อนุกรมวิธานของเชื้อสายพันธุ์ไมโครโมโนสปอราจากดินป่าพรุของประเทศไทย และสารทุติยภูมิของเชื้อไอโซเลตที่คัดเลือก

นาย จิตติ ท่าไว

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต
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TAXONOMY OF *MICROMONOSPORA* STRAINS FROM THAI PEAT SWAMP FOREST SOILS AND SECONDARY METABOLITES OF A SELECTED ISOLATE

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Thesis Title	Taxonomy of *Micromonospora* strains from Thai peat swamp forest soils and secondary metabolites of a selected isolate
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จิตติ ท่าไว : อนุกรมวิธานของเชื้อสายพันธุ์ไมโครโมโนสปอราจากดินป่าพรุของประเทศไทยและสารทุติยภูมิของเชื้อไอโซเลตที่คัดเลือก (Taxonomy of *Micromonospora* strains from Thai peat swamp forest soils and secondary metabolites of a selected isolate) อาจารย์ที่ปรึกษา : รศ. ดร. สมบูรณ์ ธนาศุภวัฒน์ อาจารย์ที่ปรึกษาร่วม : ดร. คณิต สุวรรณบริรักษ์ และ Dr. Takashi Itoh; 210 หน้า. ISBN 974-17-6077-9

ในการศึกษาเพื่อหาสายพันธุ์แอคติโนมัยซีทส์จากดินป่าพรุจังหวัดตรัง พัทลุง ยะลา และนราธิวาส พบว่าสามารถแยกเชื้อที่สร้างสปอร์เดี่ยวบนเส้นใยได้จำนวน 52 ไอโซเลต จากการศึกษาลักษณะทางฟีโนไทป์ และทางอนุกรมวิธานเคมีรวมทั้งการวิเคราะห์ลำดับเบสในช่วง 16S rDNA จึงสามารถพิสูจน์เอกลักษณ์ของเชื้อเหล่านี้ได้เป็นแบคทีเรียในสกุลไมโครโมโนสปอรา พบว่าเชื้อที่แยกได้มีกรด *meso*-diaminopimelicในผนังเซลล์ มีน้ำตาล xylose และ arabinose และพบ phospholipid ชนิด phosphatidylethanolamineเป็นองค์ประกอบหลัก รวมทั้งมีกรดไขมันส่วนใหญ่แบบ iso-$C_{16:0}$, iso-$C_{15:0}$, iso-$C_{17:0}$, anteiso-$C_{16:0}$, anteiso-$C_{15:0}$ และ anteiso-$C_{17:0}$ และมี menaquinones ชนิด MK-9(H_4), MK-9(H_6) หรือ MK-10(H_4) นอกจากนี้พบว่ามีปริมาณ G+C ของสาย DNA อยู่ในช่วง 71-73 mol% จากผลของความคล้ายคลึงทาง DNA และลักษณะทางสรีรวิทยาและชีวเคมีบางประการสามารถแบ่งแยกเชื้อเหล่านี้ได้เป็น 11 กลุ่ม และสามารถพิสูจน์เอกลักษณ์เชื้อกลุ่มที่ 1 (8 สายพันธุ์) และ กลุ่มที่ 3 (7 สายพันธุ์) เป็น *M. chalcea* และ *M. aurantiaca* ตามลำดับ สำหรับเชื้อไมโครโมโนสปอรา 9 กลุ่มที่เหลือแสดงผลของความคล้ายคลึงทาง DNA (12.9-53.1 %) และลำดับเบสในช่วง 16S rDNA (97.5-99.2 %) ในระดับต่ำรวมทั้งมีลักษณะทางฟีโนไทป์แตกต่างไปจากเชื้อไมโครโมโนสปอราที่เคยมีรายงานไว้จึงสามารถจัดเป็นเชื้อชนิดใหม่ และได้เสนอชื่อสำหรับเชื้อกลุ่มที่ 7 (2 สายพันธุ์) และกลุ่มที่ 11 (1 สายพันธุ์) เป็น *Micromonospora eburnea* และ *Micromonospora aurantionigra* ตามลำดับ

