compared with the crude extract.

CHAPTER V
CONCLUSION

In this research, seventy nine actinomycetes were isolated from twenty nine soil samples collected from Krung Ching Waterfall National Park, Angthong Islands National Park and Similan Islands National Park, in the southern part of Thailand by using starch casein nitrate agar and potato starch-glycerol agar complemented with certain antibiotics. On the basis of their phenotypic characteristics and partial 16S rRNA gene sequences analysis, these actinomycete strains were preliminary identified as the members of the genera *Streptomyces* (seventy four isolates), *Amycolatopsis* (one isolate), *Kitasatospora* (three isolates) and *Nonomuraea* (one isolate). Seventy four *Streptomyces* isolates were divided into twenty four species, three *Kitasatospora* isolates were divided into two species and the remaining two isolates were a species of *Amycolatopsis* and *Nonomuraea*.

Strains KC-031, KC-035, KC-038, KC-106 and KC-112 contained LL-DAP in cell wall peptidoglycan. These strains had MK-9(H_6) MK-9(H_4) and MK-9(H_8) as predominant menaquinones in cell membrane. They contained diphosphatidyl-glycerol (DPG), phosphatidylethanolamine (PE), phosphatidylglycerol (PG), phospha-tidylinositol (PI) and phosphatidylmethylinosides (PIMs) as phospholipid compositions. The *N*-acyl type of muramic acid was acetyl. Strains KC-031, KC-035, KC-038 and KC-112 produced spiral spore chains with smooth surface spores and strain KC-106 produced spiral spore chains with hairy surface spores. The chemotaxonomic and phenotypic features of these five strains were consistent with the genus *Streptomyces*. However, strains KC-112 (Group VIII), KC-035 (Group XI), KC-106 (Group XII) and KC-031 and KC-038 (Group XIV) exhibited less than 98.8% similarity of 16S rRNA gene sequences when compared with their closely related type strains. Therefore, the strains were proposed as novel *Streptomyces* species as follows, strains KC-031 and KC-038 as *S. siamensis*, strain KC-106 as *S. similanensis*, strain KC-035 as *S. krungchingensis* and strain KC-112 as *S. andamanensis*.

Strain KC-061 exhibited the spore morphology different from the closely related type strains of *Nonomuraea*. Therefore, this strain was subjected to complete taxonomic analysis, and was proposed as a novel species, namlely *N. thailandensis*. Strain KC-061 contained *meso*-DAP isomer as diagnostic diamino acid and galactose, mannose, madurose and ribose as diagnostic sugars in whole cells. The *N*-acyl type of muramic acid was acetyl. The predominant menaquinone was MK-9(H_4). Diphosphatidylglycerol (DPG), phosphatidylethanolamine (PE), phosphatidylmono-methylethanolamine (PME), hydroxylphosphatidylethanolamine (OH-PE), hydroxyl-phosphatidylmonomethylethanolamine (OH-PME), phosphatidylglycerol (PG), ninhydrin positive phosphoglycolipid and an unknown phospholipid (PL1) were detected as phospholipids.

In summary, *S. tendae, S. enissocaesilis, S. enissocaesilis, S. marokkonensis*, *S. parvulus*, *S. malachitospinus*, *S. diastaticus* subsp. *ardesiacus*, *S. spiralis*, *S. aureus*, *S. violarus*, *S. drozdowiczii*, *S. exfoliatus*, *S. sindenensis*, *S. cavourensis, S. iranensis*, *S. rapamycinicus*, *S. yatensis*, *S. samsunensis*, *S. cinereoruber* subsp. *cinereoruber*, *S. misakiensis* and four novel species including *S. siamensis*, *S. similanensis*, *S. krungchingensis* and *S. andamanensis* are distributed in soil samples collected in the southern Thailand. In addition, *A. keratiniphila* subsp. *keratiniphila*, *K. saccharophila* and a novel *N. thalandensis* are also distributed in soils.

In the part of screening antimicrobial activity, forty five isolates which were cultivated in production media no. 51 and 53 showed antimicrobial activity against *Kocuria rhizophila* ATCC 9341, *Bacillus subtilis* ATCC 6633, *Escherichia coli* NIHJ KB213, *Xanthomonas campestris* pv. *oryzae* KB88, *Candida albicans* KF1 and *Mucor racemosus* IFO 4581^T. Among them, twenty four *Streptomyces* were selected to cultivate in production medium no. 54 and their crude EtOAc extracts were analyzed for chemical profiles by HPLC. The results showed that the crude extracts of *S. tendae* strain KC-097 and *S. cavourensis* strain KC-121 showed interesting chemical profiles and were selected for secondary metabolite study. After chromatographic isolation, six pure compounds, including *N*-acetyltryptamine (KC097-A), phenethylacetamide (KC097-B), germicidin B (KC097-C), germicidin C (KC097-D), germicidin A (KC097-E) and isogermicidin A (KC097-F) were obtained from *S. tendae*

strain KC-097 and two pure compounds, including bafilomycin D (KC121-A) and 21-(*O*-methyl)-bafilomycin A_1 (KC121-B) from *S. cavourensis* strain KC-121. The pure compounds which obtained from *S. tendae* strain KC-097 exhibited no biological activities including anti-*B. cereus* ATCC 11778, *Candida albicans* ATCC 90028, antimalarial activity against *P. falciparum* K1, antitubercular activity against *M. tuberculosis* strain H37Ra, cytotoxicity against KB, MCF-7, NCI-H187 and Vero cells, except *N*-acetyltryptamine exhibited cytotoxicity against cancer cell NCI-H187 (IC_{50} 46.87 µg/ml). Compounds KC121-A and KC121-B obtained from *S. cavourensis* strain KC-121 were not tested for biological activities due to limited amount of the compounds. However, the original fraction KC121F2F3 containing the isolated compounds exhibited antimalarial activity against *Plasmodium falciparum* K1 (IC_{50} 0.21 µg/ml), cytotoxicity against Vero cells (IC_{50} 0.37 µg/ml) and cytotoxicity against cancer cells (KB; IC_{50} 18.39 µg/ml, MFC-7; IC_{50} 4.18 µg/ml and NCI-H187; IC_{50} 0.23 µg/ml).

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APPENDICES

APPENDIX A

Culture media

Starch casein nitrate agar

Soluble starch	10.0	g
Sodium caseinate	0.3	g
KNO_3	2.0	g
Agar	15.0	g
Distilled water	1	l
pH 7.0		

Potato starch-glycerol agar

Potato starch	10.0	g
Glycerol	10.0	g
K_2HPO_4	2.0	g
$(NH_4)_2SO_4$	2.0	g
$MgSO_4.7H_2O$	1.0	g
NaCl	1.0	g
$CaCO_3$	2.0	g
Agar	12.0	g
Distilled water	1	l
pH 7.0		

Yeast extract-malt extract agar (ISP medium 2, ISP 2)

Yeast extract	4.0	g
Malt extract	10.0	g
Glucose	4.0	g
Agar	15.0	g
Distilled water	1	l
pH 7.2-7.4		

Oatmeal agar (ISP medium 3, ISP 3)

Oatmeal	20.0	g
Trace salt solution	1.0	ml
Agar	18.0	g
Distilled water	1	l
pH 7.2		

Inorganic salts-starch agar (ISP medium 4, ISP 4)

Soluble starch	10.0	g
K_2HPO_4	1.0	g
$MgSO_4.7H_2O$	1.0	g
NaCl	1.0	g
$(NH_4)_2SO_4$	2.0	g
$CaCO_3$	2.0	g
Trace salt solution	1.0	ml
Agar	20.0	g
Distilled water	1	l
pH 7.0-7.4		

Glycerol-asparagine agar (ISP medium 5, ISP 5)

L-asparagine	1.0	g
Glycerol	10.0	g
K_2HPO_4	1.0	g
Trace salt solution	1.0	ml
Agar	20.0	g
Distilled water	1	l
pH 7.0-7.4		

Peptone-yeast extract iron agar (ISP medium 6, ISP 6)

Bacto-peptone iron agar, dehydrated (Difco)	36.0	g
Yeast extract	1.0	g
Distilled water	1	l
pH 7.0-7.2		

Tyrosine agar (ISP medium 7, ISP 7)

Glycerol	15.0	g
L-Tyrosine (Difco)	0.5	g
L-asparagine (Difco)	1.0	g
K_2HPO_4	1.0	g
$MgSO_4.7H_2O$	0.5	g
NaCl	0.5	g
$FeSO_4.7H_2O$	0.01	g
Trace salt solution	1.0	ml
Agar	20.0	g
Distilled water	1	l
pH 7.2-7.4		

Carbon utilization medium (ISP medium 9, ISP 9)

$(NH_4)_2SO_4$	2.64	g
KH_2PO_4	2.38	g
K_2HPO_4	5.65	g
$MgSO_4.7H_2O$	1.0	g
*Solution	1.0	ml
Agar	20.0	g
Distilled water	1	l
pH 6.8-7.0		

***Solution**

$CuSO_4.5H_2O$	0.64	g
$FeSO_4.7H_2O$	0.11	g
$MnCl2._4H_2O$	0.79	g
$ZnSO.7H_2O$	0.15	g
Distilled water	100	ml

Starch-yeast extract agar

Soluble starch	10.0	g
Yeast extract	2.0	g
Agar	15.0	g
Distilled water	1	l
pH 7.0		

Nutrient agar (NA)

Meat extract	10.0	g
Peptone	10.0	g
Agar	15.0	g
Distilled water	1	l
pH 7.0-7.2		

Yeast extract-glucose broth

Yeast extract	10.0	g
Glucose	10.0	g
Distilled water	1	l
pH 7.0		

301 Seed medium

Soluble starch	24.0	g
Glucose	1.0	g
Peptone	3.0	g
Meat extract	3.0	g
Yeast extract	5.0	g
$CaCO_3$	4.0	g
Distilled water	1	l
pH 7.0		

Production medium no. 51

Glucose	5.0	g
Corn steep powder	5.0	g
Oat meal	10.0	g
Pharma media	10.0	g
K_2HPO_4	5.0	g
$MgSO_4.7H_2O$	5.0	g
Trace metal solution	1.0	ml
Tap water	1	l
pH 7.0		

Production medium no. 53

Glycerol	20.0	g
Soluble starch	20.0	g
Nutrient broth	20.0	g
Defatted wheat germ	10.0	g
$CaCO_3$	3.0	g
Tap water	1	l
pH 7.0		

Production medium no. 54

Soluble starch	20.0	g
Glycerol	5.0	g
Defatted wheat germ	10.0	g
Meat extract	3.0	g
Yeast extract	3.0	g
$CaCO_3$	3.0	g
Tap water	1	l

pH 7.0

APPENDIX B

Reagents and buffers

6N HCl

Conc. HCl	60	ml
Distilled water	60	ml

Add conc. HCl into the distilled water.

2N H_2SO_4

Conc. H_2SO_4	2	ml
Distilled water	34	ml

Add conc. H_2SO_4 into the distilled water.

Aniline-butanol-phthalate reagent

Aniline	2	ml
Phthalic acid	3.25	g
Water-saturated *n*-butanol	100	ml

DON reagent

2, 7-Dihydroxynapthalene	10	mg
Conc. H_2SO_4	50	ml

Add conc. H_2SO_4 into 2,7-Dihywdroxynapthalene (DON) wait until the yellow solution to colorless (24 h). Keep this solution in refrigerator.

5% Trichloro acetic acid

Trichloro acetic acid	5	g
Distilled water	100	ml

Add conc. Trichloro acetic acid into the distilled water.

Nitrate reduction test reagents

Sulphanilic acid solution

Sulphanilic acid	0.8	g
5N Acetic acid	100	ml

***N*,*N*-dimethyl-1-naphthylamine solution**

N,*N*-dimethyl-1-naphthylamine	0.5	g
5N Acetic acid	100	ml

Reagents for fatty acid analysis

Sponification reagent

Sodium hydroxide (NaOH)	15	g
Methanol (HPLC grade)	50	ml
Milli-Q water	50	ml

Dissolve NaOH in Milli-Q water and add methanol.

Methylation reagent

6N HCl	65	ml
Methanol (HPLC grade)	55	ml

Adjust pH to below 1.5.

Extraction solvent

n-Hexane (HPLC grade)	50	ml
Methyl-3-butyl ether (HPLC grade)	50	ml

Base washing reagent

Sodium hydroxide	1.2	g
Milli-Q water	100	ml

Saturated sodium chloride solution

Sodium chloride saturated in Milli-Q

Reagents for polar lipid analysis

Anisaldehyde reagent

Ethanol	90	ml
Conc. H_2SO_4	5.0	ml
p-Anisaldehyde	5.0	ml
Acetic acid	1.0	ml

Dragendroff's reagent

Solution A

Basic bismuth nitrate	1.7	g
Acetic acid	20	ml
Distilled water	80	ml

Solution B

KI	40	g
Distilled water	100	ml

Before spraying, solution A (10 ml) plus with solution B (10 ml) and acetic acid (10 ml).

Dittmer & Lester reagent

Solution A

MoO_3	4.011	g
25N H_2SO_4	100	ml

Dissolve MoO_3 into 25N H_2SO_4 and heat.

Solution B

Molybdenum powder	0.178	g
Solution A	50	ml

Add molybdenum powder into solution A and boil it for 15 min. After cooling, remove the precipitation by decantation. Before spraying, mix solution A (50 ml) plus solution B (50 ml) and plus distilled water (50 ml)

Ninhydrin solution

Ninhydrin	0.3	g
n-Butanol	100	ml
Glacial acetic acid	3	ml

RNase A solution

RNase A	20	ng
0.15 M NaCl, pH 5.0	10	ml

Dissolve RNase A in 0.15 M NaCl, pH 5.0 and heat at 95 °C for 5-10 min. Keep RNase A solution at -20 °C

RNase T solution

RNase T	800	U
0.1 M Tris-HCl (pH 7.2)	1	ml

Mix RNase T in 0.1M Tris-HCl (pH 7.2) and heat at 95°C for 5 min. Keep RNase T solution at -20°C

Proteinase K

Proteinase K (Sigma)	4	mg
50 M Tris-HCl (pH 7.5)	1	ml

Use freshy prepared solution.

Nuclease P1 solution

Nuclease P1	0.1	mg
40 mM CH_3COONa	0.5	ml
12 mM $ZuSO_4$ (pH 5.3)	0.5	ml

Keep at 4 °C

40 mM CH_3COONa

CH_3COONa	3.2812	g
Distilled water	1	l

12 mM $ZuSO_4$ (pH 5.3)

$ZuSO_4$ (anhydrous)	1.9376	g
Distilled water	1	l
Adjust pH to 5.3		

Alkaline phosphatase solution

Alkaline phosphatase	2.4	U
0.1M Tris-HCl (pH 8.0)	1	ml

0.1 M Tris-HCl buffer, pH 9.0

Tris base	12.1	mg

Dissolve Tris base in distilled water. Stir solution and monitor the pH with a pH probe while adding conc. HCl to adjust the pH 9.0. Make up the solution to 1 l with distilled water and autoclave. Store it at room temperature.

1 M Tris-HCl buffer, pH 8.0

Tris base	121.1	mg

Dissolve Tris base in distilled water. Stir solution and monitor the pH with a pH probe while adding conc. HCl to adjust the pH 8.0. Make up the solution to 1 l with distilled water and autoclave. Store it at room temperature.

1 mM Saline-EDTA (Na_2-EDTA) pH 8.0

EDTA	0.29	g

Dissolve EDTA in 900 ml of distilled water. Stir solution and monitor the pH with a pH probe while adding NaOH pellets to adjust the pH 8.0. Make up the solution to 1 l with distilled water and autoclave.

3 M Sodium acetate

Sodium acetate trihydrate ($CH_3COONa.3H_2O$)	408.0	g

Dissolve $CH_3COONa.3H_2O$ in 400 ml of distilled water. Stir solution and monitor the pH with a pH probe while adding glacial acetic acid to adjust the pH 5.2. Make up the solution to 1 l with distilled water and autoclave.

TE buffer

10 mM Tris-HCl (pH 8.0)	10	ml
1 mM Na_2-EDTA (pH 8.0)	10	ml
Distilled water	980	ml

Sterilize the solution by autoclaving

1 mM Na_2-EDTA (pH 8.0)

EDTA	292.24	g

Dissolve EDTA in 700 ml of distilled water. Stir solution and monitor the pH with a pH probe while adding NaOH pellets to adjust the pH 8.0. Dilute the solution to 1 l with distilled water and autoclave. Store it at room temperature.

0.5 M EDTA (pH 8.0)

EDTA	186.1	g

Dissolve EDTA in 800 ml of distilled water. Stir solution and monitor the pH with a pH probe while adding NaOH pellets to adjust the pH 8.0. Dilute the solution to one liter with distilled water and autoclave. Store it at room temperature.

50X Tris-acetate (TAE) buffer

Tris Base	242.28	g
Glacial acetic acid	57.1	ml
0.5M EDTA (pH 8.0)	100	ml

Dissolve Tris Base in 600 ml of distilled water. Stir solution and add glacial acetic acid and 0.5M EDTA (pH 8.0) solution. Make up the volume to 1000 ml with distilled water. Autoclave and store at room temperature.

1X Tris-acetate (TAE) buffer

50X Tris-acetate (TAE) buffer	20	ml
Distilled water	980	ml

Ethidium bromide solution (10 mg/ml)

Ethidium bromide	1	g

Dissolve ethidium bromide in 100 ml of distilled water

0.8% Agarose gel

Agar rose	0.8	g
Distilled water	100	ml

Mix agarose and distilled water and melt the mixture with the microwave.

10% Sodium dodecyl sulphate (SDS)

Sodium dodecyl sulphate	10	g
Sterile distilled water	100	ml

Reagents and buffers for DNA-DNA hybridization

Pre-hybridization solution (10 ml)

20X SSC	1	ml
50X Denhardt's solution	1	ml
Formamide	5	ml
Sonicated salmon sperm DNA (10 mg/ml)	0.1	ml
Distilled water	2.9	ml

Hybridization solution (10 ml)

20X SSC	1	ml
50X Denhardt's solution	1	ml
Formamide	5	ml
Sonicated salmon sperm DNA (10 mg/ml)	0.1	ml
50% Dextran sulphate solution	0.5	ml
Distilled water	2.4	Ml

PBS-BSA-Triton solution (10 ml)

BSA (Bovine serum albumin)	0.05	g
Triton X	10	µl
20X PBS	0.5	ml
Distilled water	9.5	ml

SABG (Streptoavidin-β-galactosidase) solution (10 ml)

PBS-BSA-Triton solution	10	ml
SABG	10	µl

4-MUF (4 methyllumbelliferyl-*β*-D-galactoside) solution

4-MUF (10 mg/ml)	100	µl
1X PBS	10	ml

Freshly prepare

4-MUF (10 mg/ml)

4-MUF	1	mg
N-*N*-dimethylformamide	100	Ml

20X Phosphate buffered saline (PBS)

Na_2HPO_4	28.8	g
NaCl	160.0	g
KH_2PO_4	4.0	g
KCl	4.0	g

Dissolve Na_2HPO_4, NaCl, KH_2PO_4 and KCl in 800 ml of distilled water. Adjust to pH 7.2-7.4 with NaOH and volume to 1000 ml. Autoclave and store at room temperature.

20X Phosphate buffered saline (PBS)

Na_2HPO_4	28.8	g
NaCl	160.0	g
KH_2PO_4	4.0	g
KCl	4.0	g

1X Phosphate buffered saline (PBS)

20X PBS	50	ml
Sterile distilled water	950	ml

1M Magnesium chloride ($MgCl_2$)

$MgCl_2$	92.5	g

Dissolve $MgCl_2$ in distilled water and adjust volume to 1 l

Phosphate buffered saline-magnesium chloride (PBSMG) solution (10 ml)

20X PBS	0.5	ml
1M $MgCl_2$	1	ml
Distilled water	8.5	ml

Salmon sperm DNA (10 mg/ml)

Salmon sperm DNA	10	mg
TE buffer	1	ml

Dissolve salmon sperm DNA in TE buffer, boil the solution for 10 min, immediately cool in ice and sonicate for 3 min.

20X Saline sodium citrate (SSC)

NaCl	175.3	g
Sodium citrate	88.2	g
Distilled water	1	L

Dissolve NaCl and Sodium citrate in 700 ml of distilled water. Adjust pH to 7.0 with NaOH, adjust volume to 1000 ml and sterilize by autoclaving.

1X Saline sodium citrate (SSC)

20X SSC	50	ml
Sterile distilled water	950	ml

APPENDIX C

Cultural, physiological and biochemical characteristics

Table 1 Cultural characteristics of *Streptomyces* strains in group I

Media	Strains				
	KC-047	KC-054	KC-066	KC-074	KC-075
ISP 2					
Growth	Good	Good	Good	Good	Good
	Pale yellowish green	Pale greenish yellow - brilliant greenish yellow	Pale greenish yellow -light greenish yellow	Pale greenish yellow	Pale greenish yellow - light greenish yellow
Reverse	Grayish greenish yellow - moderate olive	Brilliant greenish yellow	Pale greenish yellow - light greenish yellow	Pale greenish yellow - moderate olive	Pale greenish yellow - moderate olive
Aerial mycelium	Abundant, white - bluish gray	Abundant, white	Moderate, bluish gray	Abundant, white - bluish gray	Abundant, white - bluish gray
Soluble pigment	None	None	None	None	None
ISP 3					
Growth	Good	Good	Good	Good	Good
	Grayish greenish yellow	Grayish greenish yellow	Pale greenish yellow	Grayish greenish yellow	Grayish greenish yellow
Reverse	Pale yellowish green - grayish yellowish green	Grayish greenish yellow - moderate olive	Pale greenish yellow - moderate olive	Grayish greenish yellow - moderate olive	Grayish greenish yellow - moderate olive
Aerial mycelium	Abundant, white -medium gray	Abundant, white - bluish gray	Abundant, white - bluish gray	Abundant, white - bluish gray	Abundant, white - bluish gray
Soluble pigment	None	None	None	None	None
ISP 4					
Growth	Good	Good	Good	Good	Good
	Grayish greenish yellow	Grayish greenish yellow	Pale yellowish green	Pale greenish yellow	Grayish greenish yellow
Reverse	grayish olive	Dark yellow	Grayish olive	Dark olive brown	Grayish greenish yellow
Aerial mycelium	Abundant, white - medium gray	Abundant, white - medium gray	Abundant, white - medium gray	Abundant, white - light gray	Abundant, white - medium gray
Soluble pigment	None	None	None	None	None
ISP 5					
Growth	Good	Good	Good	Good	Good
	Pale yellow	Pale yellow	Pale greenish yellow - grayish greenish yellow	Pale yellowish green	Pale yellow - dark olive brown
Reverse	Pale yellowish green - grayish yellowish green	Olive gray	Pale greenish yellow - moderate olive	Grayish greenish yellow - dark olive	Light grayish olive - grayish olive
Aerial mycelium	Abundant, white - bluish gray	Abundant, white - bluish gray	Abundant, white - bluish gray	Abundant, light greenish gray - greenish gray	Abundant, white - bluish gray
Soluble pigment	None	None	None	None	None
ISP 6					
Growth	Good	Good	Good	Good	Good
	Pale greenish yellow - light yellow	Pale greenish yellow - grayish greenish yellow	Pale greenish yellow - dark yellow	Pale greenish yellow - light yellow	Pale greenish yellow
Reverse	Light yellow	Grayish greenish yellow	Light yellow	Light yellow	Pale greenish yellow
Aerial mycelium	Moderate, white	None	None	Poor, white	None
Soluble pigment	None	None	None	None	None
ISP 7					
Growth	Good	Good	Good	Good	Good
	Pale yellowish green	Grayish greenish yellow - light olive	Moderate yellow - strong yellow	Moderate yellow - dark yellow	Dark yellow - dark grayish yellowish brown
Reverse	Pale yellowish green - dark grayish yellow	Pale greenish yellow - moderate olive	Moderate yellow - moderate yellowish brown	Moderate yellow - moderate yellowish brown	Grayish yellow - dark olive brown
Aerial mycelium	Abundant, white - bluish gray	Abundant, white - bluish gray	None	None	Grayish yellow - dark olive brown
Soluble pigment	None	None	None	None	None
YS agar					
Growth	Good	Good	Good	Good	Good
	Pale greenish yellow	Pale greenish yellow	Pale greenish yellow - light yellowish green	Pale greenish yellow - light yellowish green	Pale greenish yellow - grayish greenish yellow
Reverse	Pale yellowish green - grayish yellowish green	Pale greenish yellow	Pale greenish white - dark olive	Pale greenish white - dark olive	Pale greenish yellow - dark olive
Aerial mycelium	Abundant, white - bluish gray	Abundant, light greenish yellow	Abundant, white - bluish gray	Abundant, white - bluish gray	Abundant, greenish white - bluish gray
Soluble pigment	None	None	None	None	None
Nutrient agar					
Growth	Good	Good	Good	Good	Good
	Pale yellowish green	Pale greenish yellow	Pale greenish yellow	Pale greenish yellow	Pale greenish yellow
Reverse	Pale yellowish green - grayish yellowish green	Pale greenish yellow	Pale greenish yellow - pale yellowish green	Pale greenish yellow - pale yellowish green	Pale yellowish green - Grayish yellowish green
Aerial mycelium	Abundant, greenish white - bluish gray	Abundant, bluish gray	Abundant, greenish white - bluish gray	Abundant, greenish white - bluish gray	Abundant, greenish white - bluish gray
Soluble pigment	None	None	None	None	None

Table 1 (continued)

Media	Strains				
	KC-076	KC-095	KC-096	KC-097	KC-102
ISP 2					
Growth	Good	Good	Good	Good	Good
	Pale greenish yellow	Pale yellow	Grayish yellow	Pale yellow - light yellowish brown	Moderate olive brown
Reverse	Pale greenish yellow - dark yellow	Pale yellow - moderate olive brown	Grayish olive	Pale greenish yellow - light grayish yellowish brown	Light grayish olive - light yellow
Aerial mycelium	Abundant, light bluish gray - bluish gray	Abundant, white - bluish gray	Abundant, medium gray	Abundant, white	Abundant, medium gray
Soluble pigment	None	None	None	None	None
ISP 3					
Growth	Good	Good	Good	Good	Good
	Pale greenish yellow	Grayish greenish yellow	Light grayish olive	Greenish white	Light grayish olive
Reverse	Moderate olive	Light grayish olive	Grayish olive	Grayish olive	Grayish olive
Aerial mycelium	Abundant, white - medium gray	Abundant, white - medium gray	Abundant, white - medium gray	Abundant, greenish white – medium gray	Abundant, bluish gray
Soluble pigment	None	None	none	None	None
ISP 4					
Growth	Good	Good	Good	Good	Good
	Grayish greenish yellow	Grayish greenish yellow	Light grayish yellowish brown	Grayish yellow	Light grayish olive
Reverse	Light grayish olive	Dark yellow	Light grayish olive	Grayish yellow	Dark olive brown
Aerial mycelium	Abundant, white - medium gray	Abundant, white - medium gray	Abundant, medium gray	Abundant, greenish white	Abundant, pale blue - medium gray
Soluble pigment	None	None	None	None	None
ISP 5					
Growth	Good	Good	Good	Good	Good
	Pale yellowish green	Yellowish white - dark grayish yellow	Dark olive brown	Greenish white	Pale yellowish green - moderate olive brown
Reverse	Pale yellow - dark olive brown	Light grayish olive - grayish olive	Pale greenish yellow - grayish olive	Greenish white	Pale yellowish green - dark olive brown
Aerial mycelium	Abundant, white - bluish gray	Abundant, white - bluish gray	Abundant, greenish white	Abundant, greenish white	Moderate, bluish gray
Soluble pigment	None	None	None	None	None
ISP 6					
Growth	Good	Good	Good	Good	Good
	Pale greenish yellow - light yellow	Pale greenish yellow	Light yellow - moderate yellow	Brilliant yellow	Dark yellow - grayish greenish yellow
Reverse	Light yellow	Light yellow	Light yellow - dark yellow	Light yellow	Grayish greenish yellow
Aerial mycelium	None	Poor, white	Moderate, white - light bluish gray	None	Poor, white
Soluble pigment	None	None	None	None	None
ISP 7					
Growth	Good	Good	Good	Good	Good
	Pale yellowish green - dark yellow	Dark grayish yellow	Grayish yellow - dark olive brown	Grayish yellow	Grayish yellow - dark olive brown
Reverse	Dark grayish yellow	Dark grayish yellow	Dark grayish olive	Dark grayish yellow	Dark olive brown
Aerial mycelium	Abundant, white	Abundant, white - bluish gray	Abundant, light greenish gray	Abundant, light gray	Abundant, light greenish gray
Soluble pigment	None	None	None	None	None
YS agar					
Growth	Good	Good	Good	Good	Good
	Pale greenish yellow	Dark grayish yellow	Greenish white - grayish yellow	Greenish white	Moderate olive brown - dark olive brown
Reverse	Pale greenish yellow	Yellowish white - dark grayish yellow	Pale yellowish green - grayish olive	Grayish greenish yellow - olive gray	Light grayish olive - grayish olive
Aerial mycelium	Abundant, bluish gray	Abundant, greenish white - greenish gray	Abundant, medium gray - dark gray	Abundant, white - medium gray	Abundant, bluish gray
Soluble pigment	None	None	None	None	None
Nutrient agar					
Growth	Good	Good	Good	Good	Good
	Pale greenish yellow	Pale yellowish green	Grayish yellowish brown	Colorless	Grayish yellowish brown
Reverse	Pale greenish yellow	Light grayish olive	Grayish olive	Light grayish olive	Light olive gray
Aerial mycelium	Greenish white	Abundant, greenish white - bluish gray	Abundant, medium gray	Abundant, medium gray	Abundant, medium gray
Soluble pigment	None	None	None	None	None

Table 2 Physiological and biochemical characteristics of *Streptomyces* strains in group I

Characteristics	Strains				
	KC-047	KC-054	KC-066	KC-074	KC-075
Utilization of					
L-Arabinose	+	+	+	+	+
D-Fructose	+	+	±	+	+
D-Glucose	+	+	+	+	+
D-Mannitol	+	+	+	+	+
D-Melibiose	-	-	-	-	-
Melezitose	-	-	-	-	-
myo-Inositol	+	+	+	+	+
Raffinose	-	-	-	-	-
L-Rhamnose	+	+	+	+	+
D-Sorbitol	-	-	-	-	-
Sucrose	-	-	-	-	-
D-Xylose	+	+	±	+	+
Nitrate reduction	+	+	+	+	+
Starch hydrolysis	+	+	+	+	+
Milk peptonization	-	-	-	-	-
Milk coagulation	-	-	-	-	-
Gelatinization	-	-	-	-	-
Growth at/with:					
NaCl (%w/v)	0-8	0-8	0-7	0-9	0-7
pH	5-12	5-12	5-12	5-12	5-12
Temperature (°C)	15-37	15-40	15-37	15-37	15-40

+ = positive, ± = weakly positive, - = negative

Table 2 (continued)

Characteristics	Strains				
	KC-076	KC-095	KC-096	KC-097	KC-102
Utilization of					
L-Arabinose	+	+	+	+	+
D-Fructose	+	+	+	+	+
D-Glucose	+	+	+	+	+
D-Mannitol	+	+	+	+	+
D-Melibiose	-	-	-	-	-
Melezitose	-	-	-	-	-
myo-Inositol	+	+	+	+	+
Raffinose	-	-	-	-	-
L-Rhamnose	+	+	+	+	+
D-Sorbitol	-	-	-	-	-
Sucrose	-	-	-	-	-
D-Xylose	+	+	+	+	+
Nitrate reduction	+	-	+	-	+
Starch hydrolysis	+	+	+	+	+
Milk peptonization	-	-	-	-	-
Milk coagulation	-	-	-	-	-
Gelatinization	-	-	-	-	-
Growth at/with:					
NaCl (%)	0-7	0-10	0-10	0-9	0-10
pH	5-12	5-12	5-12	5-12	5-12
Temperature (°C)	15-40	15-40	15-37	15-37	15-40