จากการศึกษาสารทุติยภูมิของเชื้อที่คัดเลือกสายพันธุ์ TT1-11 ซึ่งแสดงฤทธิ์ต้านเชื้อจุลินทรีย์ทดสอบ พบว่าสามารถแยกสิ่งสกัดด้วยเอธิลอะซิเตทจากน้ำหมักเชื้อให้บริสุทธิ์ด้วยวิธีการทางโครมาโตกราฟีได้สารใหม่พวก polyene macrolide lactam 1 ชนิด คือ Micromonosporin A (9,11,13-trihydroxy-14,19,24-trimethyl-1-azacyclotetracosa-3,5,7,15,17,19,21-heptaen-2-one) นอกจากนี้ได้ทำการดัดแปลงโครงสร้างของสารใหม่นี้ด้วยปฏิกิริยาไฮโดรจีเนชั่นได้สารอนุพันธ์อีก 1 ชนิด คือ 9,11,13-trihydroxy-14,19,24-trimethyl-1-azacyclotetracosan-2-one การพิสูจน์โครงสร้างทางเคมีของสารเหล่านี้ใช้วิธีการวิเคราะห์ข้อมูล UV, IR, MS และ NMR spectroscopy สาร Micromonosporin A นี้ไม่เสถียรและไม่แสดงฤทธิ์ต้านเชื้อจุลินทรีย์ทดสอบ อย่างไรก็ตามสารอนุพันธ์ที่เตรียมได้แสดงฤทธิ์ต้านเชื้อจุลินทรีย์อย่างอ่อน และแสดงฤทธิ์ต้านเชื้อมาลาเรีย *Plasmodium falciparum* (K1, multidrug-resistant strain) ที่ระดับ IC_{50} = 3.1 µg/mL และเชื้อวัณโรคที่ระดับ MIC = 50 µg/mL

สาขาวิชา เภสัชเคมีและผลิตภัณฑ์ธรรมชาติ ลายมือชื่อนิสิต.....
ปีการศึกษา 2547 ลายมือชื่ออาจารย์ที่ปรึกษา.....
ลายมือชื่ออาจารย์ที่ปรึกษาร่วม.....

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CHITTI THAWAI: TAXONOMY OF *MICROMONOSPORA* STRAINS FROM THAI PEAT SWAMP FOREST SOILS AND SECONDARY METABOLITES OF A SELECTED ISOLATE. THESIS ADVISOR: ASSOCIATE PROFESSOR SOMBOON TANASUPAWAT, Ph. D., THESIS CO-ADVISOR: MR. KHANIT SUWANBORIRUX, Ph. D., and MR. TAKASHI ITOH, Ph.D., 210 pp. ISBN 974-17-6077-9.

In the course of our investigation for actinomycetes strains, fifty-two isolates which produced single non-motile spores were isolated from peat swamp forest soils in Trang, Pattaloong, Yala, and Narathiwat provinces. On the basis of the phenotypic and chemotaxonomic characteristics including the phylogenetic analysis using 16S rDNA sequences, they were identified as *Micromonospora*. The tested strains contained *meso*-diaminopimelic acid in cell wall (cell wall type II), xylose and arabinose as diagnostic whole-cell sugar pattern D, and phosphatidylethanolamine as characteristic phospholipid (type II). The major fatty acids of these strains were iso-$C_{16:0}$, iso-$C_{15:0}$, iso-$C_{17:0}$, anteiso-$C_{16:0}$, anteiso-$C_{15:0}$, and anteiso-$C_{17:0}$. Their major menaquinones were MK-9(H_4), MK-9(H_6), or MK-10(H_4). The range of G+C content of the DNA was 71-73 mol%. Based on the DNA-DNA similarity and some physiological and biochemical properties, all strains could be separated into eleven groups. The following two groups could be identified as *M. chalcea* (Group I, 8 strains) and *M. aurantiaca* (Group III, 7 strains). A low level of DNA-DNA similarity (12.9-53.1 %) and 16S rDNA similarity (97.5-99.2 %) including the phenotypic characteristics of the remaining nine groups (Groups II, IV-XI, 37 strains) indicated that these groups readily distinguished from all of validly described *Micromonospora* species and should be recognized as the new species. In this study, the names *Micromonospora eburnea* sp. nov. and *Micromonospora aurantionigra* sp. nov. are proposed for Group VII (2 strains) and Group XI (1 strains), respectively.