+ = positive, ± = weakly positive, - = negative

Table 3 Cultural characteristics of *Streptomyces* strains in group II, III and IV

Media	Strains			
	KC-020	KC-072	KC-073	KC-155
ISP 2				
Growth	Good	Good	Good	Good
	Moderate yellow	Grayish greenish yellow	Pale yellow	Pale yellow
Reverse	Moderate yellow	Light grayish olive - grayish olive	Pale yellow	Pale yellow - pale yellowish green
Aerial mycelium	Abundant, white	Abundant, white - bluish gray	None	None
Soluble pigment	None	None	None	None
ISP 3				
Growth	Good	Good	Good	Good
	Grayish yellow - moderate olive brown	Grayish yellow - dark grayish yellow	Yellowish white - pale orange yellow	Pale orange yellow
Reverse	Grayish yellow - dark yellowish brown	Dark grayish yellow - dark olive brown	Yellowish white - pale orange yellow	Pale orange yellow
Aerial mycelium	Abundant, white	Abundant, light greenish gray - greenish gray	Abundant, greenish white	Moderate, greenish white
Soluble pigment	None	Grayish yellow	None	None
ISP 4				
Growth	Good	Good	Good	Good
	Light yellowish brown - moderate yellowish brown	Grayish greenish yellow	Strong yellowish brown	Light brown
Reverse	Light grayish yellowish brown - grayish yellowish brown	Grayish greenish yellow - light grayish olive	Moderate yellowish brown	Moderate brown
Aerial mycelium	Abundant, light bluish gray - bluish gray	Abundant, bluish gray	Abundant, greenish white - light olive gray	Moderate, greenish white
Soluble pigment	Grayish yellow	None	Pale orange yellow	Pale orange yellow
ISP 5				
Growth	Good	Good	Good	Good
	Pale yellowish green	Light olive gray	Pale orange yellow - light yellowish brown	Light orange yellow
Reverse	Greenish white - pale yellowish green	Moderate yellowish brown - dark yellowish brown	Pale orange yellow - light yellowish brown	Light yellowish brown
Aerial mycelium	Abundant, greenish white	Abundant, greenish - bluish gray	Abundant, greenish white - light greenish gray	Abundant, greenish white - light greenish gray
Soluble pigment	None	Grayish yellow	Pale orange yellow	Pale orange yellow
ISP 6				
Growth	Moderate yellow	Good	Good	Good
	Grayish greenish yellow	Grayish greenish yellow	Grayish greenish yellow	Grayish greenish yellow
Reverse	Yellowish white - light yellow	Light yellow	Grayish greenish yellow	Grayish greenish yellow
Aerial mycelium	Moderate, white	Abundant, white	None	None
Soluble pigment	None	None	None	None
ISP 7				
Growth	Good	Good	Good	Good
	Pale orange yellow	Moderate yellowish brown - deep yellowish brown	Pale orange yellow - light yellowish brown	Grayish yellow - light grayish yellowish brown
Reverse	Grayish yellow	Grayish yellowish brown - dark grayish yellowish brown	Pale orange yellow - Light yellowish brown	Grayish yellow - moderate yellowish brown
Aerial mycelium	None	Abundant, white - bluish gray	Abundant, light olive gray	Abundant, light olive gray
Soluble pigment	None	Light yellowish brown	Pale orange yellow	Pale orange yellow
YS agar				
Growth	Good	Good	Good	Good
	Pale yellow	Dark grayish yellow	Light yellowish brown	Moderate yellow
Reverse	Pale yellow	Dark yellow - dark grayish yellow	Light grayish yellowish brown	Moderate yellow
Aerial mycelium	None	Abundant, white - bluish gray	Moderate, light gray	Moderate, greenish white - light greenish gray
Soluble pigment	None	Pale yellowish green	Pale orange yellow	Pale orange yellow
Nutrient agar				
Growth	Good	Good	Good	Good
	Grayish yellow	Light grayish olive	Grayish yellow	Grayish greenish yellow
Reverse	Grayish yellow	Light grayish olive - brownish black	Grayish yellow	Pale yellow
Aerial mycelium	Abundant, white	Abundant, white - medium gray	Abundant, greenish white	Abundant, greenish white
Soluble pigment	None	Grayish greenish yellow	None	None

Table 4 Physiological and biochemical characteristics of *Streptomyces* strains in group II, III and IV

Characteristics	Strains			
	KC-020	KC-072	KC-073	KC-155
Utilization of:				
L-Arabinose	+	+	+	+
D-Fructose	+	+	-	-
D-Glucose	+	+	+	+
D-Mannitol	+	+	+	+
D-Melibiose	±	+	-	-
Melezitose	-	-	-	-
myo-Inositol	+	+	±	+
Raffinose	±	+	±	±
L-Rhamnose	+	-	+	+
D-Sorbitol	-	-	-	-
Sucrose	-	+	-	-
D-Xylose	±	+	-	-
Nitrate reduction	-	-	+	+
Starch hydrolysis	+	+	+	+
Milk peptonization	+	-	-	+
Milk coagulation	-	-	-	-
Gelatinization	-	-	-	-
Growth at/with:				
NaCl (%w/v)	0-7	0-10	0-4	0-5
pH	5-12	5-12	5-12	5-11
Temperature (°C)	15-40	15-40	15-40	15-40

+ = positive, ± = weakly positive, - = negative

Table 5 Cultural characteristics of *Streptomyces* strains in group V

Media	Strains			
	KC-055	KC-090	KC-104	KC-105
ISP 2				
Growth	Good	Good	Good	Good
	Pale yellow	Grayish greenish yellow	Light yellow	Pale yellow
Reverse	Pale yellow - grayish yellow	Grayish greenish yellow	Light yellow	Pale yellow
Aerial mycelium	None	None	None	None
Soluble pigment	None	Grayish greenish yellow	None	None
ISP 3				
Growth	Good	Good	Good	Good
	Pale yellowish green - light grayish olive	Strong greenish yellow	Light olive brown	Strong greenish yellow
Reverse	Pale yellowish green - moderate olive	Brilliant greenish yellow - strong greenish yellow	Deep yellow	Moderate greenish yellow
Aerial mycelium	Abundant, greenish white - light greenish gray	Abundant, light bluish gray	Abundant, light greenish gray - greenish gray	Abundant, greenish white - bluish gray
Soluble pigment	Pale yellowish green	Light greenish yellow	Vivid greenish yellow	Light yellowish green
ISP 4				
Growth	Good	Good	Good	Good
	Dark grayish yellowish brown	Dark yellow	Strong greenish yellow	Greenish yellow
Reverse	Pale yellow - light grayish olive	Light grayish olive	Strong greenish yellow - moderate olive	Light olive brown
Aerial mycelium	Abundant, light greenish gray - medium gray	Abundant, medium gray	Abundant, greenish white - medium gray	Abundant, bluish gray
Soluble pigment	None	Pale yellowish green	Brilliant greenish yellow	Pale greenish yellow
ISP 5				
Growth	Good	Good	Good	Good
	Light grayish olive	Light grayish olive	Pale greenish yellow	Pale greenish yellow - light olive
Reverse	Pale yellow - light grayish olive	Light grayish olive	Pale greenish yellow - grayish greenish yellow	Pale yellowish green - grayish greenish yellow
Aerial mycelium	Abundant, light bluish gray	Abundant, light bluish gray	Abundant, grayish yellowish green	Poor, greenish white
Soluble pigment	Grayish yellow	Pale yellowish green	None	None
ISP 6				
Growth	Good	Good	Good	Good
	Grayish greenish yellow	Grayish greenish yellow	Grayish greenish yellow	Grayish greenish yellow
Reverse	Grayish greenish yellow	Grayish greenish yellow	Grayish greenish yellow	Grayish greenish yellow
Aerial mycelium	None	None	Poor, white	None
Soluble pigment	None	None	None	None
ISP 7				
Growth	Good	Good	Good	Good
	Grayish yellow	Grayish greenish yellow	Pale greenish yellow - grayish greenish yellow	Pale yellowish green
Reverse	Light grayish olive	Light grayish olive	Pale yellow - moderate olive	Pale yellowish green - grayish yellowish green
Aerial mycelium	Abundant, dark grayish yellow	Abundant, greenish yellow	Abundant, grayish yellowish green	Abundant, greenish white - light greenish gray
Soluble pigment	Pale yellowish green	None	Light yellowish green	None
YS agar				
Growth	Good	Good	Good	Good
	Pale yellowish green	Colorless	Strong yellow	Light olive brown
Reverse	Light yellow - deep yellow	Light yellow	Strong yellow - deep yellow	Abundant, greenish white - greenish gray
Aerial mycelium	Abundant, greenish white - light greenish gray	Abundant, white	Abundant, greenish white - greenish gray	Strong yellowish brown
Soluble pigment	Light yellow	Pale greenish yellow	Vivid yellow	Vivid yellow
Nutrient agar				
Growth	Good	Good	Good	Good
	Grayish greenish yellow - light olive gray	Grayish greenish yellow	Grayish greenish yellow	Grayish greenish yellow
Reverse	Grayish greenish yellow - moderate olive	Pale greenish yellow - grayish greenish yellow	Light greenish yellow - light olive	Pale yellowish green - moderate olive
Aerial mycelium	Abundant, greenish white - bluish gray	Abundant, white	Abundant, greenish white - medium gray	Abundant, white - bluish gray
Soluble pigment	Grayish greenish yellow	None	Strong greenish yellow	Grayish greenish yellow

Table 6 Physiological and biochemical characteristics of *Streptomyces* strains in group V

Characteristics	Strains			
	KC-055	KC-090	KC-104	KC-105
Utilization of:				
L-Arabinose	+	+	+	+
D-Fructose	+	+	+	+
D-Glucose	+	+	+	+
D-Mannitol	+	+	+	+
D-Melibiose	-	-	-	-
Melezitose	-	-	-	-
myo-Inositol	+	+	+	+
Raffinose	±	±	+	±
L-Rhamnose	+	+	+	+
D-Sorbitol	-	-	-	-
Sucrose	±	±	+	+
D-Xylose	+	+	+	+
Nitrate reduction	-	-	-	-
Starch hydrolysis	+	+	+	+
Milk peptonization	-	-	-	-
Milk coagulation	+	+	+	-
Gelatinization	-	-	-	-
Growth at/with:				
NaCl (%w/v)	0-8	0-8	0-4	0-8
pH	5-11	5-11	5-11	5-11
Temperature (°C)	20-37	20-37	20-37	20-37

+ = positive, ± = weakly positive, - = negative

Table 7 Cultural characteristics of *Streptomyces* strains in group VI

Media	Strains				
	KC-060	KC-088	KC-115	KC-118	KC-133
ISP 2					
Growth	Good	Good	Good	Good	Good
	Pale yellow	Grayish greenish yellow	Pale yellow	Grayish yellow	Pale yellow
Reverse	Pale yellow	Grayish greenish yellow	Pale yellow	Moderate olive	Pale yellow - grayish yellow
Aerial mycelium	None	None	None	Abundant, dark greenish gray	None
Soluble pigment	None	None	None	None	None
ISP 3					
Growth	Good	Good	Good	Good	Good
	Pale yellowish green	Grayish yellow - light grayish yellowish brown	Pale orange yellow	Grayish greenish yellow	Grayish greenish yellow
Reverse	Pale yellowish green	Light yellowish brown - light grayish yellowish brown	Pale orange yellow	Grayish greenish yellow - strong yellowish green	Pale yellow - grayish yellow
Aerial mycelium	Abundant, white - light greenish gray	Abundant, white - light greenish gray	Abundant, white	Abundant, bluish gray	Abundant, light greenish gray - dark greenish gray
Soluble pigment	None	Pale orange yellow	Pale orange yellow	None	None
ISP 4					
Growth	Good	Good	Good	Good	Good
	Grayish yellow	Pale yellowish green	Light grayish olive	Grayish greenish yellow	Pale yellow
Reverse	Grayish yellow	Light grayish olive	Light grayish olive	Grayish greenish yellow - light grayish olive	Pale yellow - dark yellow
Aerial mycelium	Abundant, white - greenish gray	Abundant, greenish gray	Abundant, light greenish gray	Abundant, dark greenish gray	Abundant, greenish white - dark greenish gray
Soluble pigment	None	None	None	None	None
ISP 5					
Growth	Good	Good	Good	Good	Good
	Pale yellowish green	Yellowish white	Pale yellowish green	Yellowish white	Yellowish white
Reverse	Pale yellowish green	Pale yellow - grayish yellow	Pale yellowish green	Pale yellow - light olive gray	Greenish white - light grayish olive
Aerial mycelium	Abundant, pale blue	Abundant, yellowish white	None	Moderate, white - greenish gray	Abundant, white
Soluble pigment	None	Pale orange yellow	None	None	None
ISP 6					
Growth	Good	Good	Good	Good	Good
	Grayish greenish yellow	Grayish greenish yellow	Dark yellow	Grayish greenish yellow	Light yellow
Reverse	Grayish greenish yellow	Grayish greenish yellow	Light yellow - dark yellow	Pale greenish yellow - light grayish olive	Light yellow
Aerial mycelium	None	None	Rare, white	Abundant, bluish gray	None
Soluble pigment	None	None	None	None	None
ISP 7					
Growth	Good	Good	Good	Good	Good
	Grayish yellow	Light grayish olive	Pale yellow - grayish yellow	Moderate yellowish green	Grayish yellow
Reverse	Light yellowish brown	Pale yellowish green - light grayish olive	Pale yellow - grayish yellow	Light grayish olive and dark yellowish green	Yellowish - grayish yellow
Aerial mycelium	Abundant, light greenish gray	Abundant, greenish white	None	Abundant, very pale purple	Abundant, greenish white
Soluble pigment	Grayish yellow	None	None	None	None
YS agar					
Growth	Good	Good	Good	Good	Good
	Pale yellowish green	Pale orange yellow	Grayish yellow	Pale yellowish green	Pale greenish yellow
Reverse	Pale yellowish green	Pale orange yellow - strong yellow	Grayish yellow - Dark grayish yellow	Pale yellowish green	Pale yellow
Aerial mycelium	None	Abundant, greenish white	Abundant, dark greenish gray	Abundant, very pale purple	Abundant, light greenish gray
Soluble pigment	None	Pale orange yellow	None	None	None
Nutrient agar					
Growth	Good	Good	Good	Good	Good
	Colorless	Pale yellowish green	Grayish greenish yellow	Pale greenish yellow	Pale greenish yellow
Reverse	Pale yellow – dark yellow	Pale yellowish green - grayish greenish yellow	Pale yellowish green	Grayish greenish yellow	Pale greenish yellow
Aerial mycelium	Abundant white	Abundant, white	Abundant, bluish gray	Abundant, light greenish gray - bluish gray	Moderate, white
Soluble pigment	None	None	None	None	None

Table 8 Physiological and biochemical characteristics of *Streptomyces* strains in group VI

Characteristics	Strains				
	KC-060	KC-088	KC-115	KC-118	KC-133
Utilization of:					
L-Arabinose	+	+	+	+	+
D-Fructose	-	-	+	+	+
D-Glucose	+	+	+	+	+
D-Mannitol	+	+	+	+	+
D-Melibiose	-	-	-	-	-
Melezitose	+	+	+	+	+
myo-Inositol	+	+	+	±	-
Raffinose	+	+	±	-	+
L-Rhamnose	+	+	+	+	+
D-Sorbitol	-	-	-	-	-
Sucrose	-	-	-	-	-
D-Xylose	+	+	+	+	+
Nitrate reduction	+	+	+	+	+
Starch hydrolysis	+	+	+	+	+
Milk peptonization	-	-	-	-	-
Milk coagulation	-	-	-	-	-
Gelatinization	-	-	-	-	-
Growth at/with:					
NaCl (%w/v)	0-8	0-8	0-9	0-9	0-9
pH	4-12	4-12	4-12	5-12	5-12
Temperature (°C)	15-40	15-40	15-40	15-40	15-40

+ = positive, ± = weakly positive, - = negative

Table 9 Cultural characteristics of *Streptomyces* strains in group VII

Media	Strains	
	KC-070	KC-100
ISP 2		
Growth	Good	Good
	Brownish pink - light reddish brown	Grayish greenish yellow - light reddish brown
Reverse	Dark grayish yellow - grayish reddish brown	Grayish greenish yellow - grayish reddish brown
Aerial mycelium	Abundant, white - medium gray	Abundant, bluish gray
Soluble pigment	None	None
ISP 3		
Growth	Good	Good
	Moderate reddish brown	Moderate reddish brown
Reverse	Very dark red - light grayish red	Pale yellowish green - very dark red
Aerial mycelium	Abundant, white - purplish gray	Abundant, bluish yellow
Soluble pigment	None	None
ISP 4		
Growth	Good	Good
	Light grayish yellowish brown	Dark yellow - light grayish yellowish brown
Reverse	Dark grayish yellow - grayish yellowish brown	Grayish greenish yellow - grayish yellowish brown
Aerial mycelium	Abundant, white - medium gray	Abundant, greenish white - medium gray
Soluble pigment	None	None
ISP 5		
Growth	Good	Good
	Pale yellow - grayish yellow	Dark yellow
Reverse	Pale yellowish green - light grayish olive	Moderate olive
Aerial mycelium	Abundant, light bluish gray	Abundant, bluish gray
Soluble pigment	None	Grayish yellow
ISP 6		
Growth	Good	Good
	Light yellow	Pale greenish yellow
Reverse	Light yellow - deep yellow	Pale greenish yellow
Aerial mycelium	Abundant, white	None
Soluble pigment	None	None
ISP 7		
Growth	Good	Good
	Brownish pink	Light olive brown
Reverse	Dark grayish yellow - grayish reddish brown	Moderate yellowish brown - dark yellowish brown
Aerial mycelium	Abundant, white - medium gray	Abundant, bluish gray
Soluble pigment	None	Grayish yellow
YS agar		
Growth	Good	Good
	Grayish pink - moderate red	Grayish pink - moderate red
Reverse	Reddish gray - very dark red	Reddish gray - very dark red
Aerial mycelium	Abundant, white - medium gray	Abundant, bluish gray
Soluble pigment	None	None
Nutrient agar		
Growth	Good	Good
	Pale greenish yellow	Colorless
Reverse	Pale yellow - dark grayish yellow	Pale yellowish green - dark grayish yellow
Aerial mycelium	Abundant, white - light gray	Abundant, white - bluish gray
Soluble pigment	None	None

Table 10 Physiological and biochemical characteristics of *Streptomyces* strains in group VII

Characteristics	Strains	
	KC-070	KC-100
Utilization of:		
L-Arabinose	+	+
D-Fructose	+	+
D-Glucose	+	+
D-Mannitol	+	+
D-Melibiose	-	-
Melezitose	+	±
myo-Inositol	±	+
Raffinose	±	+
L-Rhamnose	+	±
D-Sorbitol	-	-
Sucrose	-	-
D-Xylose	±	+
Nitrate reduction	+	+
Starch hydrolysis	+	+
Milk peptonization	-	-
Milk coagulation	-	-
Gelatinization	-	-
Growth at/with:		
NaCl (%w/v)	0-10	0-9
pH	5-12	5-12
Temperature (°C)	15-40	15-40

+ = positive, ± = weakly positive, - = negative

Table 11 Cultural characteristics of *Streptomyces* strains in group VIII

Media	Strains				
	KC-110	KC-111	KC-112	KC-135	KC-136
ISP 2					
Growth	Good	Good	Good	Good	Good
	Dark yellow	Colorless	Light olive brown	Vivid yellow	Light yellow
Reverse	Moderate yellowish brown	Moderate olive brown	Light olive brown - moderate olive brown	Brilliant yellow - strong yellow	Dark yellow
Aerial mycelium	Abundant, white - medium gray	Abundant, light greenish gray - medium gray	Abundant, greenish white - medium gray	None	Abundant, greenish white
Soluble pigment	Dark yellow	Dark yellow	Grayish greenish yellow	Light greenish yellow	Grayish yellow
ISP 3					
Growth	Good	Good	Good	Good	Good
	Grayish greenish yellow	Colorless	Light grayish olive	Light olive brown	Grayish greenish yellow
Reverse	Grayish yellowish green	Light grayish olive	Light grayish olive	Strong yellowish brown	Olive gray
Aerial mycelium	Abundant, white - brownish gray	Abundant, light olive gray	Abundant, white - light gray and brownish gray	Abundant, greenish gray	Abundant, brownish gray
Soluble pigment	None	None	None	Strong yellow	None
ISP 4					
Growth	Good	Good	Good	Good	Good
	Dark yellow	Colorless	Dark yellow	Strong greenish yellow	Grayish greenish yellow
Reverse	Light grayish olive	Light grayish olive	Dark grayish yellow - light grayish olive	Strong greenish yellow - moderate olive	Grayish yellow
Aerial mycelium	Abundant, white - brownish gray	Abundant, light olive gray	Abundant, white - medium gray	Abundant, greenish white - medium gray	Abundant, yellowish white - medium gray
Soluble pigment	None	None	None	Light greenish yellow	None
ISP 5					
Growth	Good	Good	Good	Good	Good
	Moderate yellowish brown	Light olive brown	Light olive brown	Grayish yellow	Light olive brown
Reverse	Pale orange yellow - deep yellowish brown	Moderate yellow - dark yellow	Pale yellow - moderate olive brown	Pale yellow - light grayish yellow	Light olive brown - moderate olive brown
Aerial mycelium	Abundant, white - greenish gray	Abundant, greenish white	Abundant, greenish white - yellowish gray	Abundant, pale yellowish green	Abundant, light greenish gray - yellowish gray
Soluble pigment	Pale orange yellow	Grayish yellow	Pale yellow	None	Grayish yellow
ISP 6					
Growth	Good	Good	Good	Good	Good
	Pale yellow	Pale greenish yellow	Pale yellow	Pale greenish yellow - grayish greenish yellow	Pale yellow
Reverse	Light yellow	Light yellow	Light yellow	Grayish greenish yellow	Pale yellow
Aerial mycelium	Abundant, white - greenish white	Abundant, greenish white	Abundant, white	Moderate, greenish white - light greenish gray	Moderate, white
Soluble pigment	Light yellow	None	None	None	None
ISP 7					
Growth	Good	Good	Good	Good	Good
	Light yellowish brown	Dark yellow - dark grayish yellow	Dark yellow	Grayish greenish yellow - strong greenish yellow	Strong yellowish brown
Reverse	Moderate yellowish brown	Moderate yellowish brown	Moderate yellowish brown	Pale yellow - moderate olive brown	Deep yellowish brown
Aerial mycelium	Abundant, greenish white - brownish gray	Abundant, greenish white - light greenish gray	Abundant, light greenish gray	Abundant, grayish yellowish green	Abundant, light greenish gray
Soluble pigment	Pale orange yellow	Light yellowish brown	Grayish yellow	Light yellowish green	Light grayish yellowish brown
YS agar					
Growth	Good	Good	Good	Good	Good
	Dark yellow	Grayish greenish yellow	Grayish greenish yellow	Strong yellow	Grayish greenish yellow
Reverse	Light grayish olive	Grayish greenish yellow - grayish olive	Light grayish olive - light olive gray	Strong yellow - deep yellow	Pale yellowish green - grayish yellowish green
Aerial mycelium	Abundant, brownish gray	Abundant, light olive gray	Abundant, white - medium gray and brownish gray	Abundant, greenish white - greenish gray	Abundant, light olive gray
Soluble pigment	None	None	None	Vivid yellow	None
Nutrient agar					
Growth	Good	Good	Good	Good	Good
	Pale yellow	Colorless	Pale yellow - grayish yellow	Grayish greenish yellow	Grayish yellow
Reverse	Pale yellow - dark yellow	Pale greenish yellow - grayish greenish yellow	Pale yellow - dark yellow	Pale greenish yellow	Light yellow - dark yellow
Aerial mycelium	Abundant, medium gray	Abundant, greenish Abundant, white - light greenish gray	Abundant, white - medium gray	Abundant, greenish white - medium gray	Abundant, white - medium gray
Soluble pigment	None	None	None	Strong greenish yellow	Pale yellow

Table 12 Physiological and biochemical characteristics of *Streptomyces* strains in group VIII

Characteristics	Strains				
	KC-110	KC-111	KC-112	KC-135	KC-136
Utilization of:					
L-Arabinose	+	+	+	+	+
D-Fructose	+	+	+	+	+
D-Glucose	+	+	+	+	+
D-Mannitol	+	+	+	+	+
D-Melibiose	-	-	-	-	-
Melezitose	-	-	-	-	-
myo-Inositol	-	-	-	-	-
Raffinose	±	-	±	±	-
L-Rhamnose	-	-	-	-	-
D-Sorbitol	-	-	-	-	-
Sucrose	-	-	-	-	-
D-Xylose	+	+	+	+	+
Nitrate reduction	-	-	-	-	-
Starch hydrolysis	-	-	-	+	-
Milk peptonization	+	+	+	-	+
Milk coagulation	-	-	-	-	-
Gelatinization	-	-	±	-	-
Growth at/with:					
NaCl (%w/v)	0-9	0-9	0-9	0-9	0-8
pH	5-12	4-12	5-12	5-12	5-12
Temperature (°C)	15-40	15-40	15-40	15-40	15-40

+ = positive, ± = weakly positive, - = negative

Table 13 Cultural characteristics of *Streptomyces* strains in group IX

Media	Strains	
	KC-062	KC-063
ISP 2		
Growth	Good	Good
	Strong greenish yellow	Strong greenish yellow
Reverse	Strong greenish yellow	Strong greenish yellow
Aerial mycelium	Abundant, white	Abundant, white
Soluble pigment	None	None
ISP 3		
Growth	Good	Good
	Moderate greenish yellow	Moderate yellowish pink - deep yellowish pink
Reverse	Pale greenish yellow - grayish greenish yellow	Strong reddish orange - deep pink
Aerial mycelium	Abundant, white	Abundant, white
Soluble pigment	None	None
ISP 4		
Growth	Good	Good
	Moderate yellow	Pale greenish yellow
Reverse	Moderate yellow	Pale yellowish green
Aerial mycelium	Abundant, white	Abundant, white
Soluble pigment	None	None
ISP 5		
Growth	Good	Good
	Light greenish yellow	Light greenish yellow
Reverse	Light greenish yellow	Light greenish yellow
Aerial mycelium	Poor, white	Abundant, white
Soluble pigment	None	None
ISP 6		
Growth	Good	Good
	Pale yellow	Grayish greenish yellow
Reverse	Pale yellow - dark yellow	Dark yellow
Aerial mycelium	None	None
Soluble pigment	None	None
ISP 7		
Growth	Good	Good
	Strong greenish yellow	Strong greenish yellow
Reverse	Strong greenish yellow	Dark yellow
Aerial mycelium	Abundant, white	Abundant, white
Soluble pigment	None	None
YS agar		
Growth	Good	Good
	Moderate greenish yellow	Moderate greenish yellow
Reverse	Light yellowish green	Moderate greenish yellow
Aerial mycelium	Abundant, white	Abundant, white
Soluble pigment	None	None
Nutrient agar		
Growth	Good	Good
	Pale greenish yellow	Light orange - moderate orange
Reverse	Pale greenish yellow	Light orange - moderate orange
Aerial mycelium	Poor, white	Poor, white
Soluble pigment	None	None

Table 14 Physiological and biochemical characteristics of *Streptomyces* strains in group IX

Characteristics	Strains	
	KC-062	KC-063
Utilization of:		
L-Arabinose	±	±
D-Fructose	+	+
D-Glucose	+	+
D-Mannitol	+	+
D-Melibiose	-	-
Melezitose	±	±
myo-Inositol	+	+
Raffinose	±	±
L-Rhamnose	±	±
D-Sorbitol	-	-
Sucrose	-	-
D-Xylose	±	±
Nitrate reduction	-	-
Starch hydrolysis	+	+
Milk peptonization	-	-
Milk coagulation	-	-
Gelatinization	-	-
Growth at/with:		
NaCl (%w/v)	0-9	0-9
pH	5-12	5-12
Temperature (°C)	15-45	15-45

+ = positive, ± = weakly positive, - = negative

Table 15 Cultural characteristics of *Streptomyces* strains in group X

Media	Strains					
	KC-004	KC-017	KC-141	KC-142	KC-152	KC-157
ISP 2						
Growth	Good	Good	Good	Good	Good	Good
	Dark yellow - dark orange yellow	Strong yellowish brown	Pale yellow	Pale yellow - grayish yellow	Pale yellow - moderate olive brown	Light grayish olive - dark olive brown
Reverse	Light yellowish brown - dark brown	Strong yellowish brown	Pale yellow	Pale yellow - dark olive brown	Dark yellow - dark olive brown	Pale yellow - grayish olive
Aerial mycelium	Abundant, white - light gray	Moderate white - light gray	None	Abundant, white - brownish black	Abundant, light bluish gray	Abundant, medium gray
Soluble pigment	None	None	None	None	None	None
ISP 3						
Growth	Good	Good	Good	Good	Good	Good
	Dark olive brown	Strong yellow	Moderate brown	Pale yellowish green	Dark olive brown	Dark grayish yellowish brown
Reverse	Dark olive brown - dark grayish olive	Strong yellow	Deep brown - dark brown	Pale yellowish green - grayish yellowish green	Dark grayish olive - dark olive brown	Dark grayish yellowish brown
Aerial mycelium	Abundant, light olive gray	Abundant, white	Abundant, light gray	Abundant, white - brownish black	Abundant, light olive gray	Abundant, light olive gray
Soluble pigment	Pale greenish yellow	Light yellow	Strong brown	None	Pale greenish yellow	None
ISP 4						
Growth	Good	Good	Good	Good	Good	Good
	Pale yellow - moderate yellow	Strong yellow	Moderate brown	Pale yellowish green	Strong yellow - grayish yellowish brown	Pale yellowish green - brownish gray
Reverse	Moderate yellow - dark grayish olive	Moderate yellow	Light olive brown - grayish brown	Pale yellow - dark grayish olive green	Moderate yellow	Pale yellowish green - dark yellowish brown
Aerial mycelium	Moderate, white - bluish gray	None	Abundant, light gray	Abundant, white - brownish black	None	None
Soluble pigment	None	None	Light grayish yellowish brown	None	None	None
ISP 5						
Growth	Good	Good	Good	Good	Good	Good
	Pale yellow - strong yellowish brown	Brilliant yellow - strong yellow	Grayish yellowish brown	Pale yellow - grayish yellow	Moderate yellow - dark yellow	Pale yellowish green
Reverse	Pale yellow - strong yellowish brown	Strong yellow - strong yellowish brown	Grayish yellowish brown - dark grayish yellowish brown	Pale yellow - brownish black	Dark yellow	Pale yellowish green
Aerial mycelium	Abundant, white - bluish gray	Moderate, white - light gray	Abundant, light gray	Moderate, white - brownish black	Moderate greenish white - light gray	None
Soluble pigment	Light yellowish green	Light yellowish green	Light grayish yellowish brown	None	None	None
ISP 6						
Growth	Good	Good	Good	Good	Good	Good
	Pale yellow - strong yellowish brown	Pale yellow - deep yellow	Pale greenish yellow	Pale yellow	Pale yellow	Pale yellow
Reverse	Pale yellow - strong yellowish brown	Deep yellow - strong yellowish brown	Pale greenish yellow	Light yellow	Light yellow	Light yellow
Aerial mycelium	None	None	None	None	None	None
Soluble pigment	None	None	None	None	None	None
ISP 7						
Growth	Good	Good	Good	Good	Good	Good
	Light yellowish brown - strong yellowish brown	Light yellow - strong yellowish brown	Dark grayish yellowish brown	Grayish yellow - dark grayish yellow	Moderate yellow - moderate olive brown	Pale yellow - dark grayish yellow
Reverse	Light yellow - strong yellowish brown	Light yellow - strong yellowish brown	Grayish yellowish brown - dark grayish yellowish brown	Grayish black - dark grayish olive	Moderate yellow - dark olive brown	Pale yellow - dark grayish yellow
Aerial mycelium	Poor, white	Poor, white	Abundant, white - light greenish gray	Abundant, brownish black	Abundant, light yellow	Moderate, light gray
Soluble pigment	None	None	Light brown	None	None	None
YS agar						
Growth	Good	Good	Good	Good	Good	Good
	Pale yellow - light olive brown	Deep yellow	Moderate yellowish brown	Dark grayish olive	Pale greenish yellow - moderate olive	Pale greenish yellow - moderate olive
Reverse	Pale yellow - dark grayish olive	Moderate yellow	Moderate yellowish brown - dark brown	Light grayish olive	Dark grayish yellow - light grayish olive	Dark grayish yellow - light grayish olive
Aerial mycelium	Abundant, white - bluish gray	None	Abundant, light gray	Abundant, white - brownish black	Abundant, white - light olive gray	Abundant, white - light olive gray
Soluble pigment	None	None	Light brown	None	None	None
Nutrient agar						
Growth	Good	Good	Good	Good	Good	Good
	Greenish white - moderate yellow	Greenish white - moderate yellow	Grayish greenish yellow	Pale yellow	Greenish white	Greenish white
Reverse	Greenish white - moderate yellow	Greenish white - moderate yellow	Dark grayish yellow	Pale greenish yellow	Greenish white - pale yellow	Greenish white - pale yellow
Aerial mycelium	None	None	Moderate, white - light bluish gray	Abundant, white	None	None
Soluble pigment	None	None	Dark grayish yellow	None	None	None