Micromonospora sp. TT1-11 was selected for secondary metabolite production since its ethylacetate crude extract showed significant antimicrobial activity. The ethyl acetate extract yielded a new polyene macrolide lactam (Micromonosporin A; 9,11,13-trihydroxy-14,19,24-trimethyl-1-azacyclotetracosa-3,5,7,15,17,19,21-heptaen-2-one). The hydrogenation reaction of this compound was performed and yielded 9,11,13-trihydroxy-14,19,24-trimethyl-1-azacyclotetracosan-2-one. The chemical structures of both compounds were elucidated through extensive analyses of their UV, IR, MS, and NMR spectroscopic data. Micromonosporin A was very unstable and has no antimicrobial activity. However, the derivative compound displayed weak antibacterial activity and also exhibited antimalarial activity at IC_{50} of 3.1 µg/mL and antimycobacterial activity with the *MIC* of 50 µg/mL.

Field of study Pharmaceutical Chemistry and Natural Products

Academic year 2004 Student's signature.....

Advisor's signature.....

Co-advisor's signature.....

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CONTENTS

CONTENTS (continued)

Page

CONTENTS (continued)

CONTENTS (continued)

LIST OF TABLES

LIST OF TABLES (continued)

LIST OF FIGURES

LIST OF FIGURES (continued)

LIST OF FIGURES (continued)

LIST OF FIGURES (continued)

LIST OF FIGURES (continued)

LIST OF SCHEMES

LIST OF ABBREVATIONS AND SYMBOLS

$[\alpha]^{28}_{D}$	=	Specific rotation at 28˚ and sodium D line (589 nm)
α	=	Alpha
Ala	=	Alanine
Ara	=	Arabinose
ATCC	=	American Type Culture Collection, Maryland, U.S.A.
$Ba(OH)_2$	=	Barium hydroxide
BC	=	Breast cancer cell line
˚C	=	Degree celsius
^{13}C-NMR	=	Carbon-13 nuclear madnetic resonance
$CDCl_3$	=	Deuterated chloroform
CFU	=	Colony forming unit
$CHCl_3$	=	Chloroform
cm	=	Centimeter
COSY	=	Correlation spectroscopy
Cz. sucrose	=	Czapek's sucrose
δ	=	Chemical shift
d	=	Doublet
DDBJ	=	DNA Data Bank of Japan
DEPT	=	Distortionless enchancement by polarization transfer
DMF	=	Dimethyl formamide
DMSO-d_6	=	Deuterated dimethylsulphoxide
DON	=	2,7-Dihydroxynapthalene
DPG	=	Diphosphatidylgylcerol
DSMZ	=	Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH
EDTA	=	Disodiumethylenediaminetetraacetate
EMBL	=	European Molecular Biology Laboratory
ESI-TOF-MS	=	Electrospray Ionization-Time of Flight Mass Spectrometry
EtOAc	=	Ethyl acetate
ε	=	Molar absorptivity
g	=	Gram
G	=	*N*-acetylglucosamine
Gal	=	Galactose

LIST OF ABBREVATIONS AND SYMBOLS (continued)

GC	=	Gas chromatography
GenBank	=	National Institute of Health genetic sequence database
Glu	=	Glutamic acid
GluNu	=	Phospholipids of unknown structure containing glucosamine
Gly	=	Glycerine
Gly.A.	=	Glycerol-asparagine agar
Glu.A.	=	Glucose-asparagine agar
h	=	Hour
HCl	=	Hydrochloric acid
HMBC	=	^{1}H-detected heteronuclear multiple bond correlation
HMQC	=	^{1}H-detected heteronuclear multiple quantum coherence
^{1}H-NMR	=	Proton nuclear magnetic resonance
HPLC	=	High performance liquid chromatography
HPTLC	=	High performance thin layer chromatograhphy
H_2O	=	Water
H_2SO_4	=	Sulfuric acid
Hz	=	Hertz
I	=	Interpeptide bridge
IC_{50}	=	Inhibition concentration
IR	=	Infrared
I.S.A	=	Inorganic salts-starch agar
ISP	=	International Streptomyces Project
J	=	Coupling constant
JCM	=	Japan Collection of Microorganisms
KB	=	A human epidermoid carcinoma cell line of the nasopharynx
KNO_3	=	Potassium nitrate
KOH	=	Potassium hydroxide
L	=	Liter
L-DA	=	L-diamino acid
lyso-PE	=	Lyso-phosphatidylethanolamine
m	=	Multiplet

LIST OF ABBREVATIONS AND SYMBOLS (continued)

mm^3	=	Cubic meter
M	=	Molar
M	=	*N*-acetylmuramic acid
$[M+H]^+$	=	Protonated molecular ion
MeCN	=	Methyl cyanide
MEGA	=	Molecular Evolutionary Genetics Analysis
MeOH	=	Methanol
meso-DAP	=	*meso*-Diaminopimelic acid
Methyl-PE	=	methyl-Phosphatidylethanolamine
µg	=	Microgram
mg	=	Milligram
$MgCl_2$	=	Magnesium chloride
MHA	=	Mueller-hinton agar
MHz	=	Megahertz
MK	=	Menaquinone
µL	=	Microliter
mL	=	Milliliter
µm	=	Micrometer
mm	=	Millimeter
MS	=	Mass spectroscopy
N	=	Normal
N.A.	=	Nutrient agar
NaCl	=	Sodium chloride
NaOH	=	Sodium hydroxide
N_2 gas	=	Nitrogen gas
nm	=	Nanometer
NMR	=	Nuclear magnetic resonance
NOESY	=	Nuclear overhouser effect correlation spectroscopy
NSS	=	Normal saline solution
nt	=	Nucleotide
OH-PE	=	Hydroxyl-phosphatidylethanolamine
O.M.	=	Oatmeal

LIST OF ABBREVATIONS AND SYMBOLS (continued)

PBS	=	Phosphate buffer saline
PC	=	Phosphatidylcholine
PCR	=	Polymerase chain reaction
Pd	=	Palladium
PE	=	Phosphatidylethanolamine
PG	=	Phosphatidylglycerol
PI	=	Phosphatidylinositol
P.I.A	=	Peptone-yeast extract iron agar
PIMs	=	Phosphatidylinositolmannoside
PME	=	Phosphatidyl-*N*-methylethylethanolamine
ppm	=	Part per million
rDNA	=	Ribosomal deoxynucleic acid
rRNA	=	Ribosomal ribonucleic acid
rpm	=	Round per minute
s	=	Singlet
SCA	=	Starch caseinate agar
SDA	=	Sabouraud's dextrose agar
SDS	=	Sodium dodexylsulfate
sec	=	Second
Si Gel	=	Silica gel
sp.	=	Species
SSC	=	Standard sodium citrate
t	=	Triplet
T.A.	=	Tyrosine agar
TAE	=	Tris-acetate
TBE	=	Tris-borate
TCA	=	Trichloroacetic acid
TLC	=	Thin layer chromatography
TOCSY	=	Total correlation spectroscophy
UV	=	Ultraviolet
Vero cell line	=	African monkey kidney cell line
Xyl	=	Xylose

LIST OF ABBREVATIONS AND SYMBOLS (continued)

YM = Yeast extract-malt extract

YMA = Yeast extract-malt extract agar

CHAPTER I

INTRODUCTION

Actinomycetes are well known as the antibiotic producers. Approximately 71.1% of them could produce the bioactive compounds except for a few of antibiotics that are produced by fungi (18.2%), e.g. penicillin and cephalosporin, and a few that are produced by bacteria (10.7%), e.g. bacitracin and polymyxin. All other antibiotics that are medically useful and have a wide application are synthesized by actinomycetes (Goodfellow, 1988). Actinomycetes have been described as the greatest source of antibiotics since Waksman introduced streptomycetes into his systematic screening program for new antibiotics in the early 1940s. They have provided about two-thirds (more than 4,000) of the naturally occurring antibiotics discovered, including many of those important in medicine, such as aminoglycosides, antracyclines, chloramphenicol, β-lactams, macrolides, and tetracyclines (Goodfellow, 1988).

Organisms belonging to the groups of common actinomycetes, *Streptomyces*, and rare actinomycetes, *Micromonospora*, *Actinoplanes*, *Dactylosporangium*, *Nocardia*, *Actinomadura*, *Microbispora*, *Streptosporangium*, *Kibdelosporangium*, and others, have been searched for antibiotic production. Among antibiotics produced from actinomycetes, *Streptomyces* strains could produce approximately 80.2% antibiotics, followed by *Micromonospora* strains, *Nocardia* strains, and the other genera that could produce about 6%, 3.7%, and 10.1%, respectively (Oki, 1994). From the above data, the antibiotics produced from *Micromonospora* seem to be a little amount when compare with the antibiotics produced from *Streptomyces*, it may be that the study in this genus is still limited.