Table 16 Physiological and biochemical characteristics of *Streptomyces* strains in group X

Characteristics	Strains					
	KC-004	KC-017	KC-141	KC-142	KC-152	KC-157
Utilization of:						
L-Arabinose	+	+	+	-	+	+
D-Fructose	+	+	+	±	+	+
D-Glucose	+	+	+	+	+	+
D-Mannitol	+	+	+	+	+	+
D-Melibiose	-	-	-	-	-	-
Melezitose	±	±	-	+	+	±
myo-Inositol	±	±	+	+	-	±
Raffinose	+	+	-	±	+	±
L-Rhamnose	+	+	+	+	+	+
D-Sorbitol	-	-	-	-	-	-
Sucrose	-	-	-	-	-	-
D-Xylose	+	+	+	-	+	+
Nitrate reduction	-	-	+	-	-	-
Starch hydrolysis	+	+	+	+	+	+
Milk peptonization	-	-	-	+	-	-
Milk coagulation	+	-	-	-	-	-
Gelatinization	-	-	-	-	-	-
Growth at/with:						
NaCl (%w/v)	0-6	0-7	0-7	0-4	0-7	0-7
pH	5-12	5-12	5-12	5-12	5-12	5-12
Temperature (°C)	15-37	15-37	15-37	15-37	15-37	15-37

+ = positive, ± = weakly positive, - = negative

Table 17 Cultural characteristics of *Streptomyces* strains in groups XI and XII

Media	Strains		
	KC-035	KC-106	KC-140
ISP 2			
Growth	Good	Good	Good
	Pale yellow	Pale yellow olive	Pale yellow olive
Reverse	Light yellow - moderate yellow	Pale yellowish brown - grayish yellowish brown	Grayish yellowish brown
Aerial mycelium	None	Abundance, white - brownish gray	Abundance, white - brownish gray
Soluble pigment	None	None	none
ISP 3			
Growth	Good	Good	Good
	Strong yellow	Grayish yellow brown	Moderate greenish yellow
Reverse	Dark orange yellow	Light brownish gray	Light brownish gray
Aerial mycelium	Abundant, yellowish white	Abundant, brownish gray	Abundant, brownish gray
Soluble pigment	Pale yellow	None	None
ISP 4			
Growth	Good	Good	Good
	Strong yellow - deep yellow	Grayish yellow brown	Grayish yellow brown
Reverse	Strong yellow - deep yellow	Yellowish gray - grayish yellow brown	Grayish yellow brown
Aerial mycelium	Abundant, yellowish white	Abundant, white - brownish gray	Abundant, white - brownish gray
Soluble pigment	Pale yellow	None	None
ISP 5			
Growth	Good	Good	Good
	Greenish white - strong yellow	Brownish white - pale yellowish brown	Pale yellowish brown
Reverse	Greenish white - moderate yellow	Brownish white - light brownish gray	Pale yellowish brown
Aerial mycelium	None	Abundant, white - brownish gray	Abundant, white
Soluble pigment	None	None	None
ISP 6			
Growth	None	Good	Good
	None	Yellowish gray	Light yellow
Reverse	None	Pale yellow	Pale greenish yellow
Aerial mycelium	None	Abundant, white	Abundant, white
Soluble pigment	None	None	None
ISP 7			
Growth	Good	Good	Good
	Light yellowish brown - brownish black	Pale yellow - pale yellowish brown	Grayish yellow
Reverse	Light yellowish brown - brownish black	Light brownish gray - pale yellowish brown	Pale yellowish brown -grayish olive
Aerial mycelium	None	Abundance, white - brownish gray	Abundant, light olive gray - brownish gray
Soluble pigment	None	None	Grayish greenish yellow
YS agar			
Growth	Good	Good	Good
	Greenish white - strong yellow	Pale yellowish brown	Pale yellowish brown
Reverse	Pale yellow - deep yellow	Grayish yellow brown	Grayish yellow brown
Aerial mycelium	Poor, yellowish white	Abundance, white - brownish gray	Abundance, white - brownish gray
Soluble pigment	Pale yellow	None	None
Nutrient agar			
Growth	Good	Good	Good
	Light yellowish brown - moderate reddish brown	Pale grayish yellow - light brownish gray	Light brownish gray
Reverse	Light yellowish brown - moderate reddish brown	Pale yellowish brown - grayish yellow brown	Pale yellowish brown - grayish yellow brown
Aerial mycelium	Abundant, greenish white	Abundance, white - brownish gray	Abundance, white - brownish gray
Soluble pigment	Moderate yellow	None	None

Table 18 Physiological and biochemical characteristics of *Streptomyces* strains in groups XI and XII

Characteristics	Strains		
	KC-035	KC-106	KC-140
Utilization of:			
L-Arabinose	-	+	+
D-Fructose	+	+	+
D-Glucose	+	+	+
D-Mannitol	+	+	+
D-Melibiose	±	-	-
Melezitose	-	-	-
myo-Inositol	+	-	-
Raffinose	+	-	-
L-Rhamnose	+	-	-
D-Sorbitol	-	-	-
Sucrose	±	-	-
D-Xylose	+	+	+
Nitrate reduction	+	+	+
Starch hydrolysis	+	+	+
Milk peptonization	-	+	+
Milk coagulation	-	-	-
Gelatinization	+	-	-
Growth at/with:			
NaCl (%w/v)	0-6	0-7	0-7
pH	5-12	5-12	4-12
Temperature (°C)	15-37	15-40	15-40

+ = positive, ± = weakly positive, - = negative

Table 19 Cultural characteristics of Streptomyces strains in group XIII

Media	Strains			
	KC-119	KC-124	KC-125	KC-134
ISP 2				
Growth	Good	Good	Good	Good
	Grayish yellow	Pale orange yellow	Pale orange yellow	Pale yellowish pink
Reverse	Dark yellow	Moderate yellow	Strong reddish orange	Strong reddish orange
Aerial mycelium	Abundant, yellowish white	Abundant, yellowish white	Abundant, yellowish white	Abundant, yellowish white
Soluble pigment	Moderate, yellow	Moderate, yellow	Moderate yellow	Moderate yellow
ISP 3				
Growth	Good	Good	Good	Good
	Yellowish white - moderate yellowish pink	Yellowish white - moderate yellowish pink	Yellowish white - moderate yellowish pink	Yellowish white - moderate yellowish pink
Reverse	Pale yellowish green	Pale yellowish green - grayish yellow	Pale yellow	Pale orange yellow - dark yellowish pink
Aerial mycelium	Abundant, yellowish white	Abundant, yellowish white	Abundant, yellowish white	Abundant, yellowish white
Soluble pigment	Pale yellow	Pale yellow	Pale yellow	Pale yellow
ISP 4				
Growth	Good	Good	Good	Good
	Grayish yellow	Grayish yellow	Grayish yellow	Grayish yellow
Reverse	Grayish yellow	Moderate yellow - strong yellow	Dark yellow	Deep yellow - moderate yellow
Aerial mycelium	Abundant, yellowish white	Abundant, yellowish white	Abundant, white - yellowish gray	Abundant, yellowish white
Soluble pigment	None	None	None	None
ISP 5				
Growth	Good	Good	Good	Good
	Pale yellow	Pale yellow	Pale yellowish green	Pale orange yellow
Reverse	Pale yellow	Pale yellowish green	Pale yellowish green	Pale yellow
Aerial mycelium	Abundant, white	Moderate, greenish white	None	None
Soluble pigment	None	Pale yellowish green	None	Pale yellow
ISP 6				
Growth	Good	Good	Good	Good
	Light olive gray	Light grayish olive	Grayish greenish yellow	Greenish gray
Reverse	Light grayish olive	Dark grayish yellow	Grayish greenish yellow	Dark olive brown
Aerial mycelium	None	None	Rare, greenish white	None
Soluble pigment	None	Moderate olive brown	None	Moderate olive brown
ISP 7				
Growth	Good	Good	Good	Good
	Light grayish olive	Light grayish olive	Olive gray	Grayish yellow
Reverse	Light grayish olive	Light grayish olive	Light grayish olive - greenish black	Grayish yellow
Aerial mycelium	None	Poor, white	Abundant, white - light gray	Poor, white
Soluble pigment	None	Light grayish olive	None	Pale yellow
YS agar				
Growth	Good	Good	Good	Good
	Greenish white	Pale yellow	Grayish yellow	Grayish yellowish pink
Reverse	Light yellowish brown	Moderate yellow	Grayish yellow	Yellowish brown
Aerial mycelium	Abundant, yellowish white	Abundant, yellowish white	Abundant, yellowish white	Abundant, yellowish white
Soluble pigment	Grayish yellow	Pale yellow	None	Pale yellow
Nutrient agar				
Growth	Good	Good	Good	Good
	Light yellowish brown	Light yellowish brown	Pale yellow	Light yellowish brown
Reverse	Moderate yellow - dark yellow	Moderate yellow	Moderate yellow - strong yellowish brown	Light olive brown
Aerial mycelium	Abundant, white	Abundant, white - yellowish white	Abundant, light greenish gray	Abundant, white - yellowish white
Soluble pigment	Moderate yellow	Deep yellow	Moderate yellow	Deep yellow

Table 20 Physiological and biochemical characteristics of *Streptomyces* strains in group XIII

Characteristics	Strains			
	KC-119	KC-124	KC-125	KC-134
Utilization of:				
L-Arabinose	+	+	+	+
D-Fructose	+	+	+	+
D-Glucose	+	+	+	+
D-Mannitol	+	+	+	+
D-Melibiose	+	+	+	+
Melezitose	-	-	-	-
myo-Inositol	+	+	+	+
Raffinose	+	+	+	+
L-Rhamnose	+	+	+	+
D-Sorbitol	+	+	+	+
Sucrose	+	+	+	+
D-Xylose	+	+	+	+
Nitrate reduction	-	-	-	-
Starch hydrolysis	+	+	+	+
Milk peptonization	+	+	+	+
Milk coagulation	+	+	+	+
Gelatinization	-	-	-	-
Growth at/with:				
NaCl (%w/v)	0-8	0-8	0-8	0-8
pH	5-12	5-12	5-12	5-12
Temperature (°C)	15-37	15-37	15-37	15-37

+ = positive, - = negative

Table 21 Cultural characteristics of *Streptomyces* strains in group XIV

Media	Strains					
	KC-031	KC-032	KC-033	KC-038	KC-150	KC-156
ISP 2						
Growth	Good	Good	Good	Good	Good	Good
	Pale yellowish brown	Pale greenish yellow - moderate greenish yellow	Grayish greenish yellow	Pale yellowish brown	Grayish greenish yellow	Strong brown
Reverse	Grayish yellow brown	Moderate olive brown	Moderate olive brown	Grayish olive - brownish gray	Moderate olive brown	Light brown - dark brown
Aerial mycelium	Moderate, white	Abundant, white - light greenish gray	Abundant, white - light olive gray	Abundant, white - brownish gray	Abundant, white - bluish gray	Abundant, light gray
Soluble pigment	None	Dark yellow	Dark yellow	None	Deep yellow	Light brown
ISP 3						
Growth	Good	Good	Good	Good	Good	Good
	Grayish yellowish brown	Grayish greenish yellow	Grayish greenish yellow - moderate greenish yellow	Light yellow - light olive	Grayish greenish yellow	Grayish greenish yellow
Reverse	Yellowish gray - pale yellowish brown	Grayish greenish yellow	Pale yellow - light grayish olive	Pale greenish yellow - grayish yellowish brown	Pale yellow - dark grayish yellow	Dark grayish yellow
Aerial mycelium	Moderate, white	Abundant, white -light olive gray	Abundant, white -light olive gray	Abundant, brownish gray	Abundant, white - light olive gray	Abundant, light gray
Soluble pigment	None	None	None	None	Grayish greenish yellow	None
ISP 4						
Growth	Good	Good	Good	Good	Good	Good
	Grayish yellowish brown - grayish yellowish brown	Moderate greenish yellow	Moderate greenish yellow	light olive	Moderate greenish yellow - moderate olive	Strong yellowish brown
Reverse	Dark yellowish brown	Moderate greenish yellow	Moderate olive brown	Grayish yellowish brown - yellowish brown	Moderate olive brown	Strong yellowish brown
Aerial mycelium	Abundant, brownish gray	Abundant, bluish gray	Abundant, bluish gray	Abundant, light brownish gray brownish gray	Abundant, bluish gray	Abundant, light gray
Soluble pigment	None	Grayish greenish yellow	Grayish greenish yellow	None	Moderate greenish yellow	Light yellowish brown
ISP 5						
Growth	Good	Good	Good	Good	Good	Good
	Brownish white	Greenish white -grayish greenish yellow	Moderate greenish yellow	Light brownish gray - light olive	Pale greenish yellow - grayish greenish yellow	Moderate yellowish brown - dark yellowish brown
Reverse	Light brownish gray	Greenish white - grayish greenish yellow	Greenish white - grayish greenish yellow	Light brownish gray - grayish yellowish brown	Greenish white - grayish greenish yellow	Moderate yellowish brown - dark brown
Aerial mycelium	Abundant, brownish white	Abundant, white	Abundant, white - light olive gray	Abundant, light brownish gray	Poor, white	Abundant, light gray
Soluble pigment	None	None	None	None	None	Dark grayish yellow
ISP 6						
Growth	Good	Good	Good	Good	Good	Good
	Grayish yellowish brown	Light greenish gray	Light greenish gray	Dark brownish gray	Light greenish gray	Strong yellow
Reverse	Grayish yellowish brown	Light olive gray	Olive gray	Dark brownish gray	Light olive gray	Strong yellow
Aerial mycelium	None	None	None	None	None	Abundant, white
Soluble pigment	Yellowish brown	Dark brown	Dark brown	Dark brown	Moderate brown	Light yellow
ISP 7						
Growth	Good	Good	Good	Good	Good	Good
	Light yellowish brown	Grayish greenish yellow	Grayish greenish yellow	Light brownish gray - pale yellow olive	Grayish greenish yellow	Dark brown
Reverse	Light brownish gray	Light grayish olive - grayish olive	Light grayish olive - grayish olive	Light brownish gray - light olive	Moderate olive brown	Dark brown
Aerial mycelium	Abundant, light brownish white	Abundant, light olive gray	Abundant, light olive gray	Abundant, light brownish gray	Light olive gray	Abundant, light gray
Soluble pigment	None	Grayish greenish yellow	Grayish greenish yellow	None	Grayish greenish yellow	Light brown
YS agar						
Growth	Good	Good	Good	Good	Good	Good
	Pale yellowish brown	Grayish greenish yellow	Grayish greenish yellow	Light brownish gray - pale olive	Grayish greenish yellow	Strong brown - dark brown
Reverse	Pale yellowish brown	Dark grayish yellow - moderate olive brown	Dark grayish yellow - moderate olive brown	Pale yellowish brown - grayish yellowish brown	Dark grayish yellow - moderate olive brown	Light brown - dark brown
Aerial mycelium	Moderate, brownish gray	Abundant, light olive gray	Abundant, light olive gray	Abundance, white - brownish gray	Abundant, light olive gray	Abundant, light gray
Soluble pigment	None	None	Pale greenish yellow	None	Grayish greenish yellow	Light brown
Nutrient agar						
Growth	Good	Good	Good	Good	Good	Good
	Pale yellow	Grayish greenish yellow	Grayish greenish yellow	Pale yellow	Pale yellow - grayish green	Colorless
Reverse	Pale yellow	Grayish greenish yellow	Grayish greenish yellow	Pale yellow - yellowish gray	Grayish greenish yellow	Grayish yellow
Aerial mycelium	None	Abundant, white	Abundant, white	Poor, white	Poor, white	Moderate, light bluish gray
Soluble pigment	None	Grayish olive	Grayish yellow	None	Grayish yellow	None

Table 22 Physiological and biochemical characteristics of *Streptomyces* strains in group XIV

Characteristics	Strains					
	KC-031	KC-032	KC-033	KC-038	KC-150	KC-156
Utilization of:						
L-Arabinose	+	+	+	+	+	+
D-Fructose	+	+	+	+	+	+
D-Glucose	+	+	+	+	+	+
D-Mannitol	+	+	+	+	+	+
D-Melibiose	+	+	+	+	+	-
Melezitose	-	-	-	-	-	-
myo-Inositol	+	+	+	+	+	+
Raffinose	±	±	±	±	±	-
L-Rhamnose	+	+	+	+	+	+
D-Sorbitol	-	-	-	-	-	-
Sucrose	-	-	-	-	-	-
D-Xylose	+	+	+	+	+	+
Nitrate reduction	+	+	+	+	+	+
Starch hydrolysis	+	+	+	+	+	+
Milk peptonization	+	+	+	+	+	+
Milk coagulation	-	-	-	-	-	-
Gelatinization	-	-	-	-	-	-
Growth at/with:						
NaCl (%w/v)	0-6	0-6	0-6	0-6	0-6	0-8
pH	5-12	5-12	5-12	5-12	5-12	5-12
Temperature (°C)	15-40	15-40	15-40	15-40	15-40	15-40

+ = positive, ± = weakly positive, - = negative

Table 23 Cultural characteristics of *Streptomyces* strains in group XV

Media	Strains			
	KC-085	KC-098	KC-101	KC-103
ISP 2				
Growth	Good	Good	Good	Good
	Pale yellow	Grayish yellow	Pale yellowish green - moderate olive brown	Pale yellowish green - moderate olive brown
Reverse	Light grayish olive - brownish black	Dark yellow	Pale yellowish green - greenish black	Pale yellowish green - greenish black
Aerial mycelium	Abundant, bluish gray	Abundant, greenish white	Abundant, pale blue	Abundant, pale blue
Soluble pigment	Dark grayish yellow	Grayish yellow	Grayish yellow	Grayish yellow
ISP 3				
Growth	Good	Good	Good	Good
	Moderate olive brown	Dark olive brown	Light grayish olive	Light grayish olive
Reverse	Dark olive brown	Brownish black	Grayish olive - dark grayish olive	Grayish olive - dark grayish olive
Aerial mycelium	Abundant, bluish gray	Abundant, bluish gray	Abundant, bluish gray	Abundant, bluish gray
Soluble pigment	Grayish yellow	None	None	None
ISP 4				
Growth	Good	Good	Good	Good
	Moderate brown	Moderate yellowish brown	Grayish greenish yellow - moderate yellowish brown	Grayish greenish yellow - moderate yellowish brown
Reverse	Grayish yellow – light olive gray	Light olive gray	Grayish yellowish brown - dark grayish yellowish brown	Grayish yellowish brown - dark grayish yellowish brown
Aerial mycelium	Abundant, light greenish gray - bluish gray	Abundant, bluish gray	Abundant, light greenish gray - bluish gray	Abundant, light greenish gray - bluish gray
Soluble pigment	Grayish yellow	Grayish yellow	Grayish yellow	Grayish yellow
ISP 5				
Growth	Good	Good	Good	Good
	Moderate olive brown - dark olive brown	Dark olive brown	Dark olive brown	Dark olive brown
Reverse	Light grayish olive - dark grayish olive	Light olive gray - greenish black	Pale yellowish green - greenish black	Pale yellowish green - greenish black
Aerial mycelium	Abundant, greenish white bluish gray	Abundant, bluish gray	Abundant, pale blue - bluish gray	Abundant, pale blue - bluish gray
Soluble pigment	None	None	None	None
ISP 6				
Growth	Good	Good	Good	Good
	Pale yellow	Pale yellow	Grayish greenish yellow	Grayish greenish yellow
Reverse	Pale yellow - dark grayish yellow	Pale yellow - dark grayish yellow	Pale yellow - dark yellow	Pale yellow - dark yellow
Aerial mycelium	Abundant, greenish white - light greenish gray	Abundant, greenish white - light greenish gray	Abundant, white	Abundant, white
Soluble pigment	None	None	None	None
ISP 7				
Growth	Good	Good	Good	Good
	Dark olive brown	Moderate olive brown - dark olive brown	Dark olive brown	Dark olive brown
Reverse	Light olive gray - greenish black	Light olive gray - greenish black	Pale yellowish green - greenish black	Pale yellowish green - greenish black
Aerial mycelium	Abundant, bluish gray	Abundant, bluish gray	Abundant, pale blue - bluish gray	Abundant, pale blue - bluish gray
Soluble pigment	Grayish yellowish green	Grayish yellowish green	None	None
YS agar				
Growth	Good	Good	Good	Good
	Dark grayish yellow	Pale yellowish green - light grayish olive	Moderate olive brown - dark olive brown	Moderate olive brown - dark olive brown
Reverse	Dark grayish yellow - dark olive brown	Light grayish olive	Light grayish olive - greenish black	Light grayish olive - greenish black
Aerial mycelium	Abundant, greenish white - light greenish gray	Abundant, light greenish gray	Abundant, light greenish gray - greenish gray	Abundant, light greenish gray - greenish gray
Soluble pigment	Light olive brown	Pale greenish yellow	Grayish greenish yellow	Grayish greenish yellow
Nutrient agar				
Growth	Good	Good	Good	Good
	Dark grayish yellow	Dark grayish yellow	Moderate olive brown	Moderate olive brown
Reverse	Abundant, light bluish gray - bluish gray	Light grayish olive - dark grayish yellowish brown	Light grayish olive - greenish black	Light grayish olive - greenish black
Aerial mycelium	Light grayish olive	Abundant, light bluish gray	Abundant, bluish gray	Abundant, bluish gray
Soluble pigment	Grayish greenish yellow	Grayish greenish yellow	Grayish greenish yellow	Grayish greenish yellow

Table 24 Physiological and biochemical characteristics of *Streptomyces* strains in group XV

Characteristics	Strains			
	KC-085	KC-098	KC-101	KC-103
Utilization of:				
L-Arabinose	+	+	+	+
D-Fructose	-	±	-	±
D-Glucose	+	+	+	+
D-Mannitol	-	-	-	-
Melezitose	-	-	-	-
D-Melibiose	+	+	+	+
myo-Inositol	-	-	±	±
Raffinose	+	+	±	+
L-Rhamnose	+	+	±	+
D-Sorbitol	-	-	-	-
Sucrose	-	-	-	-
D-Xylose	+	+	+	+
Nitrate reduction	+	+	+	+
Starch hydrolysis	+	+	+	+
Milk peptonization	+	+	+	+
Milk coagulation	-	±	-	±
Gelatinization	-	-	-	-
Growth at/with:				
NaCl (%w/v)	0-8	0-7	0-8	0-7
pH	5-12	5-12	5-12	5-12
Temperature (°C)	15-37	15-37	15-37	15-37

+ = positive, ± = weakly positive, - = negative

Table 25 Cultural characteristics of *Streptomyces* strains in groups XVI and XVII

Media	Strains			
	KC-034	KC-036	KC-117	KC-128
ISP 2				
Growth	Good	Good	Good	Good
	Dark grayish yellow	Dark grayish yellow	Vivid yellow	Vivid yellow
Reverse	Moderate olive brown	Moderate olive brown	Vivid yellow	Vivid yellow
Aerial mycelium	Abundant, light bluish gray - bluish gray	Abundant, light bluish gray - bluish gray	Abundant, white	Abundant, white
Soluble pigment	Grayish greenish yellow	Grayish greenish yellow	Vivid greenish yellow	Vivid greenish yellow
ISP 3				
Growth	Good	Good	Good	Good
	Dark grayish yellow	Dark grayish yellow	Light greenish yellow	Light greenish yellow
Reverse	Pale yellowish green - light grayish olive	Light grayish olive - grayish olive	Light greenish yellow	Light greenish yellow
Aerial mycelium	Abundant, white - light bluish gray	Abundant, light gray - medium gray	Abundant, white	Abundant, white
Soluble pigment	None	None	Light greenish yellow	Light yellow
ISP 4				
Growth	Good	Good	Good	Good
	Light olive gray	Dark grayish olive	Moderate olive brown	Light olive brown
Reverse	Light olive gray	Light grayish olive - light olive gray	Moderate greenish yellow	Grayish white
Aerial mycelium	Abundant, bluish gray	Abundant, light gray - medium gray	Abundant, light greenish gray	Abundant, greenish white
Soluble pigment	None	None	None	None
ISP 5				
Growth	Good	Good	Good	Good
	Light grayish olive	Light grayish olive	Brilliant yellow	Brilliant yellow
Reverse	Light olive gray	Light grayish olive - grayish olive	Brilliant orange	Brilliant yellow
Aerial mycelium	Abundant, light bluish gray	Abundant, medium gray	Abundant, white	Abundant, white
Soluble pigment	None	None	Light orange yellow	Light orange yellow
ISP 6				
Growth	Good	Good	Good	Good
	Dark yellow	Moderate yellow	Grayish greenish yellow	Grayish greenish yellow
Reverse	Dark yellow	Dark yellow	Grayish greenish yellow	Grayish greenish yellow
Aerial mycelium	None	None	Poor, white	None
Soluble pigment	None	None	None	None
ISP 7				
Growth	Good	Good	Good	Good
	Dark yellow	Dark grayish yellow	Pale yellowish green	Grayish greenish yellow
Reverse	Pale yellow - dark grayish yellow	Light grayish olive - grayish olive	Pale yellowish green	Dark grayish yellow
Aerial mycelium	Abundant, white	Abundant, medium gray	None	Abundant, white
Soluble pigment	None	None	None	None
YS agar				
Growth	Good	Good	Good	Good
	Dark grayish yellow	Dark grayish yellow	Brilliant greenish yellow	Brilliant greenish yellow
Reverse	Pale yellowish green - dark olive brown	Light grayish olive - grayish olive	Vivid greenish yellow	Vivid greenish yellow
Aerial mycelium	Abundant, white - bluish gray	Abundant, light bluish gray - medium gray	Abundant, white	Abundant, white
Soluble pigment	None	None	Brilliant greenish yellow	Brilliant greenish yellow
Nutrient agar				
Growth	Good	Good	Good	Good
	Grayish greenish yellow	Grayish greenish yellow	Colorless	Colorless
Reverse	Pale yellow - moderate olive brown	Light grayish olive - dark olive brown	Pale greenish yellow	Pale greenish yellow
Aerial mycelium	Abundant, medium gray	Abundant, light bluish gray - bluish gray	Abundant, white	Abundant, white
Soluble pigment	Grayish greenish yellow	Grayish greenish yellow	None	None

Table 26 Physiological and biochemical characteristics of *Streptomyces* strains in groups XVI and XVII

Characteristics	Strains			
	KC-034	KC-036	KC-117	KC-128
Utilization of:				
L-Arabinose	-	-	-	-
D-Fructose	-	-	+	+
D-Glucose	+	+	+	+
D-Mannitol	-	-	+	+
D-Melibiose	-	-	-	-
Melezitose	-	-	-	-
myo-Inositol	-	-	-	-
Raffinose	-	-	-	-
L-Rhamnose	-	-	-	-
D-Sorbitol	-	-	-	-
Sucrose	-	-	-	-
D-Xylose	-	-	±	±
Nitrate reduction	+	+	+	+
Starch hydrolysis	+	+	+	+
Milk peptonization	±	±	+	+
Milk coagulation	-	-	+	-
Gelatinization	-	-	-	-
Growth at/with:				
NaCl (%w/v)	0-4	0-4	0-7	0-7
pH	5-12	5-12	5-12	5-12
Temperature (°C)	15-37	15-37	15-37	15-37

+ = positive, ± = weakly positive, - = negative

Table 27 Cultural characteristics of *Streptomyces* strains in group XVIII

Media	Strains			
	KC-120	KC-121	KC-122	KC-130
ISP 2				
Growth	Good	Good	Good	Good
	Light olive brown	Dark yellow	Strong yellowish brown	Dark yellow
Reverse	Dark yellow	Light olive brown	Light olive brown	Dark yellow
Aerial mycelium	Abundant, greenish white	Abundant, greenish white	Abundant, greenish white	Abundant, greenish white
Soluble pigment	Deep yellow	Dark yellow	Light olive brown	None
ISP 3				
Growth	Good	Good	Good	Good
	Grayish greenish yellow	Grayish greenish yellow	Light olive brown	Grayish greenish yellow
Reverse	Grayish greenish yellow	Grayish greenish yellow	Moderate olive brown	Dark yellow
Aerial mycelium	Abundant, greenish white - light greenish gray	Abundant, greenish white - light greenish gray	Abundant, greenish white - light greenish gray	Abundant, greenish white
Soluble pigment	None	None	None	None
ISP 4				
Growth	Good	Good	Good	Good
	Dark grayish olive	Grayish greenish yellow	Moderate olive brown	Grayish greenish yellow - dark yellow
Reverse	Light olive brown	Moderate olive brown	Pale orange yellow - light greenish gray	Dark grayish yellow
Aerial mycelium	Abundant, greenish white - light greenish gray	Abundant, greenish white - light greenish gray	Abundant, greenish white - light greenish gray	Abundant, greenish white
Soluble pigment	None	None	None	None
ISP 5				
Growth	Good	Good	Good	Good
	Grayish greenish yellow	Pale yellowish green	Grayish greenish yellow	Grayish greenish yellow
Reverse	Grayish greenish yellow	Pale yellowish green - pale greenish yellow	Pale yellow - dark yellow	Grayish greenish yellow
Aerial mycelium	Abundant, light greenish gray	Moderate, greenish white	Abundant, greenish white	None
Soluble pigment	None	None	Pale yellowish green	None
ISP 6				
Growth	Good	Good	Good	Good
	Colorless	Colorless	Colorless	Grayish greenish yellow
Reverse	Light grayish olive	Dark brown	Moderate olive brown	Pale greenish yellow - grayish greenish yellow
Aerial mycelium	Abundant, greenish white - light greenish gray	Abundant, greenish white	Abundant, light greenish gray	Abundant, greenish white
Soluble pigment	Dark grayish yellow	Dark brown	Dark grayish yellow	None
ISP 7				
Growth	Good	Good	Good	Good
	Light grayish olive	Grayish yellow	Dark grayish yellow	Grayish greenish yellow
Reverse	Light grayish olive	Dark grayish yellow	Dark grayish yellow	Light olive gray
Aerial mycelium	Abundant, greenish white - light greenish gray	Abundant, yellowish gray	Abundant, light gray	None
Soluble pigment	Dark grayish yellow	None	None	None
YS agar				
Growth	Good	Good	Good	Good
	Grayish greenish yellow	Grayish greenish yellow	Moderate olive brown	Grayish greenish yellow
Reverse	Grayish greenish yellow	Moderate olive brown	Light olive brown	Grayish greenish yellow
Aerial mycelium	Abundant, greenish white - light greenish gray	Abundant, light greenish gray	Abundant, light greenish gray	Abundant, greenish white
Soluble pigment	Grayish greenish yellow	Dark yellow	Moderate yellow	Grayish greenish yellow
Nutrient agar				
Growth	Good	Good	Good	Good
	Colorless	Colorless	Colorless	Grayish greenish yellow
Reverse	Light olive brown	Light olive brown	Abundant, greenish white	Abundant, greenish white
Aerial mycelium	Abundant, greenish white	Abundant, greenish white	Dark yellow	Moderate olive brown
Soluble pigment	Dark yellow	Dark yellow	Dark yellow	Grayish greenish yellow

Table 28 Physiological and biochemical characteristics of *Streptomyces* strains in group XVIII

Characteristics	Strains			
	KC-120	KC-121	KC-122	KC-130
Utilization of:				
L-Arabinose	-	-	-	-
D-Fructose	+	+	+	+
D-Glucose	+	+	+	+
D-Mannitol	+	+	+	+
D-Melibiose	-	-	-	-
Melezitose	-	-	-	-
myo-Inositol	-	-	-	-
Raffinose	-	-	-	-
L-Rhamnose	-	-	-	-
D-Sorbitol	-	-	-	-
Sucrose	-	-	-	-
D-Xylose	+	+	+	+
Nitrate reduction	+	+	+	+
Starch hydrolysis	+	+	+	+
Milk peptonization	+	+	+	+
Milk coagulation	-	-	-	-
Gelatinization	-	-	-	+
Growth at/with:				
NaCl (%w/v)	0-8	0-8	0-8	0-7
pH	5-12	5-12	5-12	5-12
Temperature (°C)	15-37	15-37	15-37	15-37

Table 29 Cultural characteristics of *Streptomyces* strains in groups XIX, XX and XXI

Media	Strains			
	KC-087	KC-037	KC-058	KC-079
ISP 2				
Growth	Good	Good	Good	Good
	Pale yellow	Moderate yellow - strong greenish yellow	Dark grayish yellow	Pale grayish yellow
Reverse	Dark yellow	Moderate yellow - deep yellow	Light grayish olive - moderate grayish olive	Strong greenish yellow
Aerial mycelium	Abundant, white - bluish gray	Abundant, greenish white - moderate greenish gray	Abundant, greenish white – moderate greenish gray	Abundant, greenish white
Soluble pigment	Grayish yellow	Strong greenish yellow	None	None
ISP 3				
Growth	Good	Good	Good	Good
	Pale yellow	Pale yellow	Grayish greenish yellow	Pale yellowish green
Reverse	Moderate yellow	Pale yellow	Grayish olive green - dark grayish olive green	Pale yellowish green
Aerial mycelium	Abundant, white - medium gray	Abundant, greenish white – moderate greenish gray	Abundant, greenish white – moderate greenish gray	Abundant, greenish white
Soluble pigment	Grayish yellow	None	None	Pale yellowish green
ISP 4				
Growth	Good	Good	Good	Good
	Grayish greenish yellow	Pale greenish yellow	Pale yellow - grayish yellow	Grayish greenish yellow
Reverse	Pale yellow - dark grayish yellow	Light yellowish brown	Light grayish olive - dark greenish gray	Grayish greenish yellow
Aerial mycelium	Abundant, greenish white - light gray	Abundant, white	Abundant, greenish white - moderate greenish gray	Abundant, olive gray
Soluble pigment	None	None	None	None
ISP 5				
Growth	Good	Good	Good	Good
	Pale yellow	Pale greenish yellow	Greenish white	Pale yellowish green
Reverse	Pale yellow - grayish yellow	Pale greenish yellow	Pale yellowish green	Pale greenish yellow
Aerial mycelium	Abundant, white	Abundant, white – light greenish gray	Abundant, greenish white	None
Soluble pigment	None	None	None	Pale yellowish green
ISP 6				
Growth	Good	Good	Good	Good
	Grayish yellow	Grayish yellow	Pale yellow	Pale yellow
Reverse	Moderate yellow	Moderate yellow	Strong greenish yellow	Light yellow
Aerial mycelium	None	None	None	None
Soluble pigment	None	None	None	None
ISP 7				
Growth	Good	Good	Good	Good
	Light olive brown	Light yellowish brown	Moderate yellow	Moderate olive brown
Reverse	Light olive brown	Light yellowish brown	Dark yellow - dark grayish yellow	Moderate olive brown
Aerial mycelium	None	Abundant, greenish white	Abundant, greenish white - moderate greenish gray	Abundant, greenish white - olive gray
Soluble pigment	None	None	Pale yellow	Grayish greenish yellow
YS agar				
Growth	Good	Good	Good	Good
	Pale yellow	Strong greenish yellow	Grayish greenish yellow	Pale greenish yellow
Reverse	Pale yellow - dark yellow	Pale greenish yellow – moderate greenish yellow	Grayish greenish yellow - dark greenish gray	Pale greenish yellow - moderate greenish yellow
Aerial mycelium	Abundant, greenish white - brownish black	Abundant, greenish white – light greenish gray	Abundant, greenish white - greenish black	Abundant, greenish white - light greenish gray
Soluble pigment	None	None	None	Pale greenish yellow
Nutrient agar				
Growth	Good	Good	Good	Moderate
	Colorless	Greenish white	Pale yellowish green	Pale yellow
Reverse	Pale greenish yellow	Pale greenish yellow	Pale greenish yellow	Pale yellow
Aerial mycelium	Abundant, white	Abundant, white	Abundant, white	Abundant, greenish white
Soluble pigment	None	None	None	None

Table 30 Physiological and biochemical characteristics of *Streptomyces* strains in groups XIX, XX and XXI

Characteristics	Strains			
	KC-087	KC-037	KC-058	KC-079
Utilization of:				
L-Arabinose	±	±	±	+
D-Fructose	+	+	+	+
D-Glucose	+	+	+	+
D-Mannitol	+	+	+	+
Melezitose	-	-	-	-
D-Melibiose	+	+	+	+
myo-Inositol	+	+	+	+
Raffinose	±	+	+	+
L-Rhamnose	+	+	+	+
D-Sorbitol	-	-	-	-
Sucrose	±	-	-	-
D-Xylose	+	+	+	+
Nitrate reduction	+	+	+	+
Starch hydrolysis	+	+	+	+
Milk peptonization	±	-	-	-
Milk coagulation	-	-	-	-
Gelatinization	-	+	+	+
Growth at/with:				
NaCl (%w/v)	7	7	7	4
pH	5-12	5-12	5-11	5-12
Temperature (°C)	15-37	15-37	15-37	15-37

+ = positive, ± = weakly positive, - = negative

Table 31 Cultural characteristics of *Streptomyces* strains in group XXII

Media	Strains				
	KC-080	KC-093	KC-094	KC-138	KC-151
ISP 2					
Growth	Good	Good	Good	Good	Good
	Grayish greenish yellow	Colorless	Grayish greenish yellow	Grayish greenish yellow	Dark yellow - light olive gray
Reverse	Light yellow	Light olive brown	Grayish greenish yellow	Grayish greenish yellow	Dark yellow - light olive gray
Aerial mycelium	Abundant, greenish white	Abundant, greenish white - light greenish gray	Abundant, medium gray	Abundant, greenish white - light greenish gray	Abundant, medium gray
Soluble pigment	None	Deep yellow	None	None	None
ISP 3					
Growth	Good	Good	Good	Good	Good
	Light olive gray	Colorless	Greenish white	Grayish greenish yellow	Light grayish olive - olive gray
Reverse	Greenish gray - greenish black	Dark yellow	Light yellowish green	Light grayish olive - olive gray	Light grayish olive - olive gray
Aerial mycelium	Abundant, dark grayish yellowish brown - greenish black	Abundant, greenish white - light greenish gray	Abundant, medium gray	Abundant, greenish white - brownish black	Abundant, bluish gray
Soluble pigment	None	Dark yellow	None	None	None
ISP 4					
Growth	Good	Good	Good	Good	Good
	Light grayish olive	Dark grayish yellow	Grayish yellow	Colorless	Grayish greenish yellow - light olive brown
Reverse	Light grayish olive - dark greenish gray	Moderate olive brown	Grayish yellow	Light grayish olive - olive gray	Grayish greenish yellow - dark grayish yellow
Aerial mycelium	Abundant, light olive gray - greenish black	Abundant, greenish white - light greenish gray	Abundant, brownish gray	Light olive gray	Abundant, bluish gray
Soluble pigment	None	Dark grayish yellow	None	None	None
ISP 5					
Growth	Good	Good	Good	Good	Good
	Light grayish olive	Pale yellow	Grayish greenish yellow	Pale yellowish green	Pale yellow - light yellow
Reverse	Light greenish gray - dark grayish olive	Grayish yellow	Grayish greenish yellow	Pale yellow	Pale yellow - light yellow
Aerial mycelium	Abundant, light greenish gray - brownish black	Abundant, greenish white - light greenish gray	None	Moderate, greenish white	None
Soluble pigment	None	None	None	None	None
ISP 6					
Growth	Good	Good	Good	Good	Good
	Grayish greenish yellow	Colorless	Grayish greenish yellow	Grayish greenish yellow	Pale yellow – grayish yellow
Reverse	Pale greenish yellow - dark yellow	Dark yellow	Grayish greenish yellow	Grayish greenish yellow	Light yellow – dark yellow
Aerial mycelium	Abundant, greenish white	Abundant, greenish white	Abundant, greenish white	Poor, greenish white	Abundant, greenish white
Soluble pigment	None	Moderate olive brown	None	None	None
ISP 7					
Growth	Good	Good	Good	Good	Good
	Grayish greenish yellow	Grayish yellow	Grayish greenish yellow - moderate olive	Moderate olive brown	Moderate yellow - dark yellow
Reverse	Grayish greenish yellow - light grayish olive	Grayish yellow	Moderate olive	Moderate olive brown	Dark yellow - dark grayish yellow
Aerial mycelium	Abundant, greenish white	Abundant, white	Abundant, medium gray	Poor, white	Abundant, light gray - medium gray
Soluble pigment	None	None	None	Grayish greenish yellow	None
YS agar					
Growth	Good	Good	Good	Good	Good
	Light grayish olive	Grayish greenish yellow	Greenish white - grayish greenish yellow	Colorless	Light grayish olive
Reverse	Light grayish olive - dark greenish gray	Dark yellow	Grayish greenish yellow	Grayish greenish yellow	Light grayish olive - grayish olive
Aerial mycelium	Abundant, brownish black - greenish black	Abundant, greenish white	Abundant, medium gray	Abundant, white - olive gray	Abundant, light olive gray
Soluble pigment	None	Dark yellow	None	None	None
Nutrient agar					
Growth	Good	Good	Good	Good	Good
	Light grayish olive	Pale greenish yellow	Grayish greenish yellow	Colorless	Grayish greenish yellow
Reverse	Light grayish olive - olive gray	Dark yellow	Grayish greenish yellow	Pale yellowish green - olive gray	Grayish greenish yellow
Aerial mycelium	Abundant, light olive gray - greenish black	Abundant, greenish white	Abundant, light gray - medium gray	Abundant, white - brownish black	Abundant, light olive gray
Soluble pigment	None	Dark yellow	None	None	None

Table 32 Physiological and biochemical characteristics of *Streptomyces* strains in group XXII

Characteristics	Strains				
	KC-080	KC-093	KC-094	KC-138	KC-151
Utilization of:					
L-Arabinose	+	+	+	+	+
D-Fructose	+	+	+	+	+
D-Glucose	+	+	+	+	+
D-Mannitol	+	+	+	+	+
Melezitose	-	-	-	-	-
D-Melibiose	+	+	+	+	+
myo-Inositol	+	+	+	+	-
Raffinose	-	-	-	+	+
L-Rhamnose	+	+	+	+	+
D-Sorbitol	-	-	-	-	-
Sucrose	-	-	-	-	-
D-Xylose	+	+	+	+	+
Nitrate reduction	+	+	+	+	+
Starch hydrolysis	+	+	+	+	+
Milk peptonization	+	+	+	+	+
Milk coagulation	-	-	-	-	-
Gelatinization	-	-	-	-	-
Growth at/with:					
NaCl (%w/v)	0-5	0-8	0-5	0-4	0-8
pH	5-12	5-12	5-12	5-12	5-12
Temperature (°C)	15-37	15-37	15-37	15-37	15-37

+ = positive, - = negative

Table 33 Cultural characteristics of *Streptomyces* strains in groups XXIII and XXIV

Media	Strains	
	KC-145	KC-003
ISP 2		
Growth	Good	Good
	Light yellowish brown	Moderate yellowish brown
Reverse	Light olive brown	Dark brown
Aerial mycelium	Abundant, light greenish gray	Abundant, light greenish gray - greenish gray
Soluble pigment	Dark yellow	Strong yellowish brown
ISP 3		
Growth	Good	Good
	Moderate pink - dark pink	Grayish greenish yellow
Reverse	Moderate pink - dark pink	Light grayish olive - grayish olive
Aerial mycelium	Abundant, pinkish white - pinkish gray	Abundant, light yellowish green
Soluble pigment	Pale pink	None
ISP 4		
Growth	Good	Good
	Moderate yellowish brown	Grayish greenish yellow
Reverse	Light olive gray - grayish brown	Greenish white
Aerial mycelium	Abundant, light greenish gray - greenish gray	Abundant, bluish gray
Soluble pigment	Grayish yellow	None
ISP 5		
Growth	Good	Good
	Pale yellowish green	Moderate olive brown
Reverse	Pale yellow	Dark grayish yellow - dark olive brown
Aerial mycelium	Abundant, white - greenish white	Abundant, greenish gray
Soluble pigment	Pale yellowish green	Brownish pink
ISP 6		
Growth	Good	Good
	Grayish greenish yellow	Dark yellow
Reverse	Grayish greenish yellow	Grayish yellowish brown
Aerial mycelium	Abundant, greenish white	None
Soluble pigment	Grayish greenish yellow	Deep yellowish brown
ISP 7		
Growth	Good	Good
	Dark grayish yellow	Dark grayish yellow
Reverse	Grayish yellowish brown	Grayish brown
Aerial mycelium	Abundant, light greenish gray - greenish gray	Abundant, white - light greenish gray
Soluble pigment	Grayish yellow	Brownish pink
YS agar		
Growth	Good	Good
	Dark pink - strong yellowish brown	Grayish greenish yellow
Reverse	Light reddish brown - reddish brown	Grayish yellowish green
Aerial mycelium	Abundant, pinkish white - pinkish gray	Abundant, bluish gray
Soluble pigment	Brownish pink	None
Nutrient agar		
Growth	Good	Good
	Moderate olive brown	Grayish greenish yellow
Reverse	Dark yellowish brown	Moderate olive brown
Aerial mycelium	Abundant, greenish gray	Abundant, white - pale blue
Soluble pigment	Dark yellow	Dark grayish yellow

Table 34 Physiological and biochemical characteristics of *Streptomyces* strains in groups XXIII and XXIV

Characteristics	Strains	
	KC-145	KC-003
Utilization of:		
L-Arabinose	-	-
D-Fructose	-	-
D-Glucose	+	+
D-Mannitol	-	-
Melezitose	-	-
D-Melibiose	-	-
myo-Inositol	-	-
Raffinose	-	-
L-Rhamnose	-	-
D-Sorbitol	-	-
Sucrose	-	+
D-Xylose	-	-
Nitrate reduction	-	-
Starch hydrolysis	+	-
Milk peptonization	+	-
Milk coagulation	±	-
Gelatinization	-	+
Growth at/with:		
NaCl (%w/v)	0-7	0-2
pH	5-12	5-12
Temperature (°C)	15-40	15-37

+ = positive, ± = weakly positive, - = negative

Table 35 Cultural characteristics of *Amycolatopsis* strain in group A and *Kitasatospora* strains in groups 1 and 2

Media	Strains			
	KC-132	KC-001	KC-005	KC-143
ISP 2				
Growth	Good	Good	Good	Good
	Light yellowish pink	Yellowish white	Yellowish white	Grayish greenish yellow - moderate olive
Reverse	Light orange yellow	Light olive brown - moderate olive brown	Moderate olive brown	Grayish yellow - dark olive brown
Aerial mycelium	Abundant, white	Abundant, light greenish gray	Abundant, light greenish gray	Abundant, light greenish gray - greenish gray
Soluble pigment	Light orange	Dark yellow	Dark grayish yellow	Strong greenish yellow
ISP 3				
Growth	Good	Good	Good	Good
	Light yellowish pink	Greenish white	Greenish white	Dark grayish yellow
Reverse	Light orange yellow	Grayish greenish yellow	Grayish greenish yellow	Light grayish olive
Aerial mycelium	Abundant, white	Abundant, bluish gray	Abundant, bluish gray	Abundant, white - light yellowish green
Soluble pigment	Pale yellowish pink	None	None	Light grayish olive
ISP 4				
Growth	Good	Good	Good	Good
	Pale yellowish pink	Pale yellowish green - pale yellow	Pale yellowish green - pale yellow	Light grayish olive
Reverse	Pale orange yellow	Moderate olive brown	Moderate olive brown	Greenish white
Aerial mycelium	Abundant, white	Abundant, light greenish gray	Abundant, light greenish gray	Abundant, greenish white - bluish gray
Soluble pigment	Pale yellowish pink	None	None	None
ISP 5				
Growth	Good	Good	Good	Good
	Light brown	Greenish white - yellowish white	Greenish white - yellowish white	Greenish black
Reverse	Brownish orange	Greenish white	Greenish white	light olive gray - greenish black
Aerial mycelium	Abundant, white	Abundant, greenish white - light greenish gray	Abundant, greenish white - light greenish gray	Abundant, pale blue
Soluble pigment	Light orange yellow	None	None	None
ISP 6				
Growth	Good	Good	Good	Good
	Pale yellow	Greenish white	Greenish white	Pale yellow
Reverse	Pale yellow	Pale greenish yellow	Pale greenish yellow	Pale yellow
Aerial mycelium	Abundant, white	Abundant, white	Abundant, white	None
Soluble pigment	Light yellowish pink	None	None	None
ISP 7				
Growth	Good	Good	Good	Good
	Strong brown	Greenish white	Greenish white	Light olive gray - dark olive brown
Reverse	Light reddish purple - very deep reddish purple	Greenish white - light greenish gray	Greenish white - light greenish gray	Light olive gray - greenish black
Aerial mycelium	Abundant, white	Abundant, light bluish gray	Abundant, light bluish gray	Abundant, light greenish gray
Soluble pigment	Deep reddish purple	None	None	Light grayish olive
YS agar				
Growth	Good	Good	Good	Good
	Light yellowish pink	Greenish white	Greenish white	Grayish greenish yellow
Reverse	Light yellowish pink	Pale greenish yellow	Pale greenish yellow	Light grayish olive
Aerial mycelium	Abundant, white	Abundant, white	Abundant, white	Abundant, white - bluish gray
Soluble pigment	Light yellowish pink	None	None	None
Nutrient agar				
Growth	Good	Good	Good	Good
	Pale greenish yellow	Greenish white	Greenish white	Grayish greenish yellow
Reverse	Pale greenish yellow	Grayish greenish yellow	Grayish greenish yellow	Dark grayish yellow
Aerial mycelium	Abundant, white	Abundant, light greenish gray	Abundant, light greenish gray	Abundant, white - light greenish gray
Soluble pigment	None	None	None	Dark grayish yellow

Table 36 Physiological and biochemical characteristics of *Amycolatopsis* strain in group A and *Kitasatospora* strains in groups 1 and 2

Characteristics	Strains			
	KC-132	KC-001	KC-005	KC-143
Utilization of:				
L-Arabinose	+	-	-	-
D-Fructose	+	-	-	-
D-Glucose	+	+	+	+
D-Mannitol	+	-	-	-
Melezitose	±	-	-	-
D-Melibiose	+	-	-	-
myo-Inositol	+	-	-	-
Raffinose	+	-	-	-
L-Rhamnose	-	-	-	-
D-Sorbitol	-	-	-	-
Sucrose	-	-	-	-
D-Xylose	+	+	+	+
Nitrate reduction	+	-	-	-
Starch hydrolysis	+	+	+	-
Milk peptonization	+	+	+	+
Milk coagulation	+	-	-	-
Gelatinization	+	-	-	-
Growth at/with:				
NaCl (%w/v)	0-7	0-3	0-3	0-4
pH	5–12	5-12	5-12	5-12
Temperature (°C)	15–40	15-37	15-37	15-37

+ = positive, ± = weakly positive, - = negative

APPENDIX D

16S rRNA gene sequences of novel species

S. siamensis strain KC-031

GACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGATGAAGCCCTTCGGGGTGGATTAGTGGCGAAC
GGGTGAGTAACACGTGGGCAATCTGCCCTTCACTCTGGGACAAGCCCTGGAAACGGGGTCTAATACCGGATAA
CACTCCTGCCTGCATGGGCGGGGGTTAAAAGCTCCGGCGGTGAAGGATGAGCCCGCGGCCTATCAGCTTGTTG
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ACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGAAAGCCTGATGCAGCGAC
GCCGCGTGAGGGATGACGGCCTTCGGGTTGTAAACCTCTTTCAGCAGGGAAGAAGCGAAAGTGACGGTACCTG
CAGAAGAAGCGCCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGCGCAAGCGTTGTCCGGAATTAT
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GAGGAACACCGGTGGCGAAGGCGGATCTCTGGGCCATTACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAA
CAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGGTGGGCACTAGGTGTTGGCGACATTCCACGTCGTCGG
TGCCGCAGCTAACGCATTAAGTGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGG
GGGCCCGCACAAGCAGCGGAGCATGTGGCTTAATTCGACGCAACGCGAAGAACCTTACCAAGGCTTGACATAC
GCCGGAAAACCCTGGAGACAGGGTCCCCCTTGTGGTCGGTGTACAGGTGGTGCATGGCTGTCGTCAGCTCGTG
TCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTTCTGTGTTGCCAGCATGCCCTTCGGGGTG
ATGGGGACTCACAGGAGACTGCCGGGGTCAACTCGGAGGAAGGTGGGGACGACGTCAAGTCATCATGCCCCT
TATGTCTTGGGCTGCACACGTGCTACAATGGCAGGTACAAAGAGCTGCGAAGCCGCGAGGCGGAGCGAATCTC
AAAAAGCCTGTCTCAGTTCGGATTGGGGTCTGCAACTCGACCCCATGAAGTCGGAGTTGCTAGTAATCGCAGA
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CCGAAGCCGGTGGCCCAACCCCTTGTGGGAGGGAGCTGTCGAAGGTGGGACTGGCGATTGGGACGAAGTCGT
AACAAGGTAGCCGTACCGGAAGGTGCGGCTGGATCACCTCC

S. krungchingensis strain KC-035

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GGCGAACGGGTGAGTAACACGTGGGCAATCTGCCCTTCACTCTGGGACAAGCCCTGGAAACGGGGTCTAATAC
CGGATACGACCTGCCGAGGCATCTCGGCGGGTGGAAAGCTCCGGCGGTGAAGGATGAGCCCGCGGCCTATCA
GCTTGTTGGTGAGGTAGTGGCTCACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACACTG
GGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGAAAGCCTGAT
GCAGCGACGCCGCGTGAGGGATGACGGCCTTCGGGTTGTAAACCTCTTTCAGCAGGGAAGAAGCGAAAGTGA
CGGTACCTGCAGAAGAAGCGCCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGCGCAAGCGTTGT
CCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGCTTGTCGCGTCGGTTGTGAAAGCCCGGGGCTTAACCCCG
GGTCTGCAGTCGATACGGGCAGGCTAGAGTGTGGTAGGGGAGATCGGAATTCCTGGTGTAGCGGTGAAATGC
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CACGTCGTCGGTGCCGCAGCTAACGCATTAAGTGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAA
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GGCTTGACATACGCCGGAAAACCCTGGAGACAGGGTCCCCCTTGTGGTCGGTGTACAGGTGGTGCATGGCTGT
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AGTAATCGCAGATCAGCATTGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACGTCACGAA
AGTCGGTAACACCCGAAGCCGGTGGCCCAACCCCTTGTGGGAGGGAGCTGTCGAAGGTGGGACTGGCGATTG
GGACGAAGTCGTAACAAGGTAGCCGTACCGGAAGGTGCGGCTGG

S. siamensis strain KC-038

GACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGATGAAGCCCTTCGGGGTGGATTAGTGGCGAAC
GGGTGAGTAACACGTGGGCAATCTGCCCTTCACTCTGGGACAAGCCCTGGAAACGGGGTCTAATACCGGATAA
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GCCGCGTGAGGGATGACGGCCTTCGGGTTGTAAACCTCTTTCAGCAGGGAAGAAGCGAAAGTGACGGTACCTG
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TGGGCGTAAAGAGCTCGTAGGCGGCTTGTCACGTCGATTGTGAAAGCTCGGGGCTTAACCCCGAGTCTGCAGT
CGATACGGGCTAGCTAGAGTGTGGTAGGGGAGATCGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAG
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AAAAAGCCTGTCTCAGTTCGGATTGGGGTCTGCAACTCGACCCCATGAAGTCGGAGTTGCTAGTAATCGCAGA
TCAGCATTGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACGTCACGAAAGTCGGTAACAC
CCGAAGCCGGTGGCCCAACCCCTTGTGGGAGGGAGCTGTCGAAGGTGGGACTGGCGATTGGGACGAAGTCGT
AACAAGGTAGCCGTACCGGAAGGTGCGGCTGGATCACCTCCT

S. similanensis strain KC-106

GACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGATGAACCACTTCGGTGGGGATTAGTGGCGAAC
GGGTGAGTAACACGTGGGCAATCTGCCCTTCACTCTGGGACAAGCCCTGGAAACGGGGTCTAATACCGGATAC
AACCGCTGACCGCATGGTCGGGCGGTGGAAAGCTCCGGCGGTGAAGGATGAGCCCGCGGCCTATCAGCTTGT
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AGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGAAAGCCTGATGCAGCG
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GTCGATACGGGCAGGCTAGAGTGTGGTAGGGGAGATCGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATC
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ACACCGGAAAGCATTAGAGATAGTGCCCCCCTTGTGGTCGGTGTACAGGTGGTGCATGGCTGTCGTCAGCTCG
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GATCAGCATTGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACGTCACGAAAGTCGGTAAC
ACCCGAAGCCGGTGGCCCAACCCCTTGTGGGAGGGAGCTGTCGAAGGTGGGACTGGCGATTGGGACGAAGTC
GTAACAAGGTAGCCGTACCGGAAGGTGCGGCTGGATCACCTCCT

S. andamanensis strain KC-112

GCTCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGATGAACCACTTCGGTGGGGATTAGTG
GCGAACGGGTGAGTAACACGTGGGCAATCTGCCCTGCACTCTGGGACAAGCCCTGGAAACGGGGTCTAATACC
GGATACGAGTCTCCAAGGCATCTTGGGGACTGTAAAGCTCCGGCGGTGCAGGATGAGCCCGCGGCCTATCAG
CTTGTTGGTGAGGTAGTGGCTCACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACACTGG
GACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGAAAGCCTGATG
CAGCGACGCCGCGTGAGGGATGACGGCCTTCGGGTTGTAAACCTCTTTCAGCAGGGAAGAAGCGAAAGTGAC
GGTACCTGCAGAAGAAGCGCCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGCGCAAGCGTTGTC
CGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGCTTGTCACGTCGGTTGTGAAAGCCCGGGGCTTAACCCCGG
GTCTGCAGTCGATACGGGCAGGCTAGAGTTCGGTAGGGGAGATCGGAATTCCTGGTGTAGCGGTGAAATGCGC
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TCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCCCGTGTTGCCAGCAGGCC
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GCGAATCTCAAAAAGCCGGTCTCAGTTCGGATTGGGGTCTGCAACTCGACCCCATGAAGTCGGAGTCGCTAGT
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CGGTAACACCCGAAGCCGGTGGCCCAACCCCTTGTGGGAGGGAGCTGTCGAAGGTGGGACTGGCGATTGGGA
CGAAGTCGTAACAAGGTAGCCGTACCGGAAGGTGCGGCTGGATCACCTC

N. thailandensis **strain KC-061**

GACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAGCGGAAAGGCCCTTCGGGGTACTCGAGCGGCGAA
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GGTGGGGTAGTGGCCTACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACACTGGGACTGA
GACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCGCAATGGGCGGAAGCCTGACGCAGCGA
CGCCGCGTGGGGGATGACGGCCTTCGGGTTGTAAACCTCTTTCAGCAGGGACGAAGTTGACGTGTACCTGCAG
AAGAAGCGCCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGCGCAAGCGTTGTCCGGAATTATTGG
GCGTAAAGAGCTCGTAGGTGGCTTGTCGCGTCGGATGTGAAAGCCCGCAGCTTAACTGCGGGTCTGCATTTGA
TACGGGCAGGCTAGAGGTAGGTAGGGGAGAACGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGA
GGAACACCGGTGGCGAAGGCGGTTCTCTGGGCCTTACCTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACA
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CGGAGCTAACGCATTAAGCGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGG
GCCCGCACAAGCGGCGGAGCATGTTGCTTAATTCGACGCAACGCGAAGAACCTTACCAAGGTTTGACATCACC
CGGAAACCTGCAGAGATGTGGGCCTCTTCGGACTGGGTGACAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCG
TGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGCTCCATGTTGCCAGCAGGCGCTTCGGCGTGCTG
GGGACTCATGGGGGACTGCCGGGGTCAACTCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGCCCCTTAT
GTCTTGGGCTGCAAACATGCTACAATGGCCGGTACAGAGGGTTGCGATACCGTGAGGTGGAGCGAATCCCTAA
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GCAATGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACGTCACGAAAGTCGGCAACACCCG
AAGCCCGTGGCCCAACCCGTAAGGGGGGGAGCGGTCGAAGGTGGGGCTGGCGATTGGGACGAAGTCGTAACA
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APPENDIX E

HPLC profiles, Mass and NMR spectra

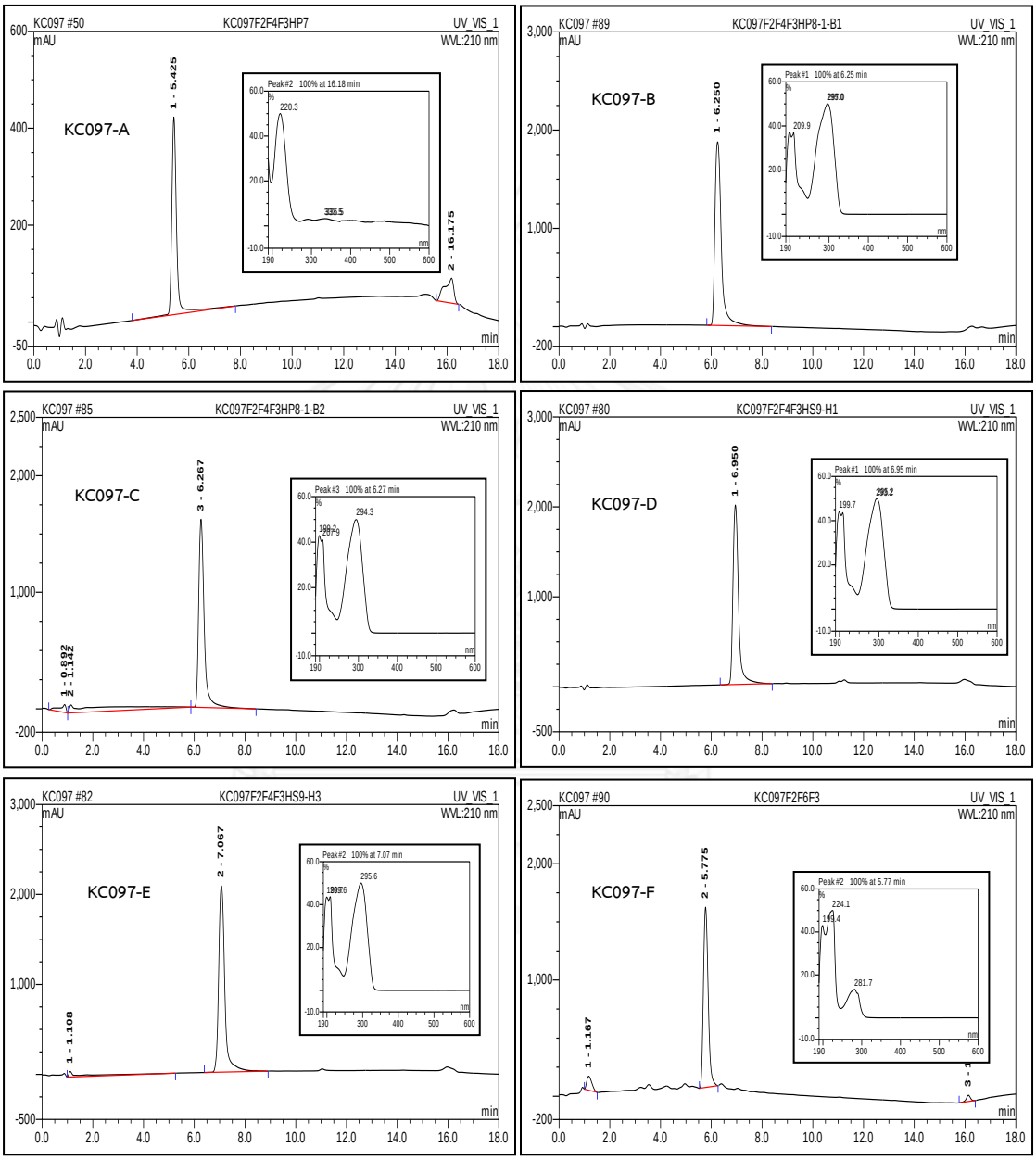

Figure 1 The HPLC profiles of six pure compounds from *S. tendae* strain KC-097

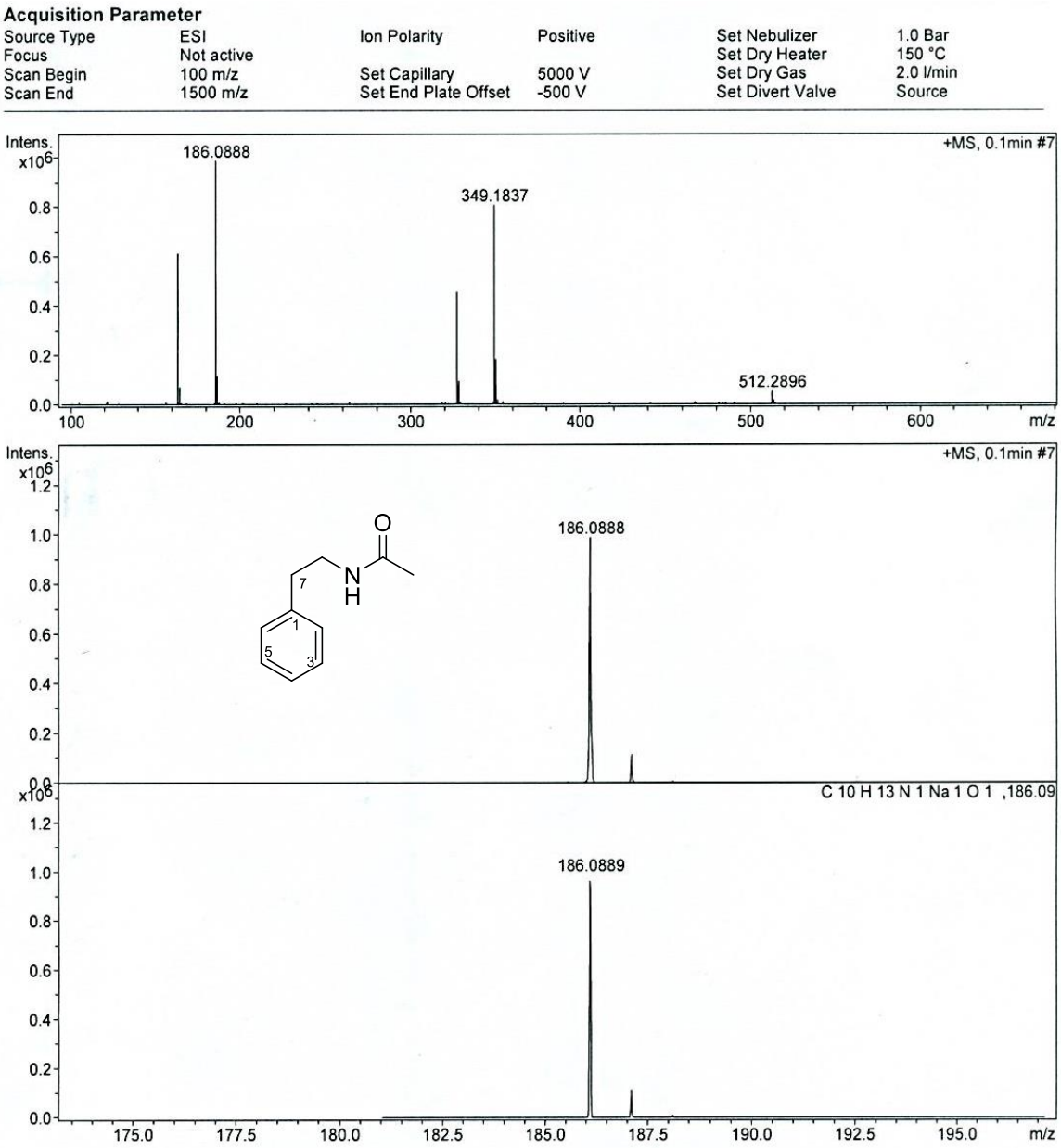

Figure 2 Mass spectrum of KC097-A

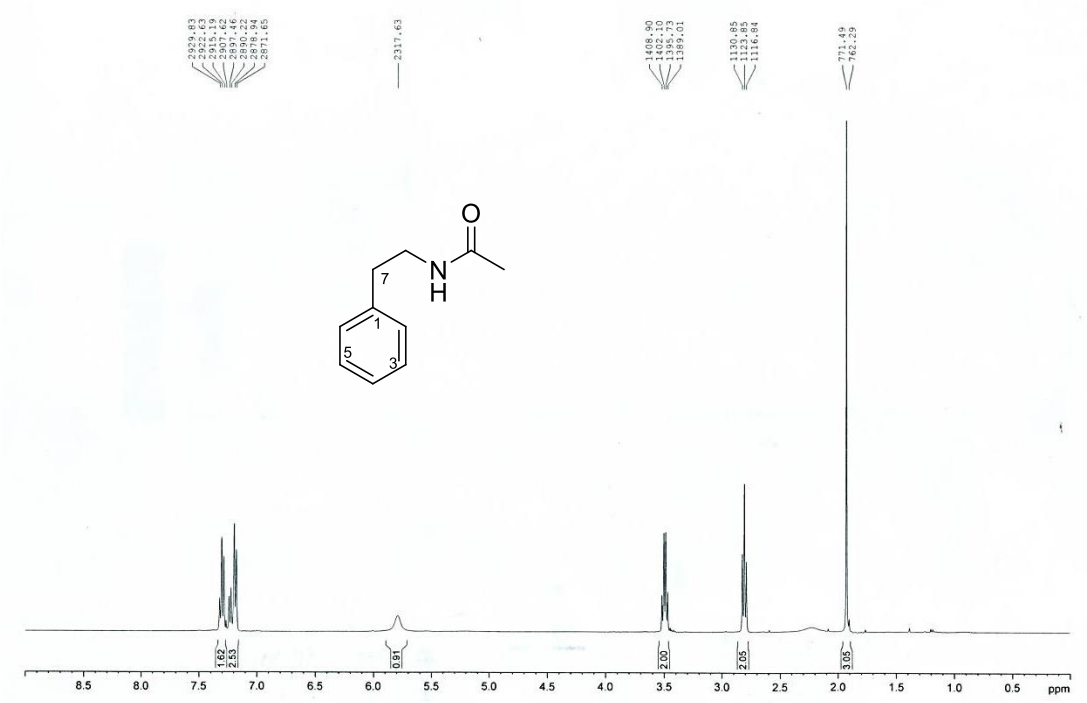

Figure 3 ^{1}H NMR spectrum (400 MHz, in $CDCl_3$) of KC097-A

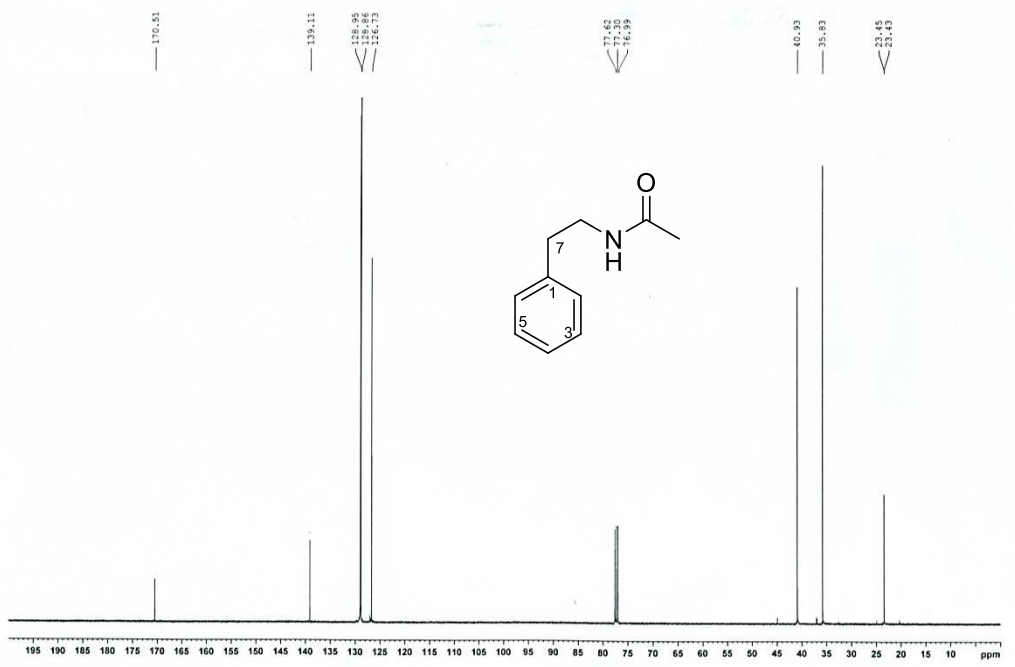

Figure 4 ^{13}C NMR spectrum (400 MHz, in $CDCl_3$) of KC097-A

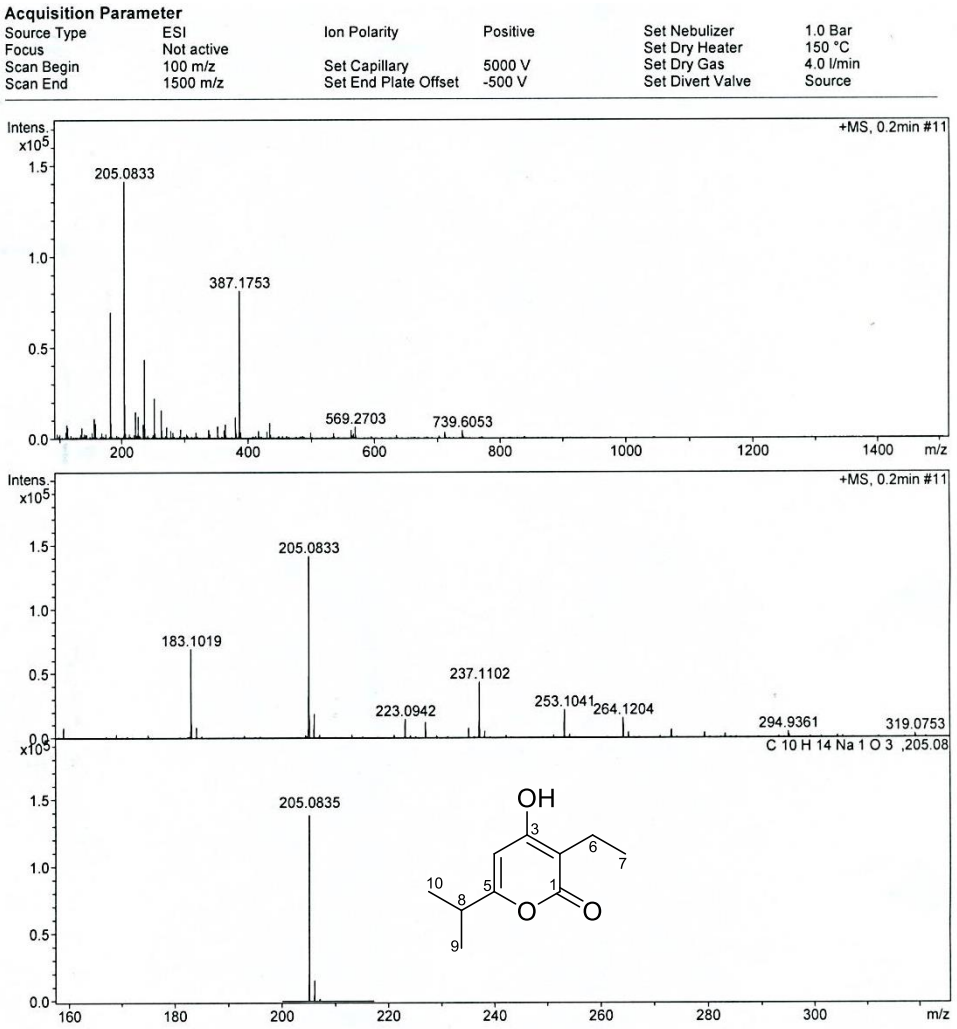

Figure 5 Mass spectrum of KC097-B

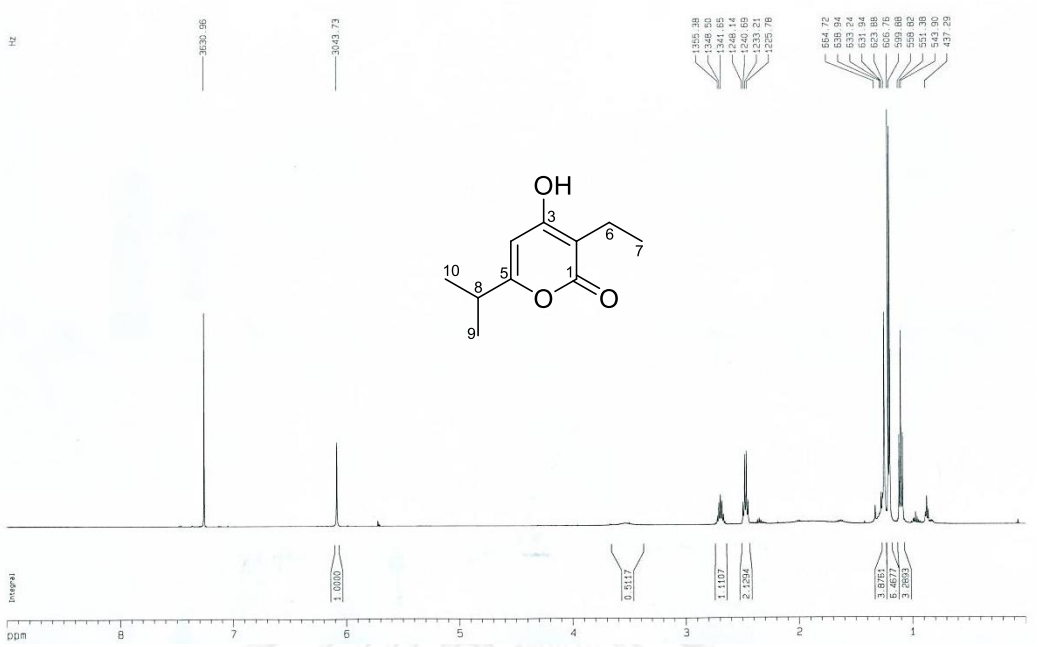

Figure 6 ^{1}H NMR spectrum (500 MHz, in $CDCl_3$) of KC097-B

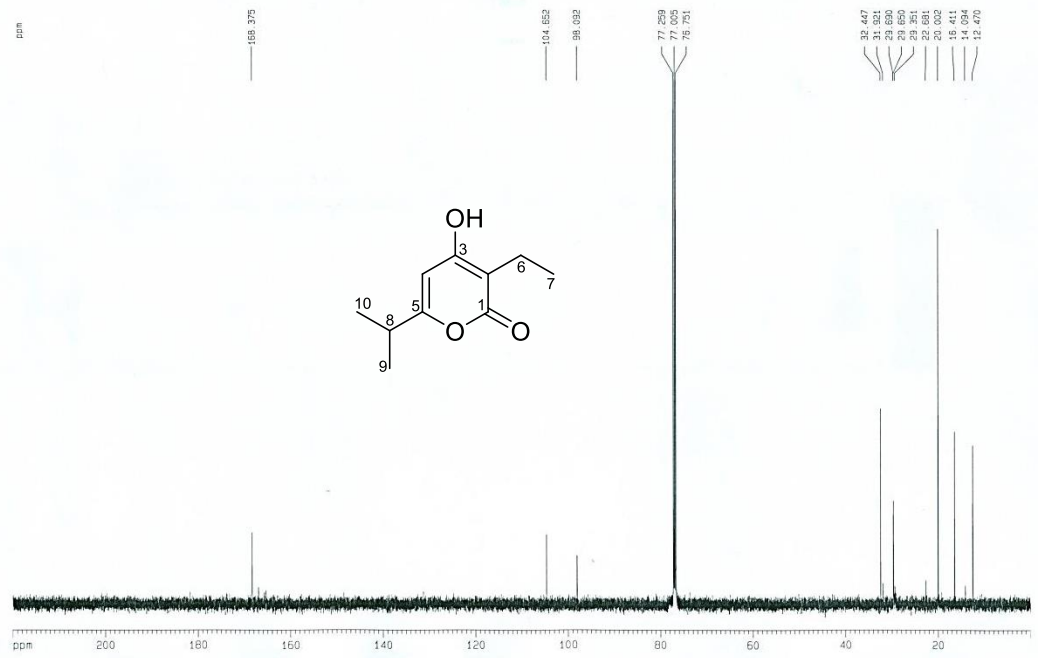

Figure 7 ^{13}C NMR spectrum (500 MHz, in $CDCl_3$) of KC097-B

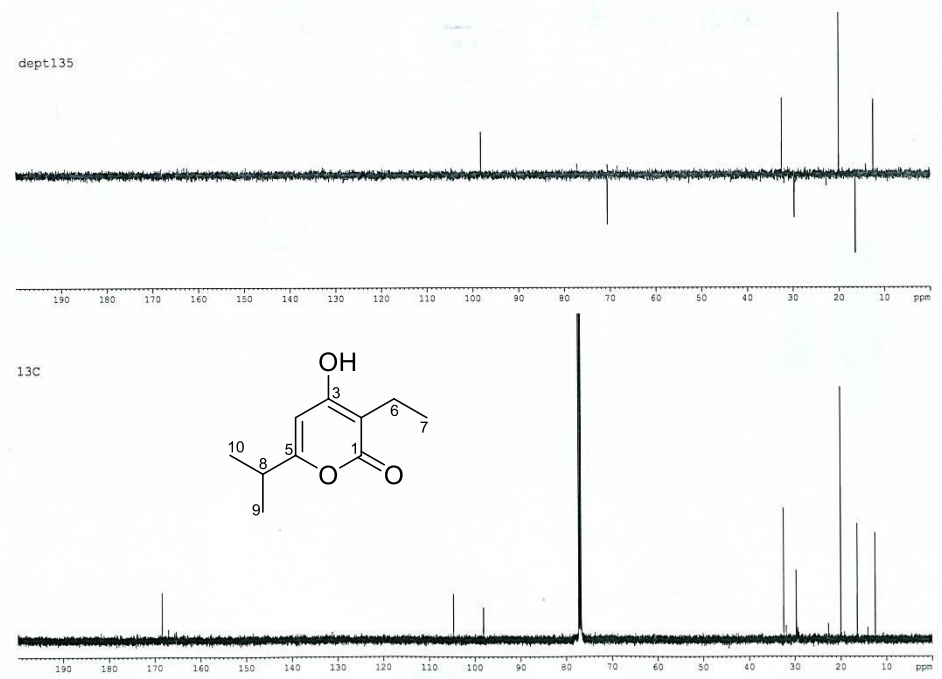

Figure 8 DEPT 135 spectrum of KC097-B (500 MHz, in $CDCl_3$)

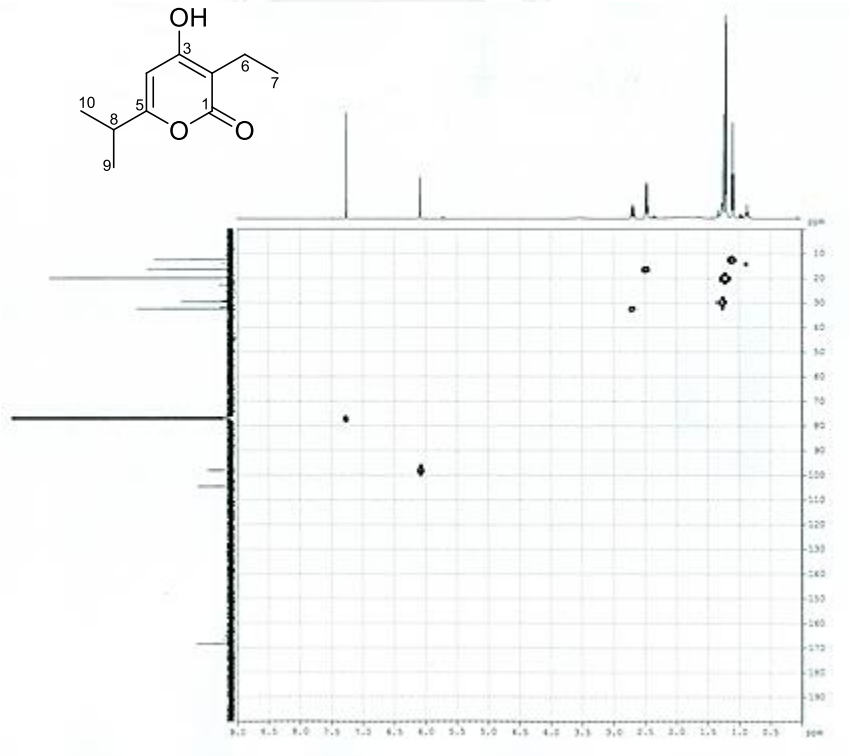

Figure 9 HMQC spectrum (500 MHz, in $CDCl_3$) of KC097-B

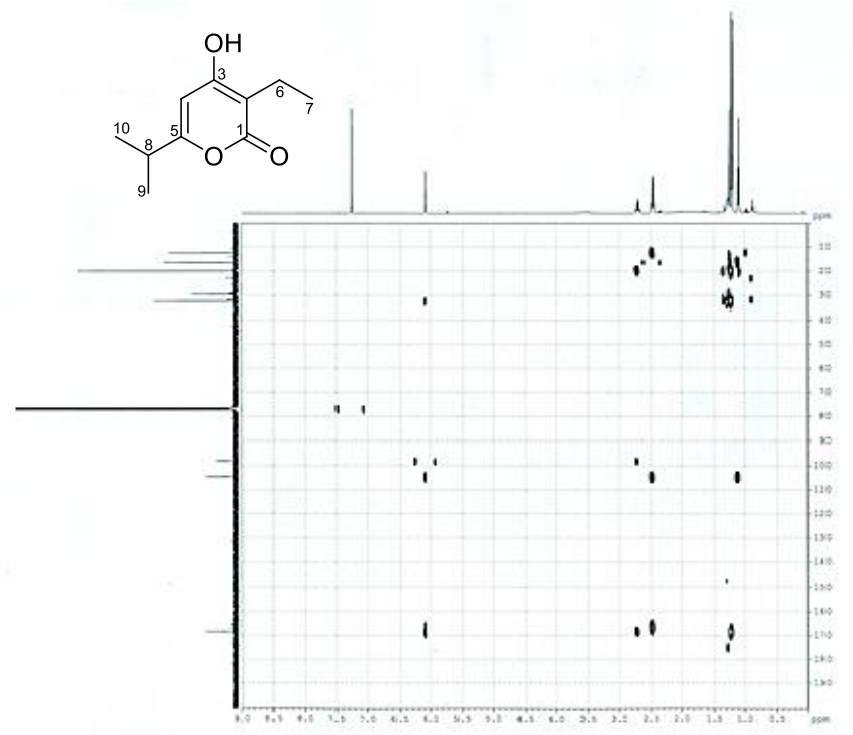

Figure 10 HMBC spectrum (500 MHz, in $CDCl_3$) of KC097-B

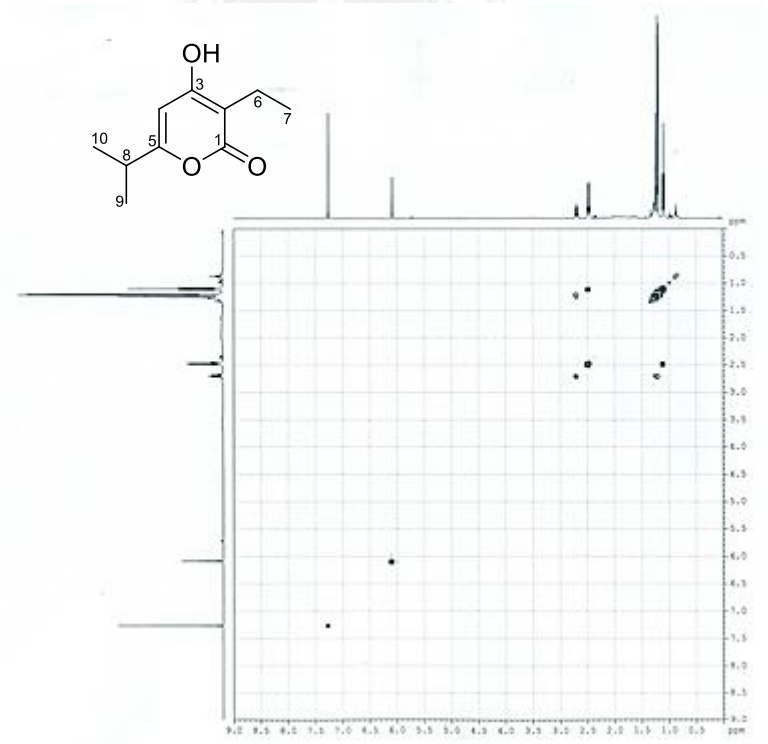

Figure 11 COSY spectrum (500 MHz, in $CDCl_3$) of KC097-B

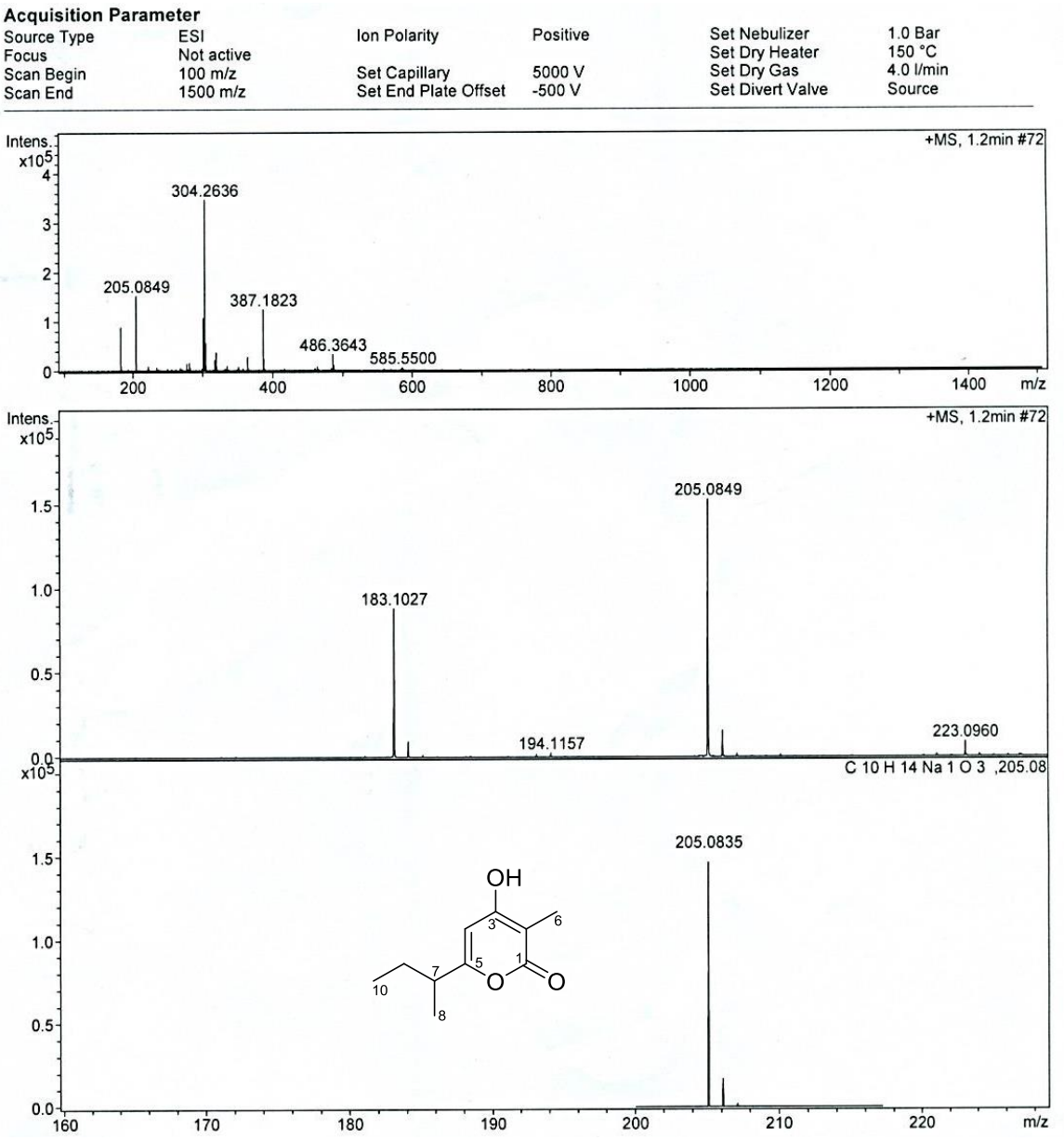

Figure 12 Mass spectrum of KC097-C

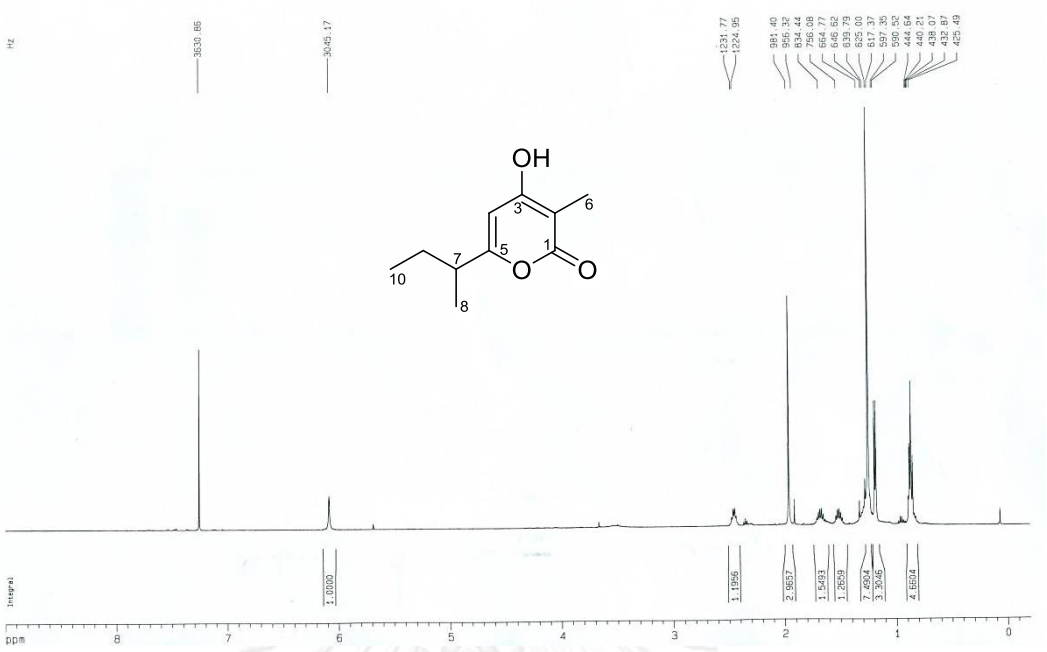

Figure 13 ^{1}H NMR spectrum (500 MHz, in $CDCl_3$) of KC097-C

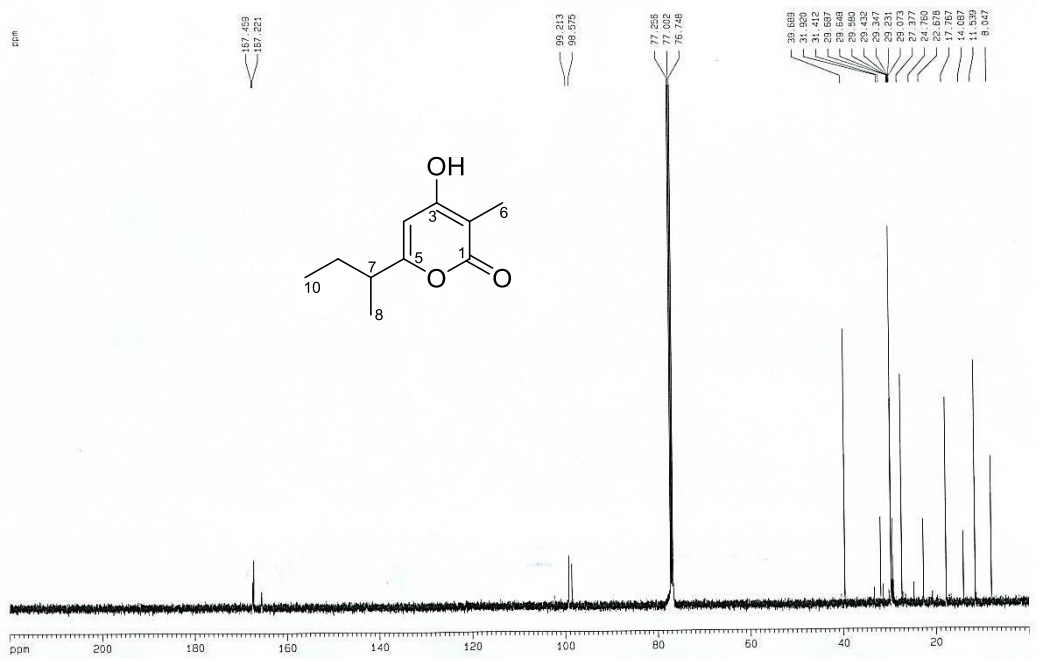

Figure 14 ^{13}C NMR spectrum (500 MHz, in $CDCl_3$) of KC097-C

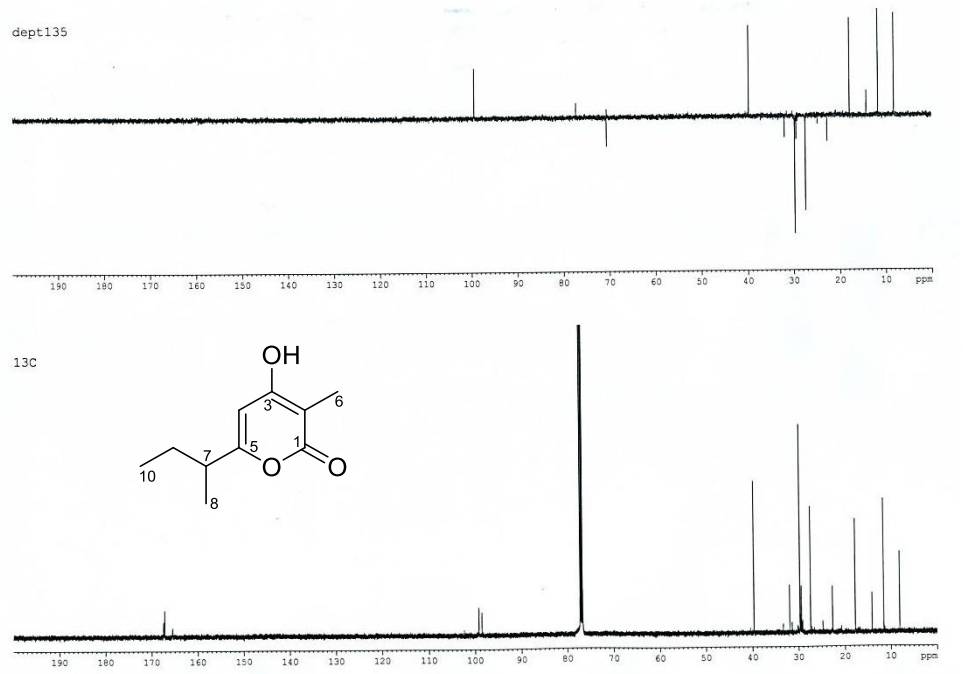

Figure 15 DEPT 135 spectrum (500 MHz, in $CDCl_3$) of KC097-C

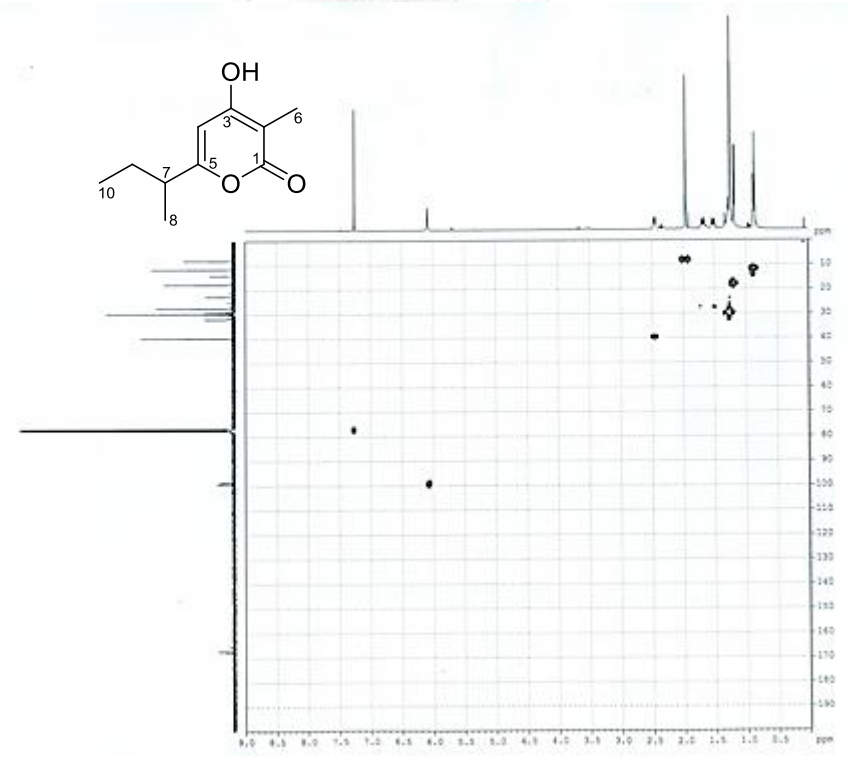

Figure 16 HMQC spectrum (500 MHz, in $CDCl_3$) of KC097-C

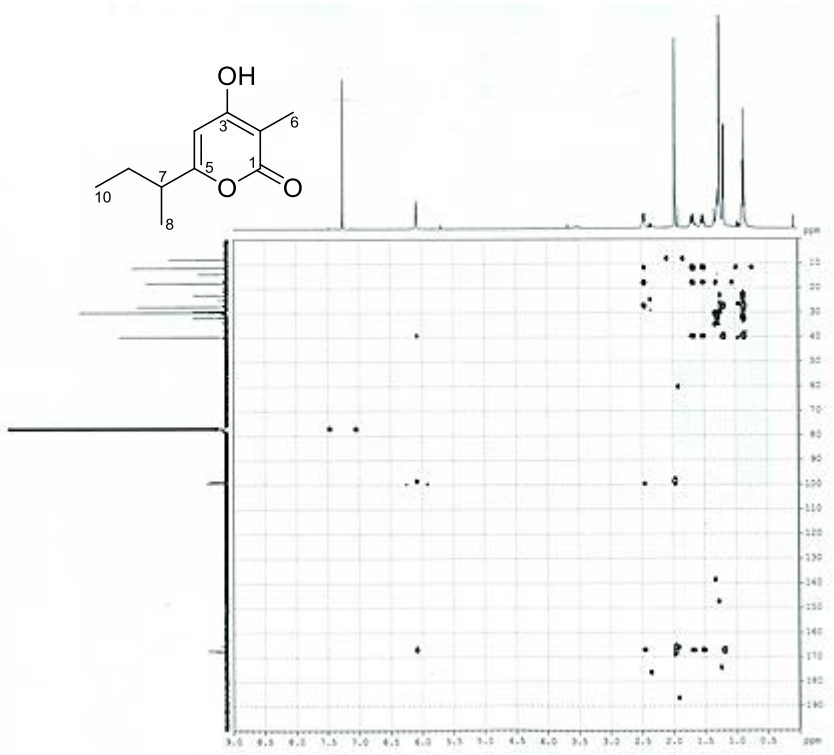

Figure 17 HMBC spectrum (500 MHz, in $CDCl_3$) of KC097-C

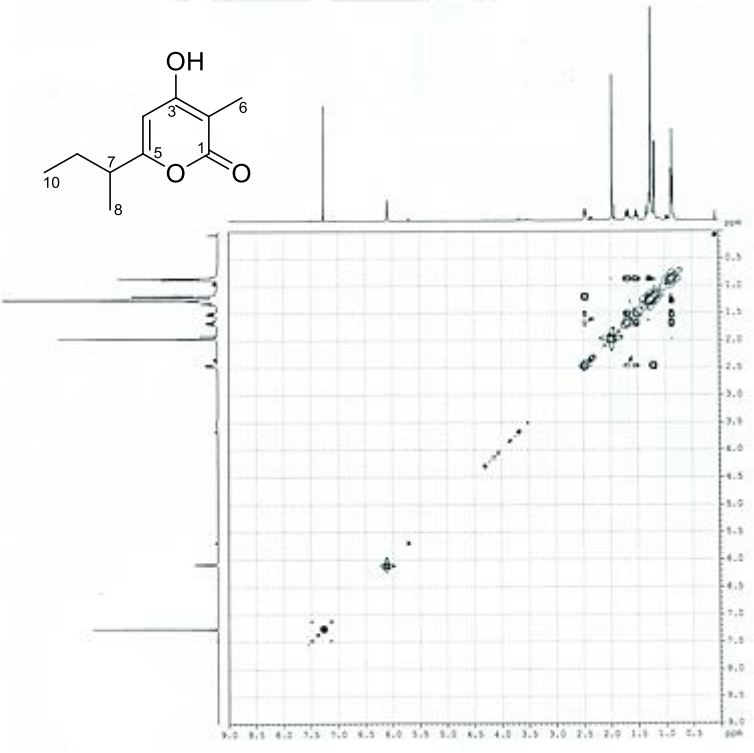

Figure 18 COSY spectrum (500 MHz, in $CDCl_3$) of KC097-C

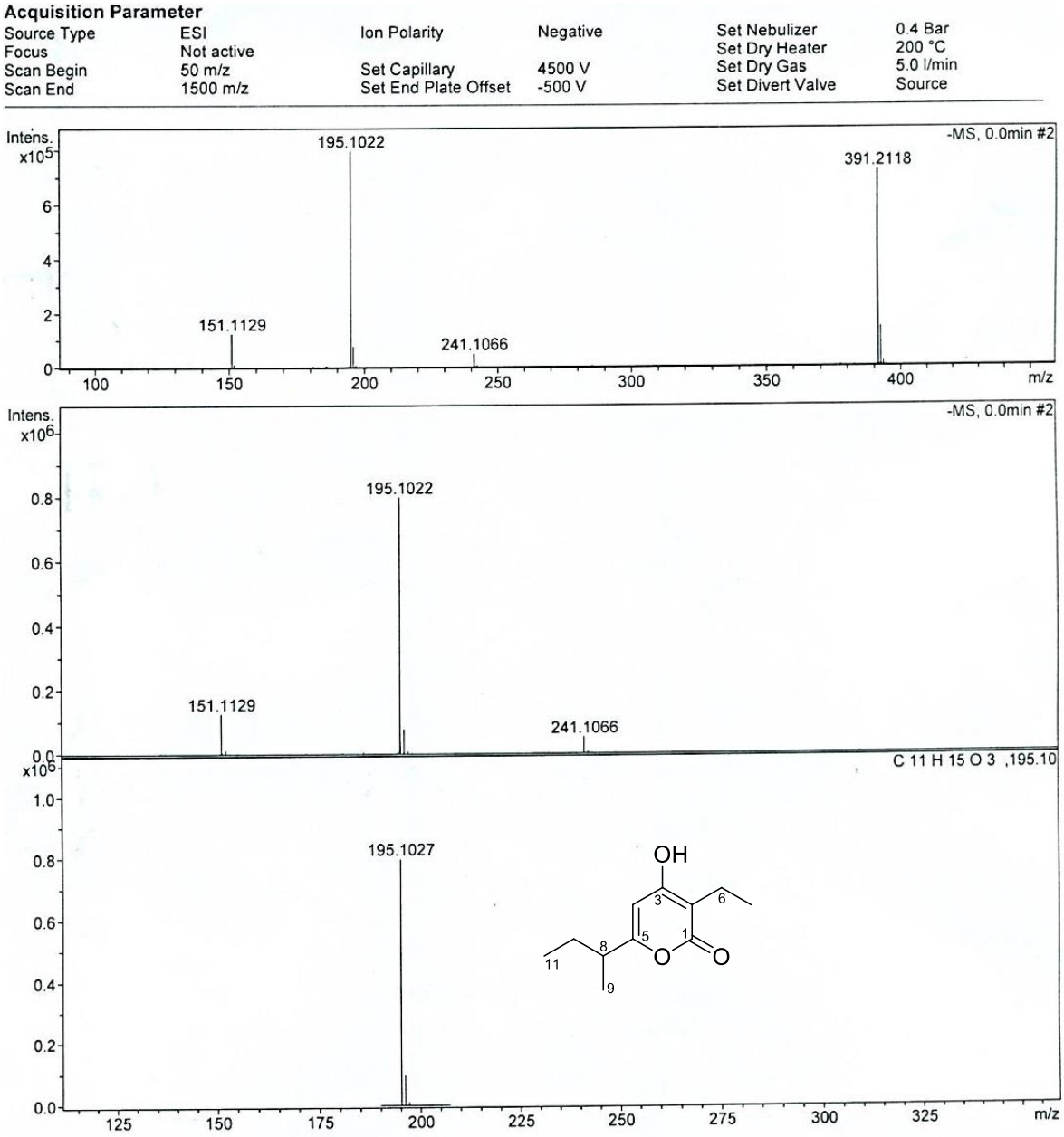

Figure 19 Mass spectrum of KC097-D

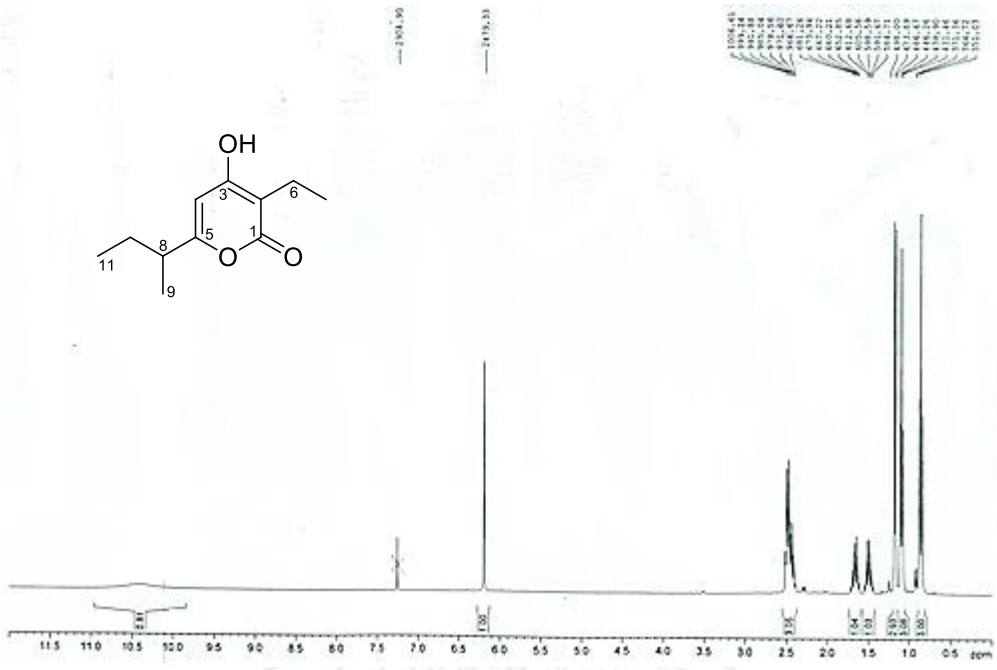

Figure 20 ^{1}H NMR spectrum (500 MHz, in $CDCl_3$) of KC097-D

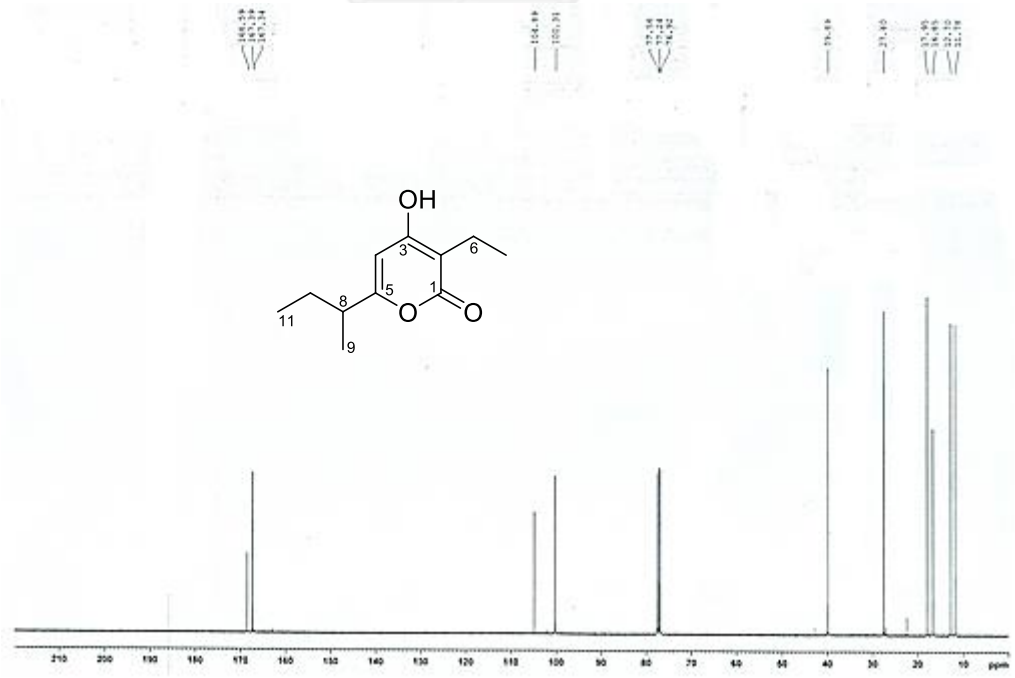

Figure 21 ^{13}C NMR spectrum (500 MHz, in $CDCl_3$) of KC097-D

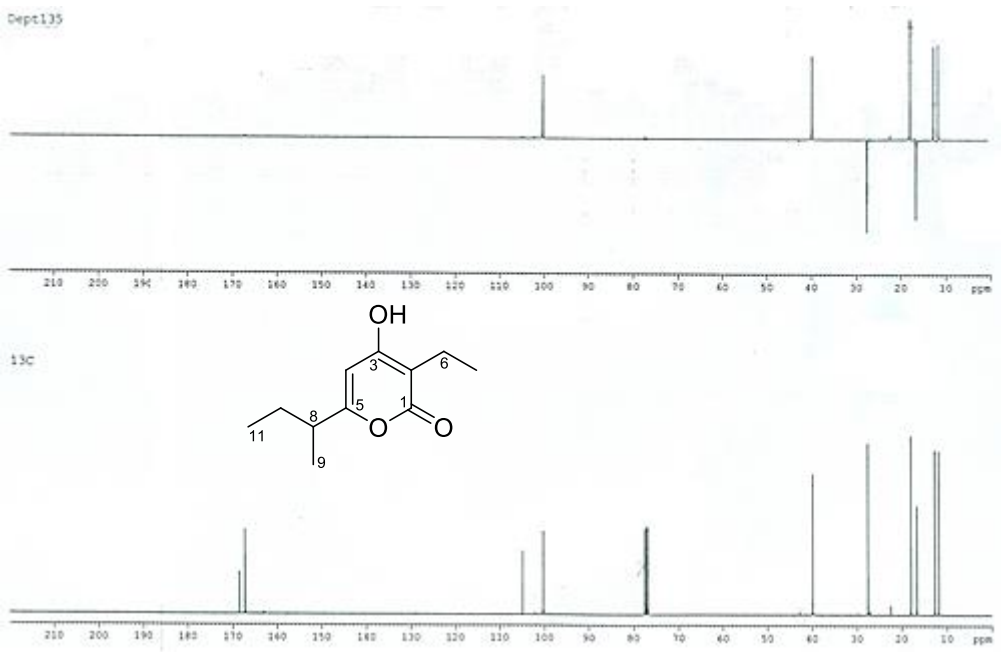

Figure 22 DEPT 135 spectrum (500 MHz, in $CDCl_3$) of KC097-D

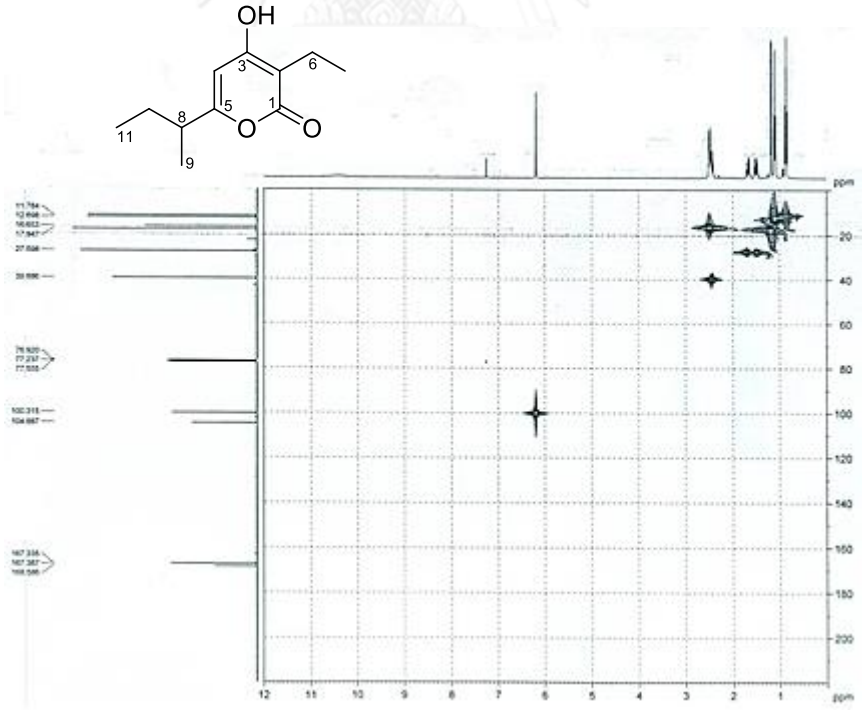

Figure 23 HMQC spectrum (500 MHz, in $CDCl_3$) of KC097-D

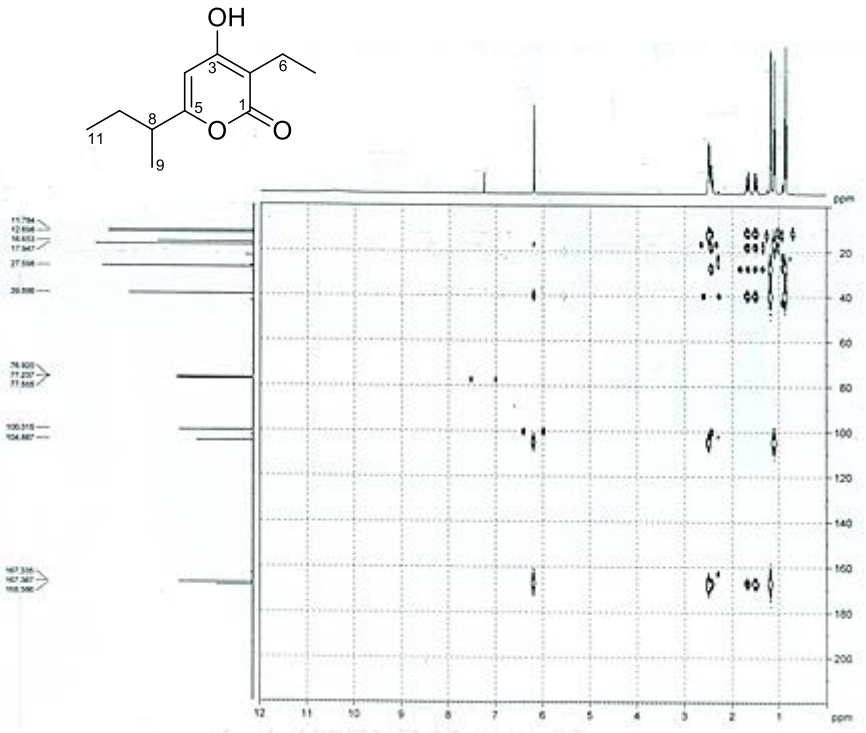

Figure 24 HMBC spectrum (500 MHz, in $CDCl_3$) of KC097-D

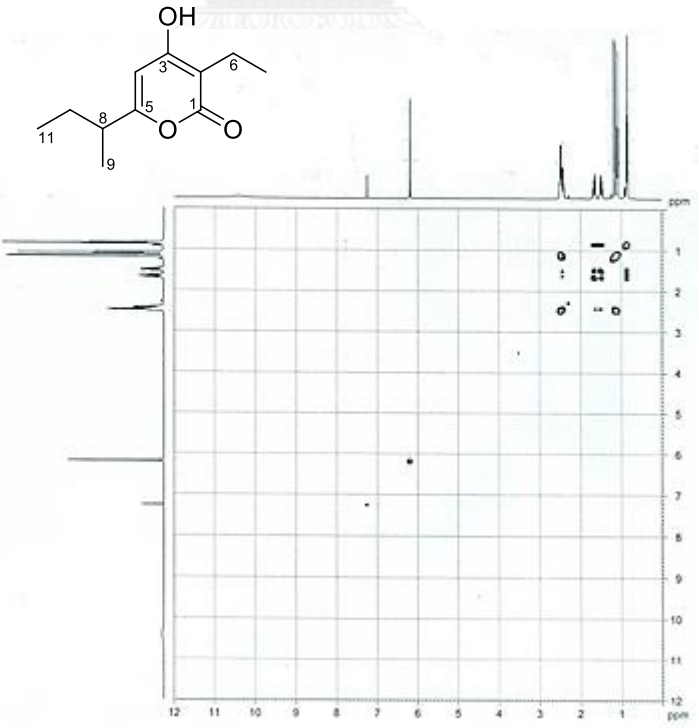

Figure 25 COSY spectrum (500 MHz, in $CDCl_3$) of KC097-D

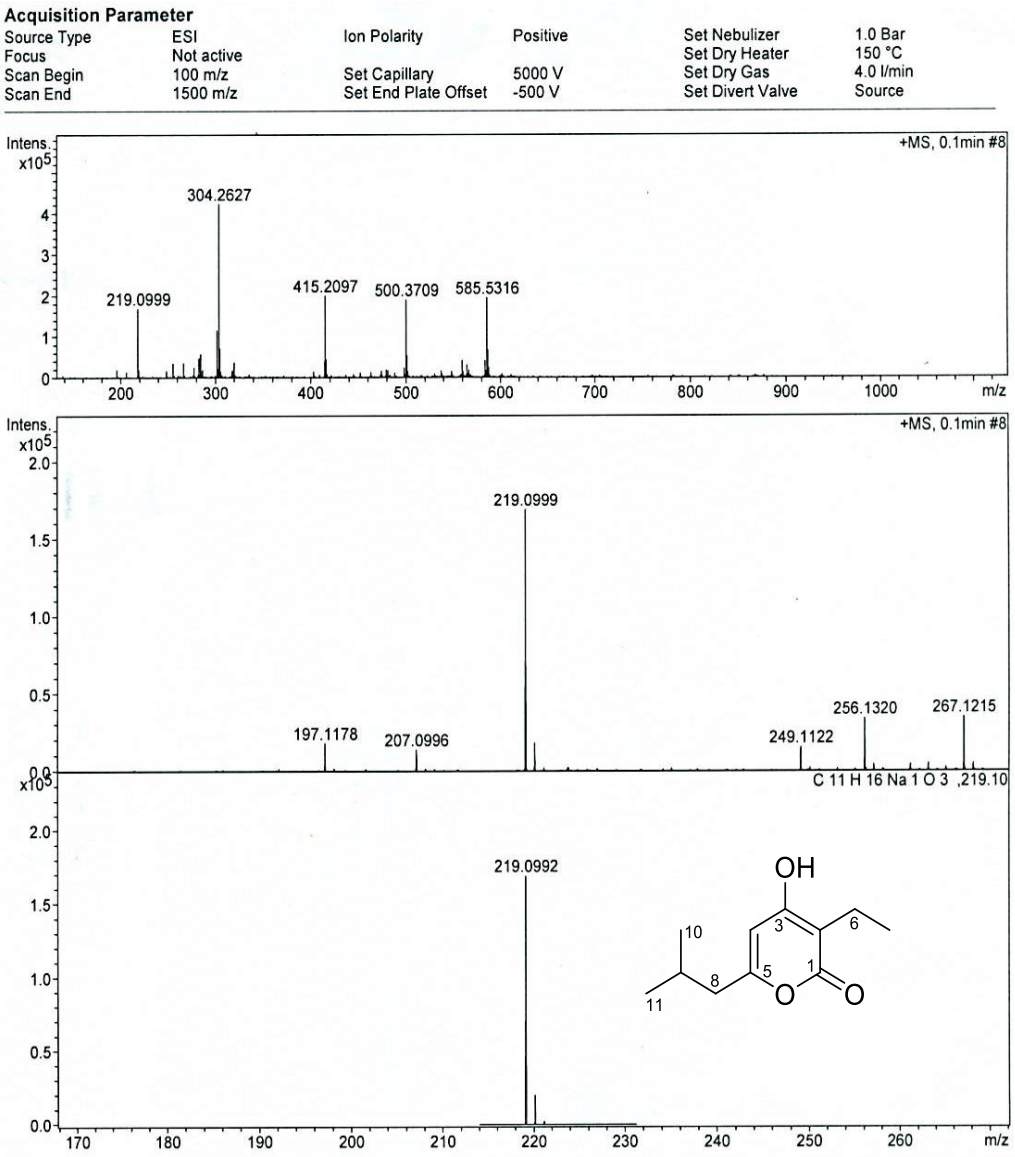

Figure 26 Mass spectrum of KC097-E

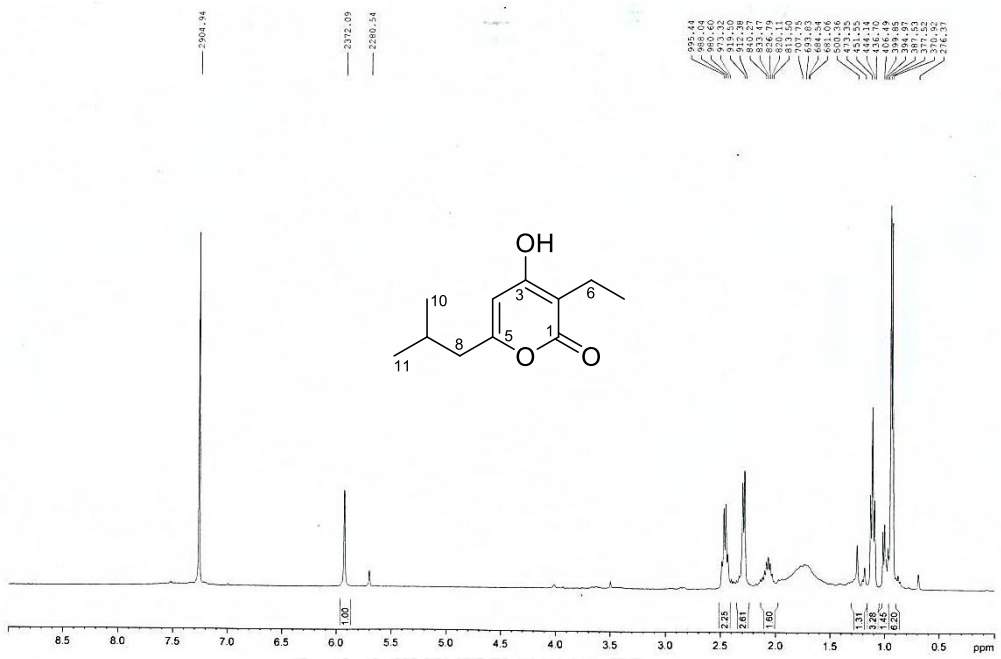

Figure 27 ^{1}H NMR spectrum (400 MHz, in $CDCl_3$) of KC097-E

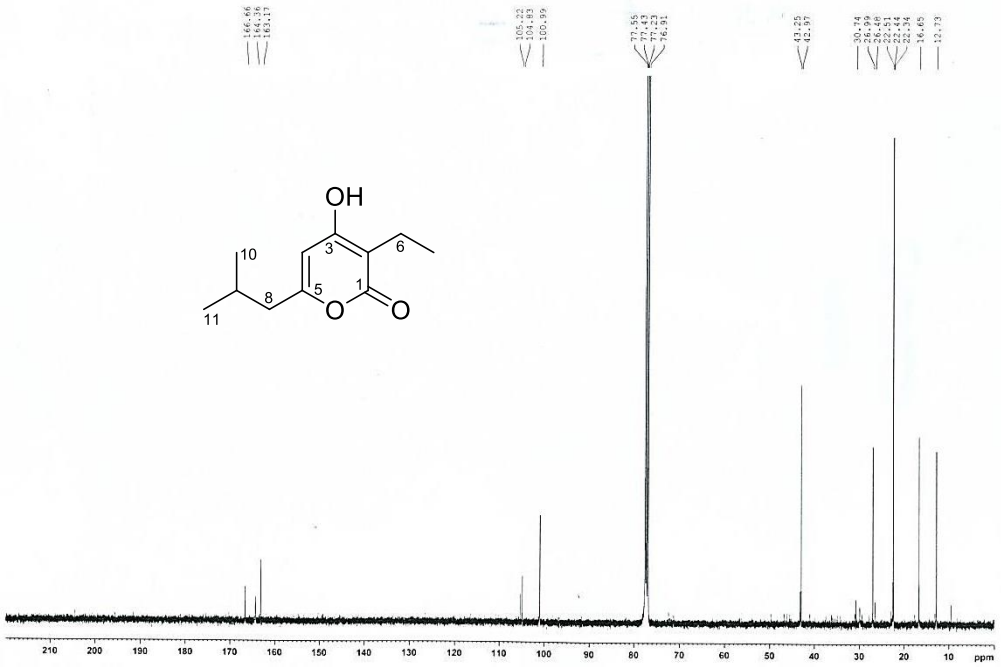

Figure 28 ^{13}C NMR spectrum (400 MHz, in $CDCl_3$) of KC097-E

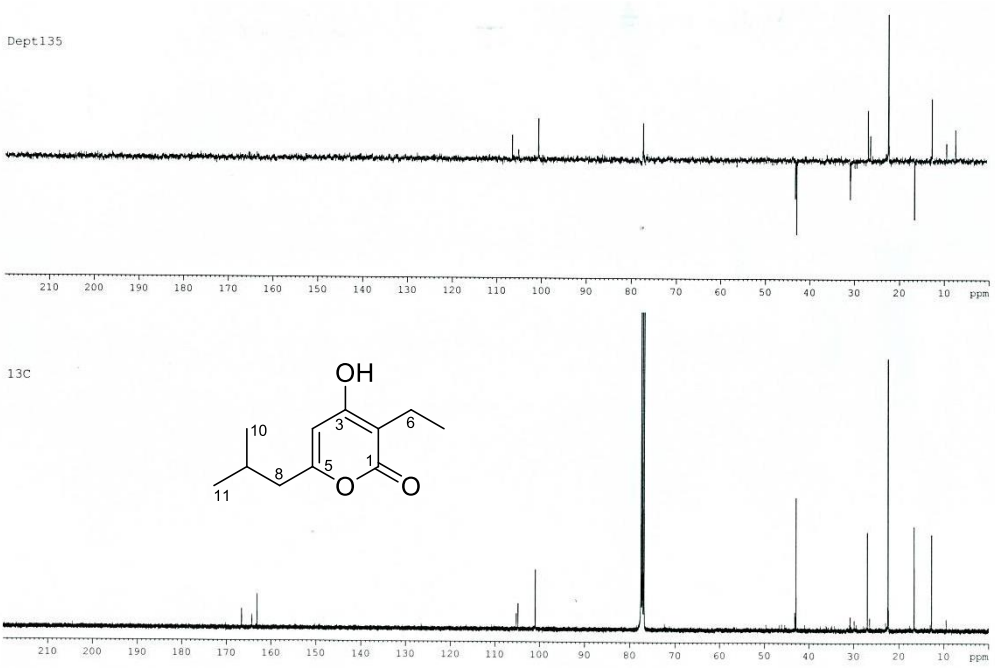

Figure 29 DEPT 135 spectrum (400 MHz, in $CDCl_3$) of KC097-E

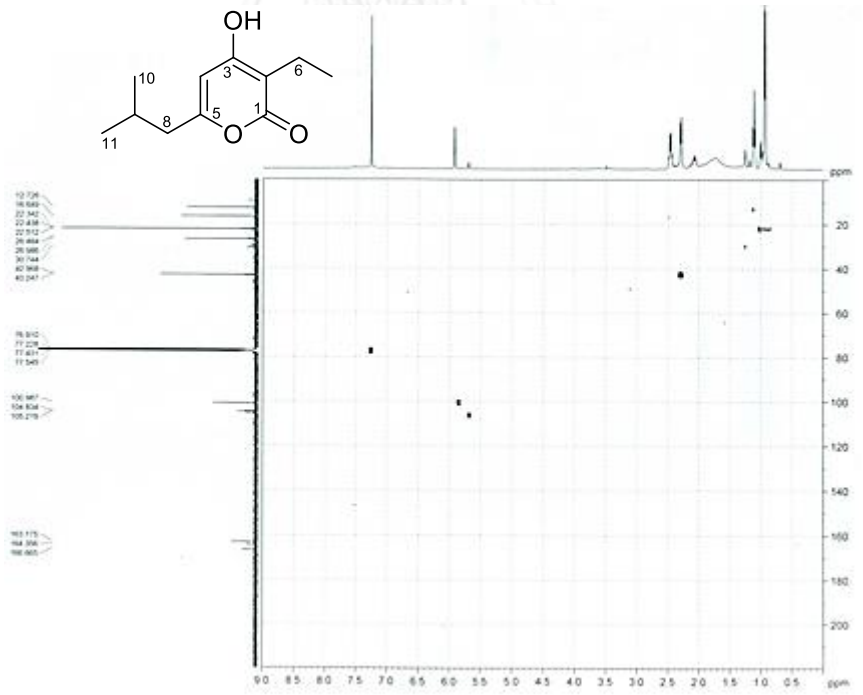

Figure 30 HMQC spectrum (400 MHz, in $CDCl_3$) of KC097-E

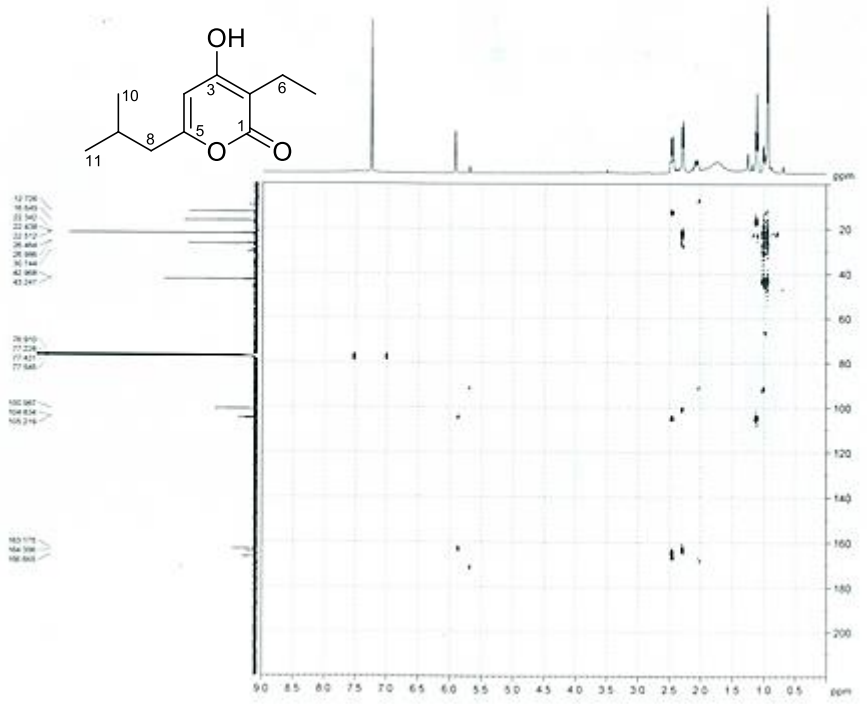

Figure 31 HMBC spectrum (400 MHz, in $CDCl_3$) of KC097-E

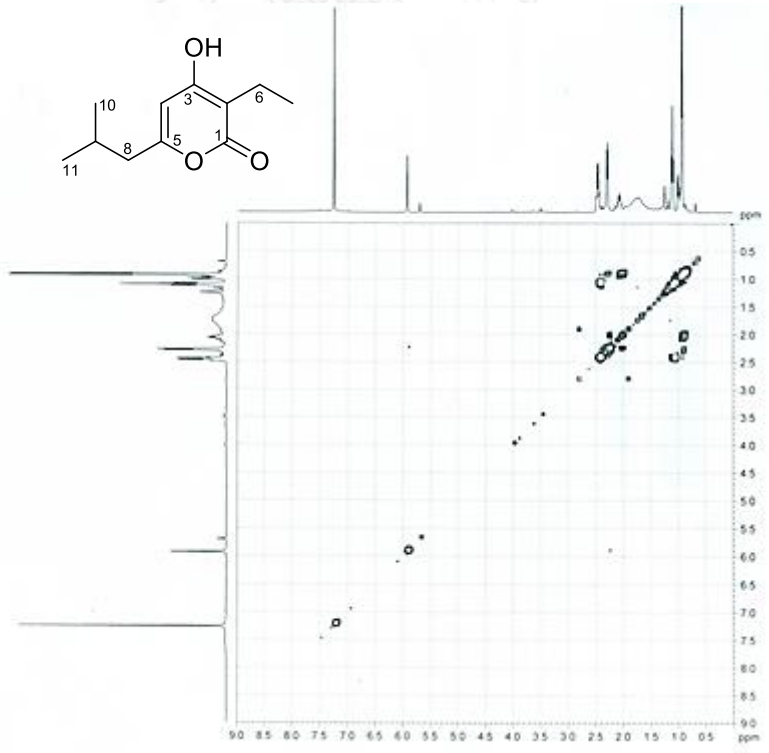

Figure 32 COSY spectrum (400 MHz, in $CDCl_3$) of KC097-E

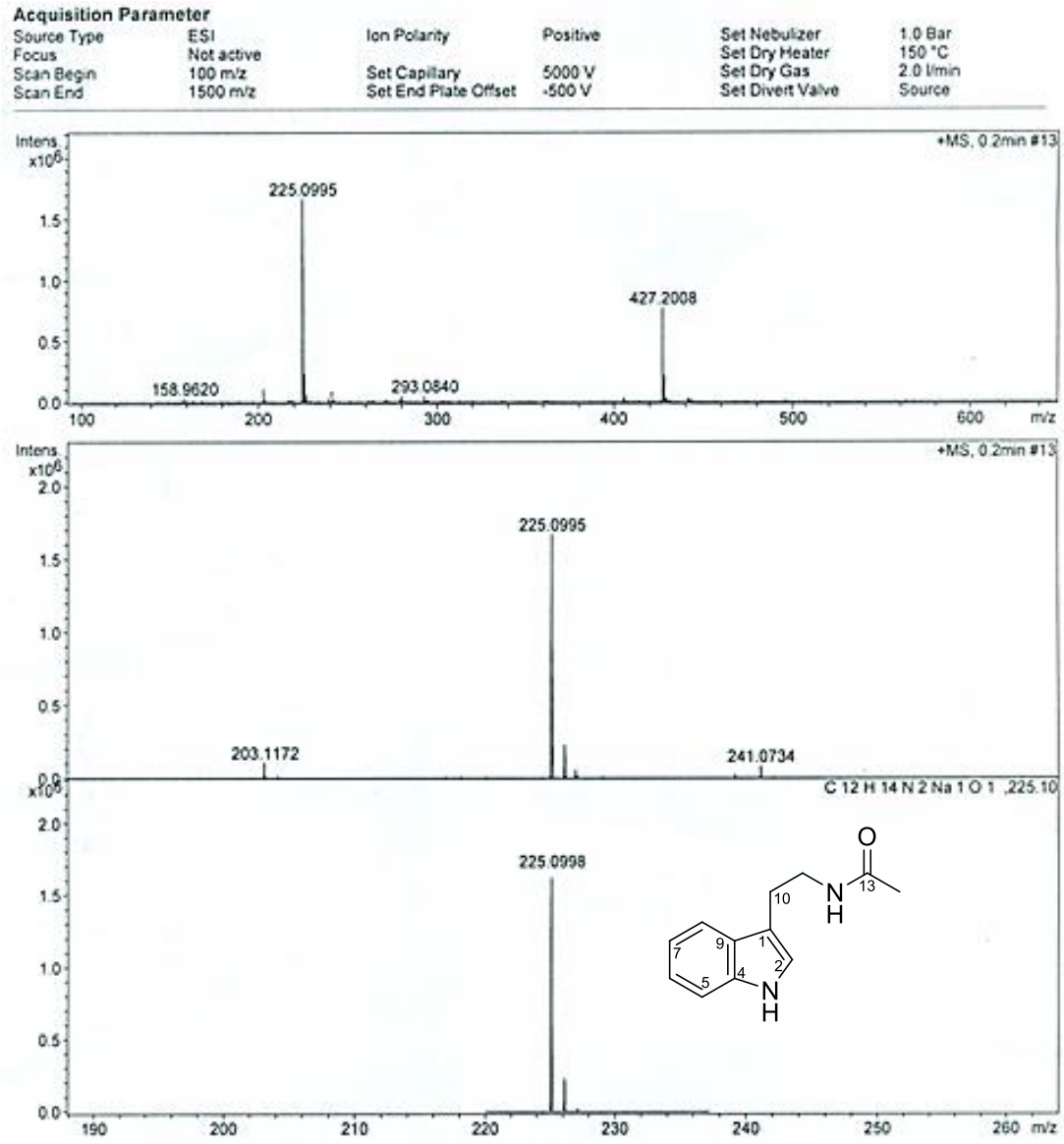

Figure 33 Mass spectrum of KC097-F

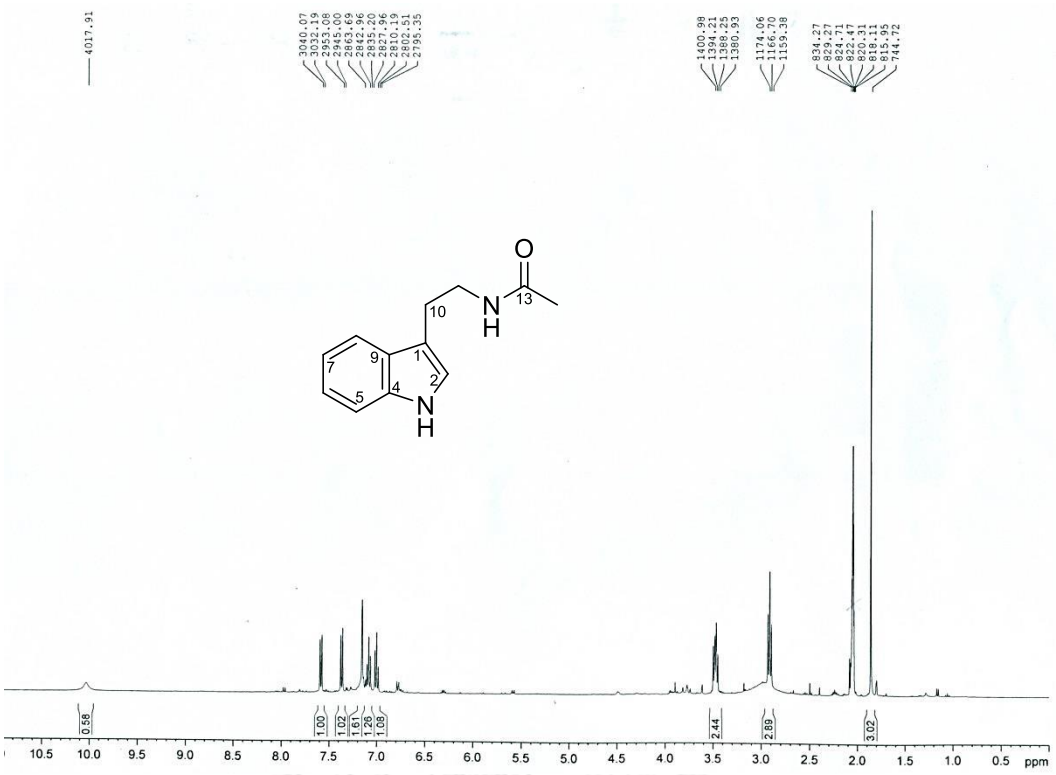

Figure 34 ^{1}H NMR spectrum (400 MHz, in acetone-d_6) of KC097-F

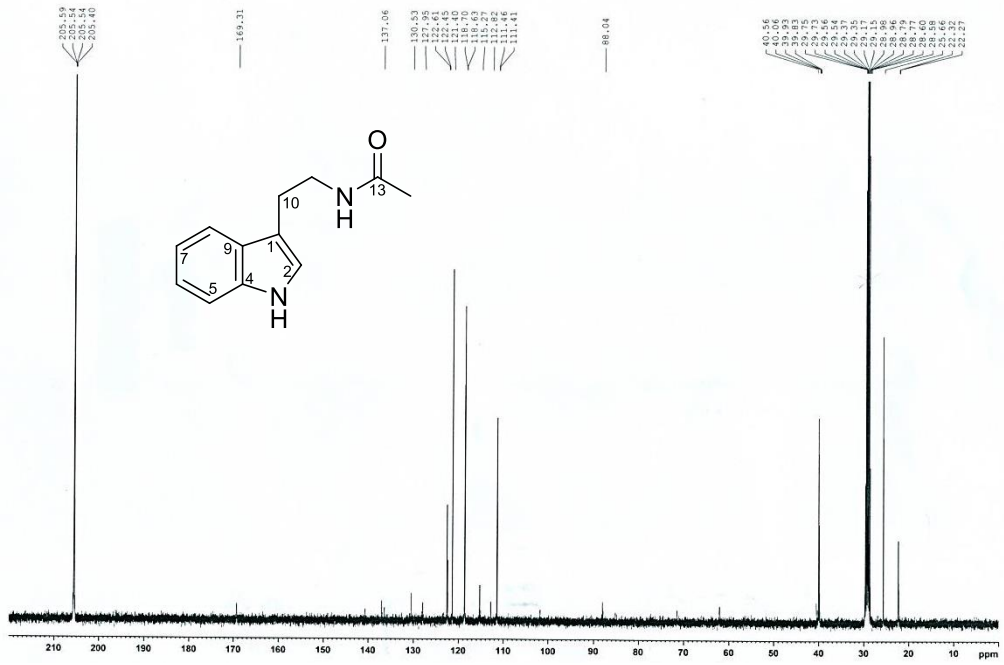

Figure 35 ^{13}C NMR spectrum (400 MHz, in acetone-d_6) of compound KC097-F

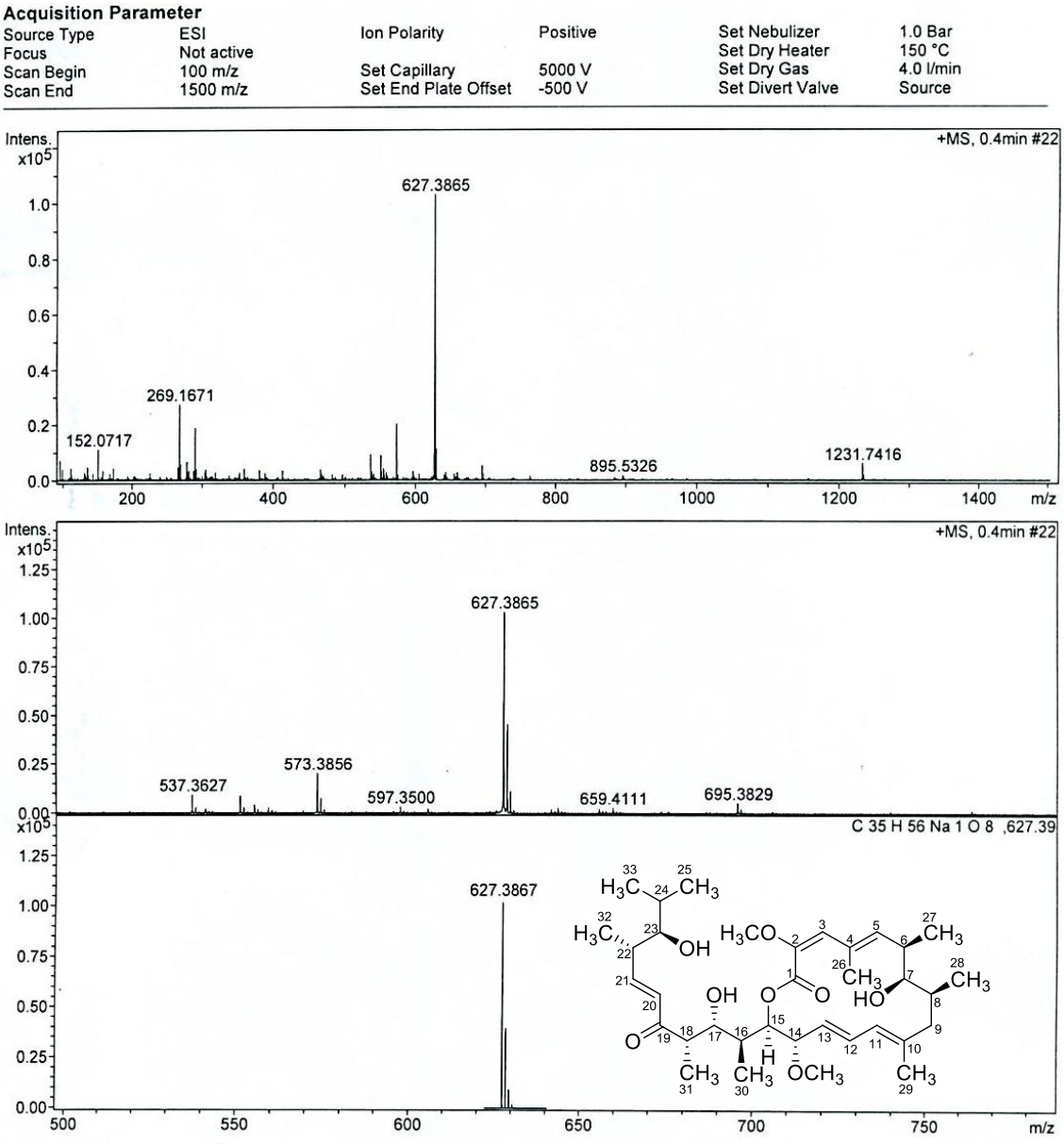

Figure 36 Mass spectrum of KC121-A

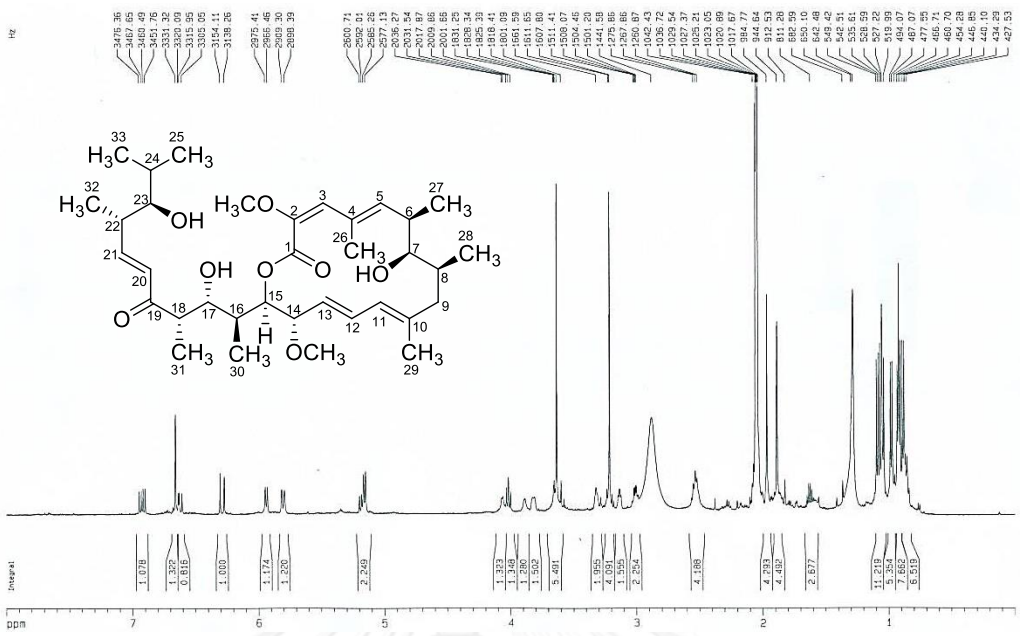

Figure 37 ^{1}H NMR spectrum (500 MHz, in acetone-d_6) of KC121-A

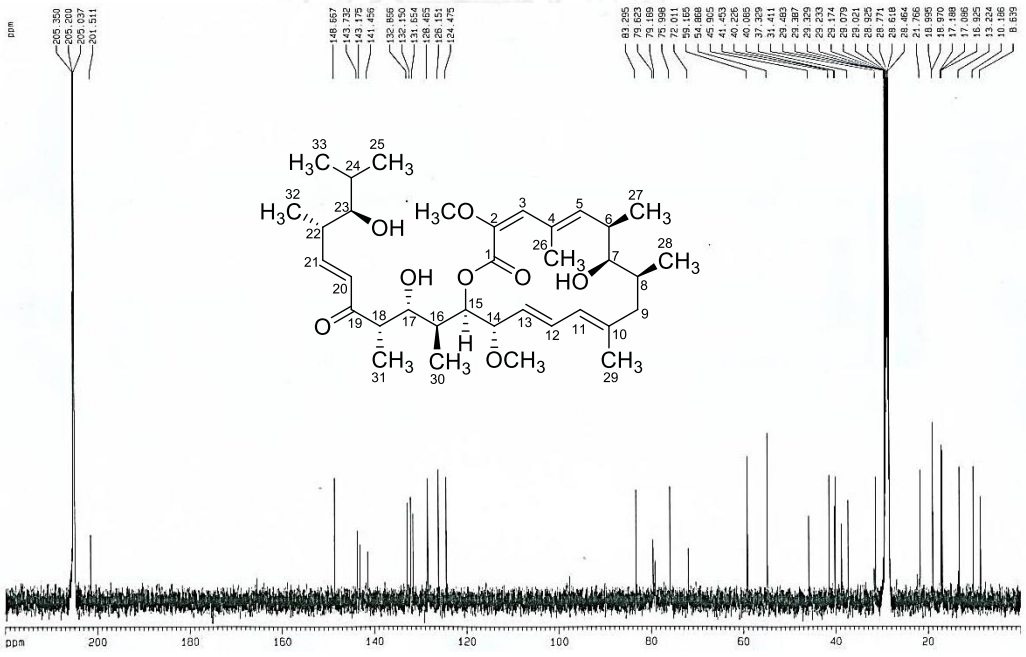

Figure 38 ^{13}C NMR spectrum (500 MHz, in acetone-d_6) of KC121-A

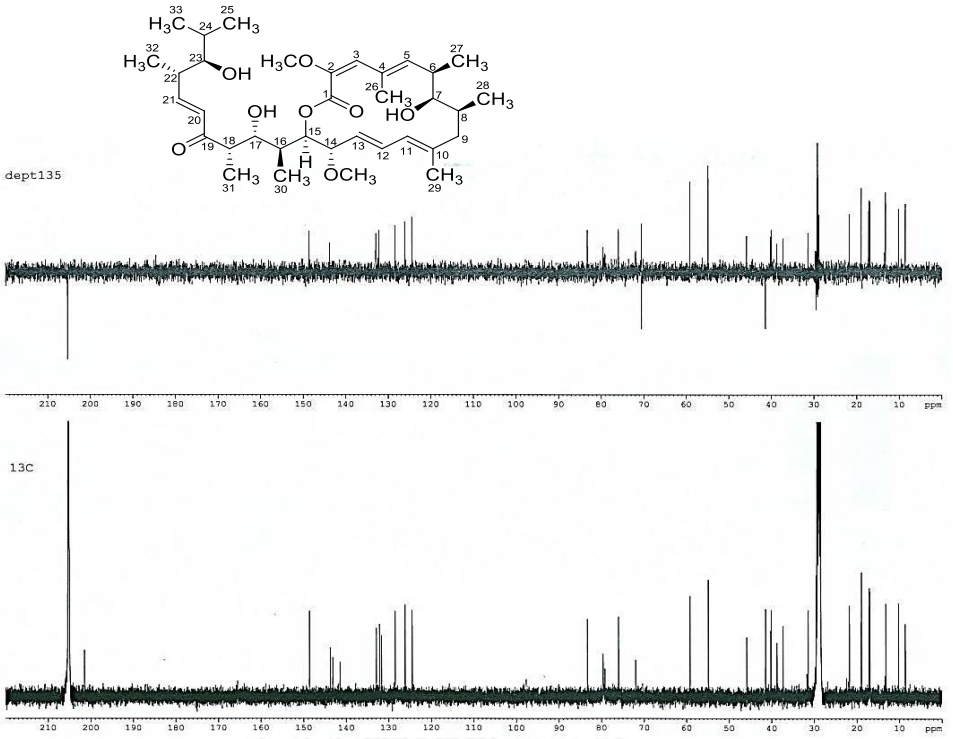

Figure 39 DEPT 135 spectrum (500 MHz, in acetone-d_6) of KC121-A

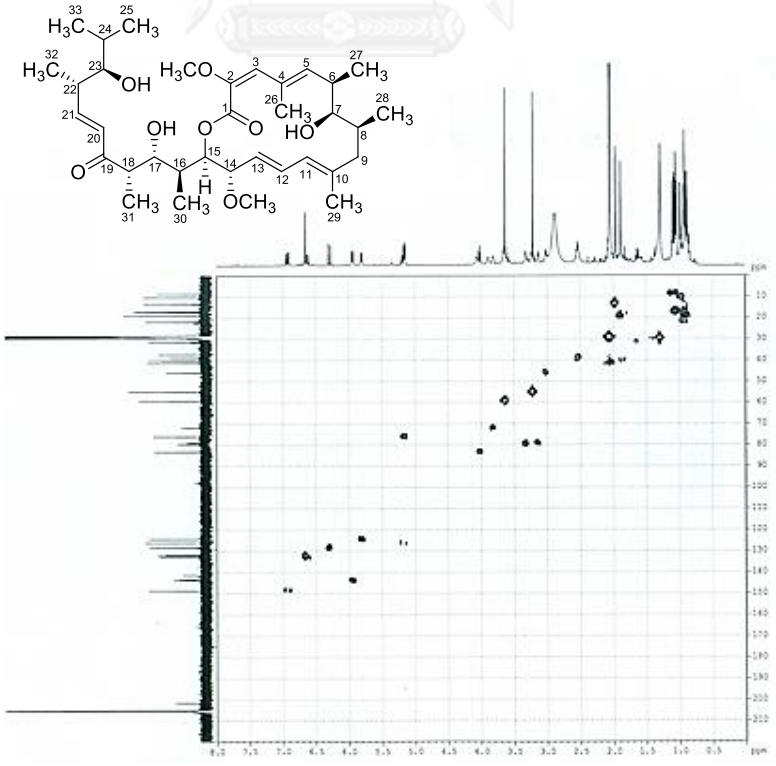

Figure 40 HMQC spectrum (500 MHz, in acetone-d_6) of KC121-A

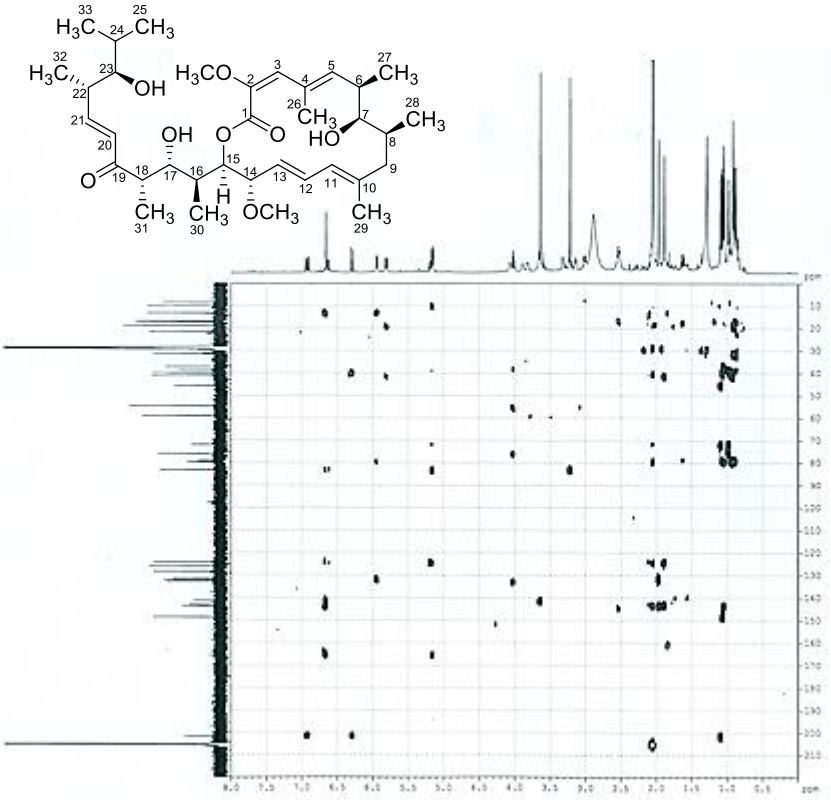

Figure 41 HMBC spectrum (500 MHz, in acetone-d_6) of KC121-A

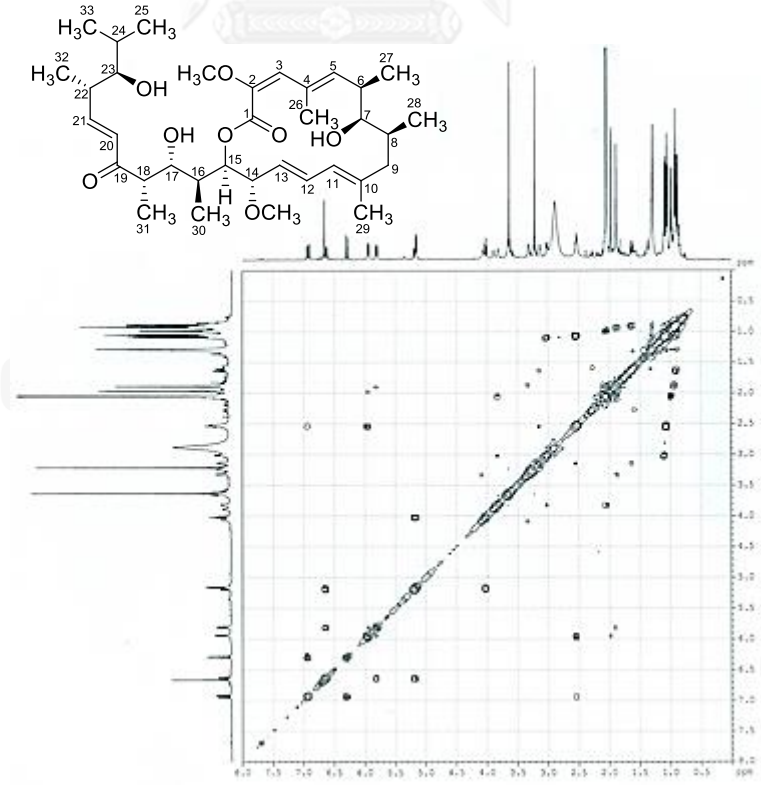

Figure 42 COSY spectrum (500 MHz, in acetone-d_6) of KC121-A

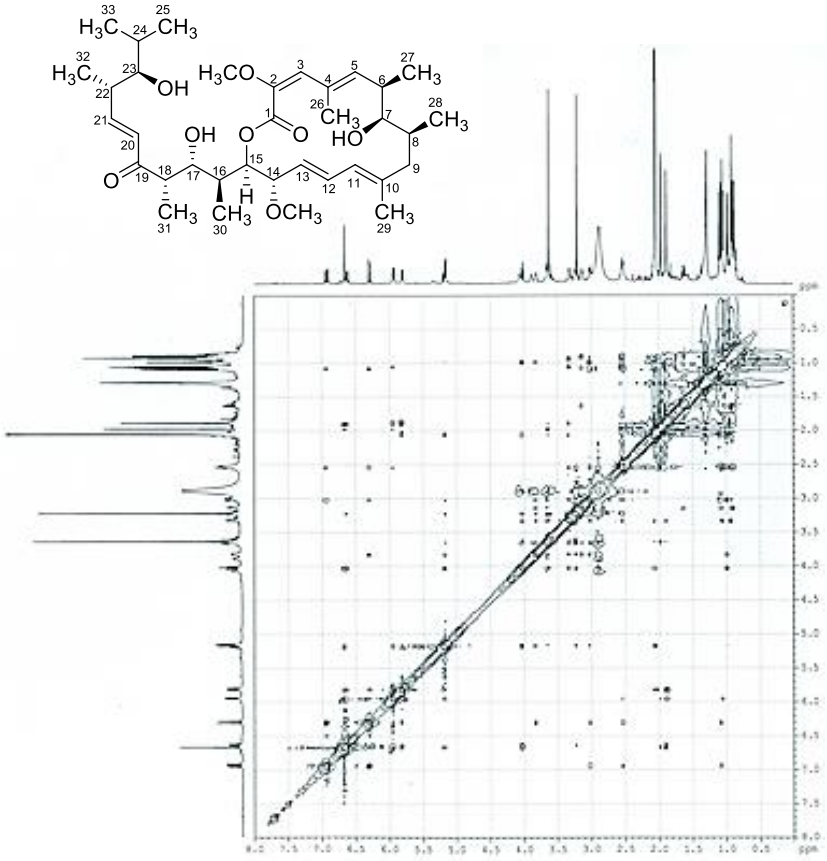

Figure 43 NOESY spectrum (500 MHz, in acetone-d_6) of KC121-A

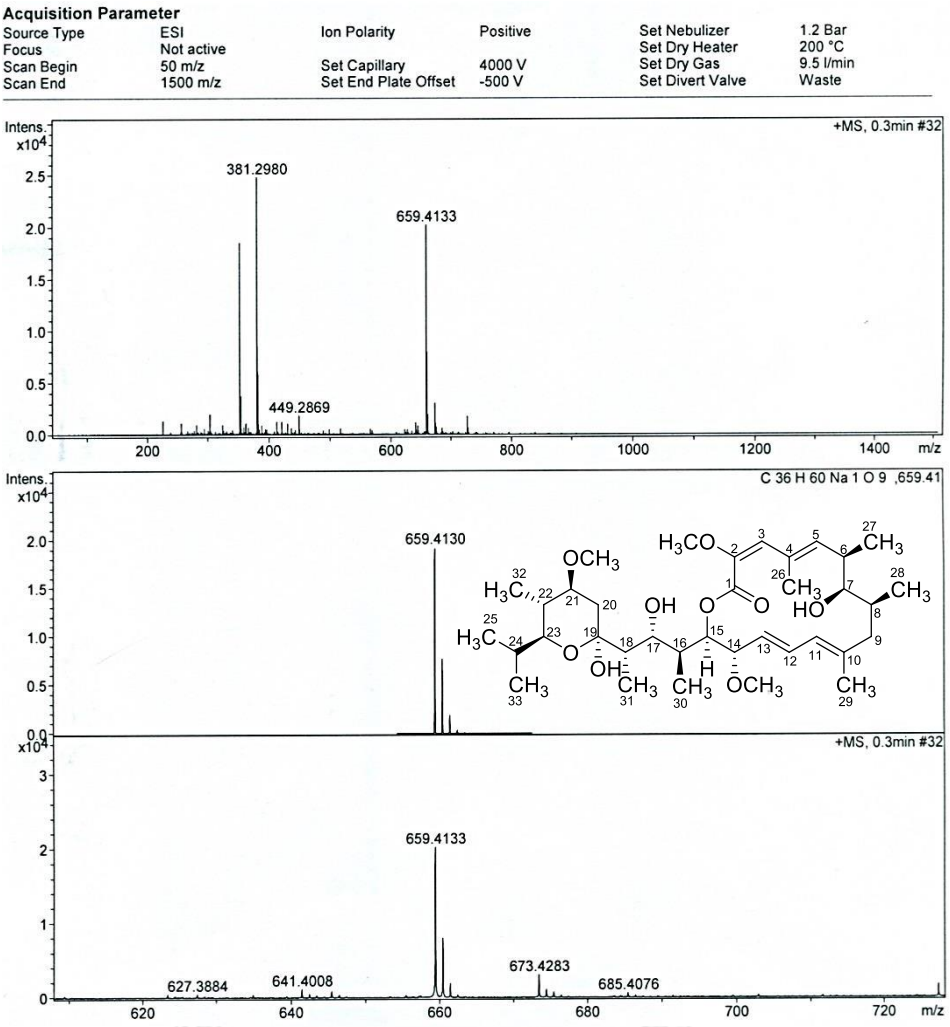

Figure 44 Mass spectrum of KC121-B

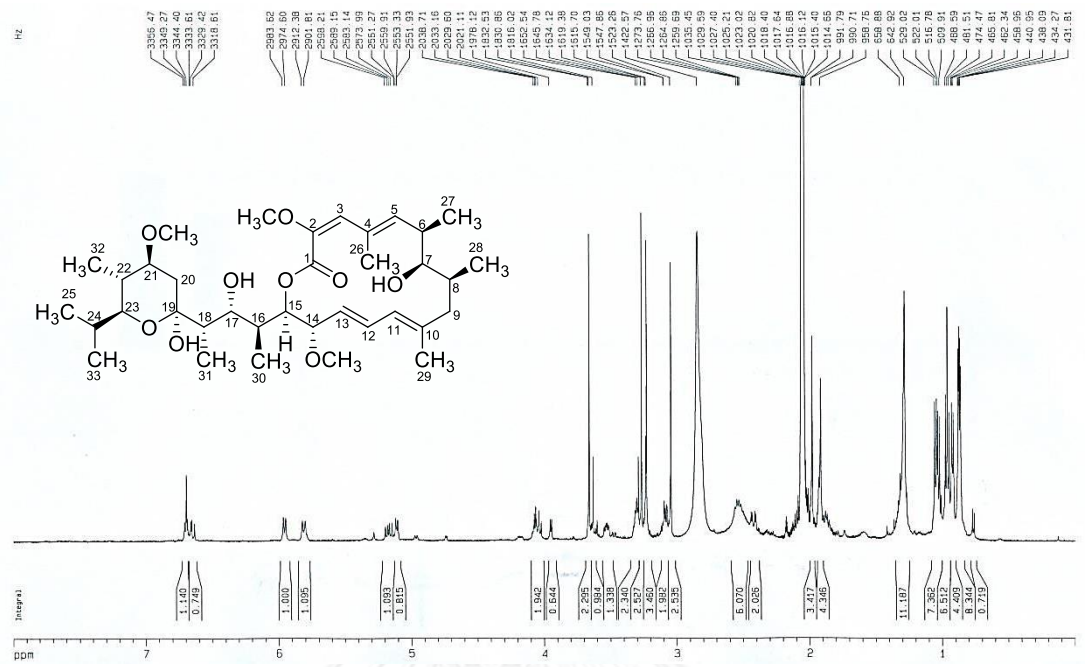

Figure 45 ^{1}H NMR spectrum (500 MHz, in acetone-d_6) of KC121-B

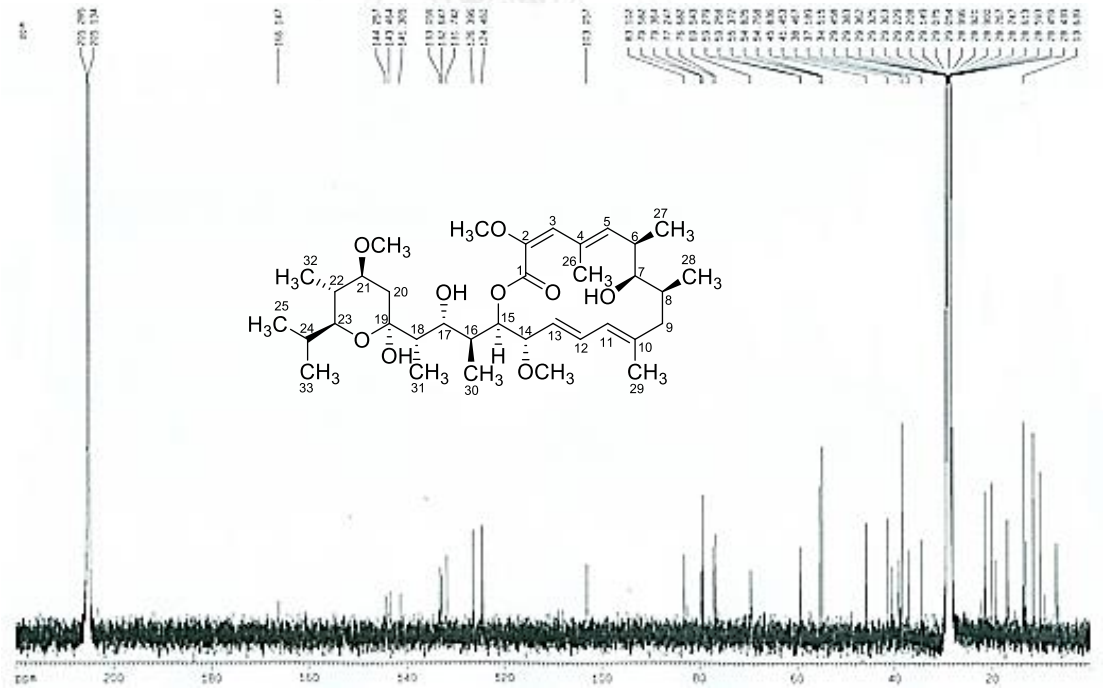

Figure 46 ^{13}C NMR spectrum (500 MHz, in acetone-d_6) of KC121-B

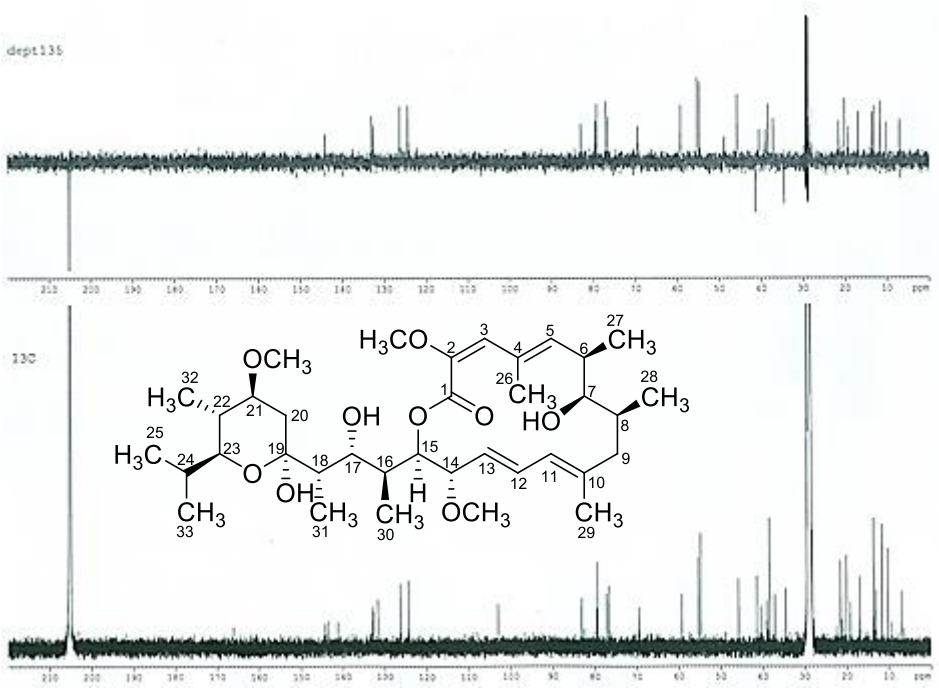

Figure 47 DEPT 135 spectrum (500 MHz, in acetone-d_6) of KC121-B

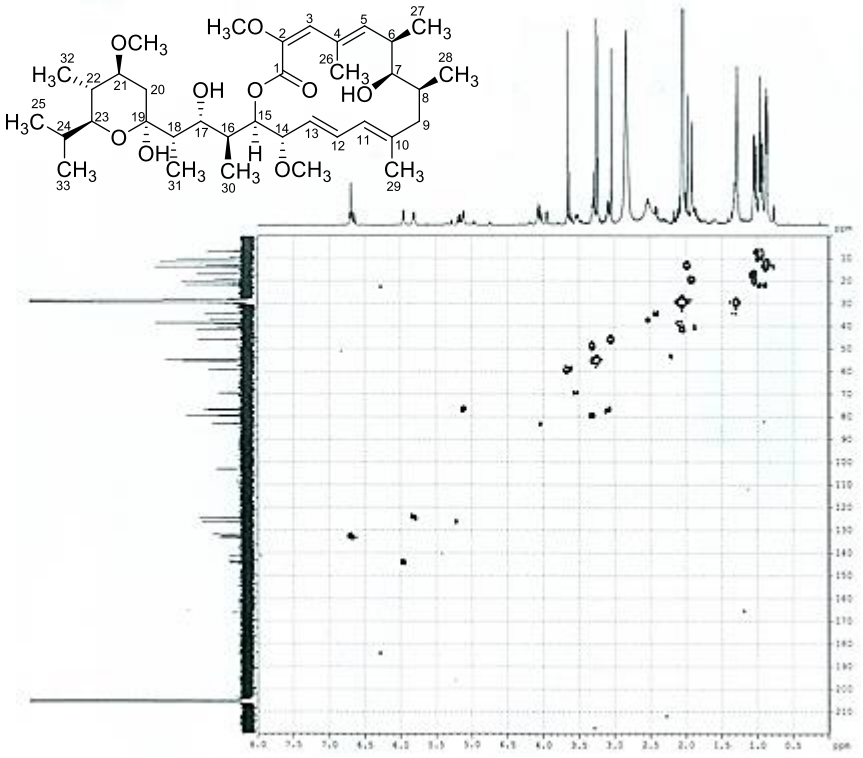

Figure 48 HMQC spectrum (500 MHz, in acetone-d_6) of KC121-B

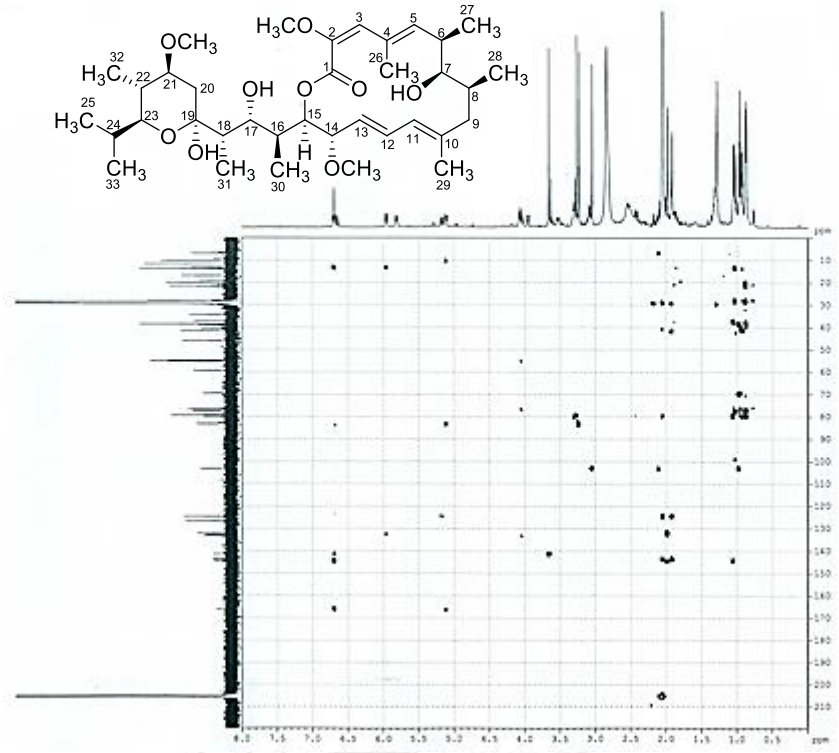

Figure 49 HMBC spectrum (500 MHz, in acetone-d_6) of KC121-B

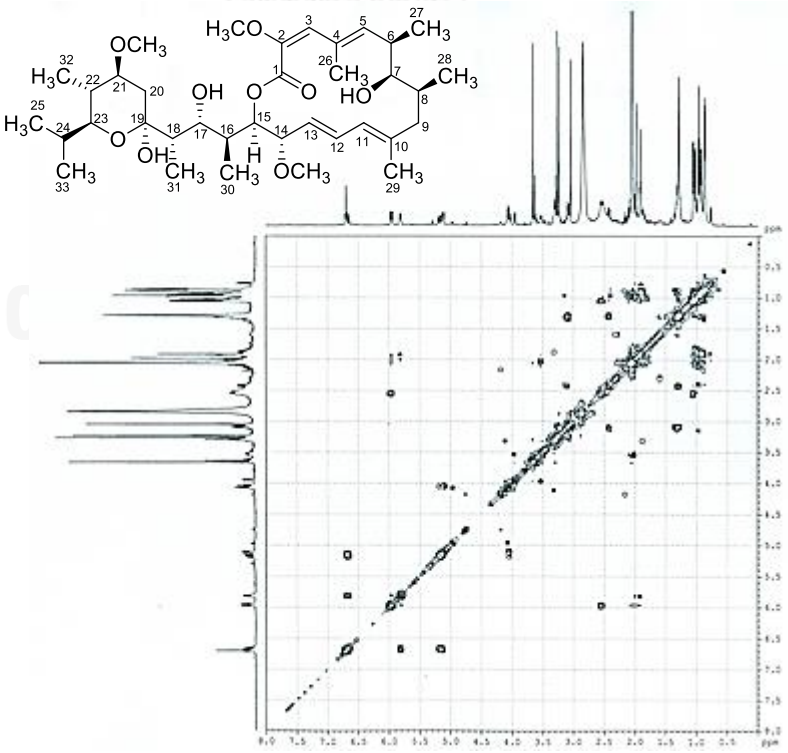

Figure 50 COSY spectrum (500 MHz, in acetone-d_6) of KC121-B

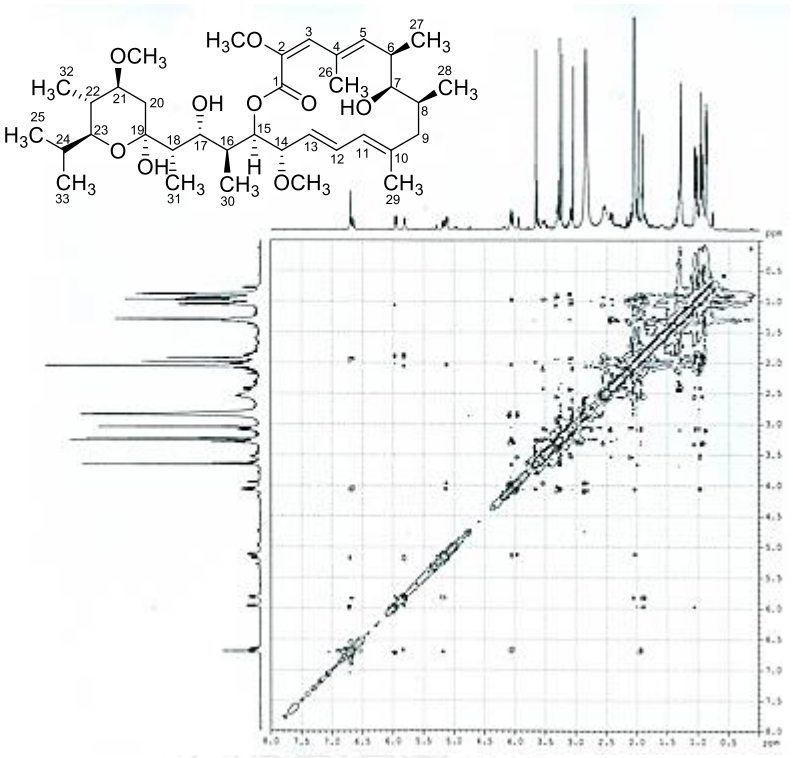

Figure 51 NOESY spectrum (500 MHz, in acetone-d_6) of KC121-B

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PUBLICATIONS:

1. Sripreechasak, P., Tanasupawat, S., Suwanborirux, K., Inahashi, Y., Matsumoto, A., Shiomi, K. & Takahashi, Y. (2013). Nonomuraea thailandensis sp. nov. isolated from Thai soil. J Antibiot (Tokyo), 66, 79-84.

2. Sripreechasak, P., Matsumoto, A., Suwanborirux, K., Inahashi, Y., Shiomi, K. Tanasupawat, S. & Takahashi, Y. (2013). Streptomyces siamensis sp. nov., and Streptomyces similanensis sp. nov., isolated from Thai soils. J Antibiot (Tokyo), 66, 633-640.

3. Sripreechasak, P., Tanasupawat, S., Matsumoto, A., Inahashi, Y., Suwanborirux, K. & Takahashi, Y. (2013). Identification and antimicrobial activity of actinobacteria from soils in southern Thailand. Trop Biomed, 30, 46-55.

POSTER PRESENTATIONS:

1. Sripreechsak, P., Sripairoj, P., Suwanborirux, K. & Tanasupawat, S. Identification and antimicrobial activity of Amycolatopsis strains from soils. The 21st Annual Meeting and International Conference of Thai Society for Biotechnology: Biotechnology: A Solution to the Global Economic Crisis?: 24-25 September 2009, Queen Sirikit National Convention Center, Bangkok, Thailand.

2. Sripreechasak, P., Matsumoto, A., Tanasupawat, S., Inahashi, Y. & Takahashi, Y. Identification and antimicrobial activity of actinomycete strains from soils in southern Thailand. The 23rd Annual Meeting of Thai Society of Biotechnology

Systems Biotechnology: 27-28 October 2011, The Imperial Queen's Park Hotel Bangkok, Thailand.

3. Sripreechasak, P., Matsumoto, A., Suwanborirux, K., Inahashi, Y., Shiomi., K. Tanasupawat, S. & Takahashi, Y. Streptomyces siamensis sp. nov., and Streptomyces similanensis sp. nov., isolated from Thai soil. RGJ Seminar Series LXXXVIII Microbial Resources: Their Biodiversity and Utilization: 24 August 2012, Kasetsart University, Bangkok, Thailand.

4. Sripreechasak, P., Tanasupawat, S., Suwanborirux, K., Inahashi, Y., Matsumoto, A. & Takahashi, Y. Identification and antimicrobial activity of Streptomyces strains from soils in Angthong Islands National Park, Surat Thani. The 39th Congress on Science and Technology of Thailand: 21-23 October 2013, Bangkok International Trade & Exhibition Centre (BITEC) Bangna, Bangkok, Thailand.