Micromonospora strains are potential actinomycetes that seemingly untimated capacity to produce secondary metabolites with diverse chemical structures, and biological activities, e.g. gentamicin, sagamicin, megalomicin, mycinamicin, halomicin, mutamicin, everninomicin, mycinamicin (Glasby, 1993). The actinomycete antibiotics are not restricted to antibacterial antibiotics but include

antifungal, anticancer, antiviral antibiotics, and also produce a number of biologically active substances such as enzyme inhibitors.

Micromonosporae are aerobic, gram-positive, mesophilic, non-motile actinomycete which produces single non-motile spore directly from substrate hyphae in generally indicated orange color. Cell wall contained glutamic acid, glycine, alanine, *meso*-diaminopimelic acid, and glycolylmuramic acid. The diagnostic whole-cells were xylose and arabinose. The phospholipid patterns were diphosphatidylglycerol, phosphatidylinositol, phosphatidylinositolmannosides, and phosphatidylethanolamine, but not phosphatidylcholine. Major cellular fatty acids were iso-$C_{16:0}$ and iso-$C_{15:0}$ and small amount of iso-$C_{17:0}$, anteiso-$C_{17:0}$, and anteiso-$C_{15:0}$ were also present. Mycolic acids were absent. The predominant menaquinones were MK-9, MK-10, and MK-12. The range of G+C content of the DNA was 71-73 mol%.

Members of the genus *Micromonospora* can be commonly isolated from neutral and alkaline soils. However, their predominant incidence seems to be in aquatic ecosystems, including both freshwater and marine habitats. Nowadays, *Micromonospora* isolated from tropical peat swamp forests are still limited. In Thailand, research on taxonomy and antibiotic production of *Micromonospora* so far received little attention. New species of *Micromonospora* are of great interest because they may be able to produce new antibiotics. Therefore, extensive screening and discovery of new species are important for medical and industrial applications. At present, the reports on new antibiotics from *Micromonospora* are limited. In this study, the attempt to sampling the soil samples in the unique sources is focused.

Peat swamp forest is a very special type of the evergreen forests that differs from other forests because it occurs in fresh-water marshy land. The soil that covered in this forest, is formed by a thick layer of peat, 0.5 to 5 meters deep or more with acidic condition in which the range of soil pH is from 4.5 to 6.0. Peat swamp forest is an interested source for soil sampling because it is much different from the normal soil. Most of peat swamp forests in Thailand are located in the south and a few were found in the north and north-east. In this study, *Micromonospora* strains isolated from peat swamp forest in the southern areas of Thailand were characterized and identified by both classical and molecular techniques, and the investigation of a secondary metabolites is also performed.

The main objectives of this investigation are as follows:

1. To isolate and screen *Micromonospora* strains from soils of Thai peat swamp forest.

2. To identify and characterize *Micromonospora* strains.

3. To isolate secondary metabolites from the selected *Micromonospora* isolate.

4. To elucidate chemical structures of the isolated secondary metabolites.

5. To evaluate biological activities of the isolated secondary metabolites.

CHAPTER II

HISTORICAL

There was a good description by Coyne (1999) on how the actinomycetes are close friends to human and they can influence our daily life style. "Take a handful of garden or field soil, hold it close to your nose, and breathe deeply. What do you smell? It's not obvious that you should smell anything based simply on the composition of soil, because most soils are primarily made up of inert materials such as sand, silt, and clay. But you probably do smell something: an earthy, musty, smell. Maybe it's a smell that brings back old memories of cutting grass in spring or burning leaves in fall. The smell is real even if the images it evokes are just memories. What your sense of smell detects are microbial products called geosmins (1,10-dimethyl-9-decalols). Geosmins produce the smell of freshly plowed soils and musty cellars—the smells that remind city folk of country life. Geosmins are produced by the group of microorganisms---THE ACTINOMYCETES" (Coyne, 1999).

1. CHARACTERISTICS OF ACTINOMYCETES

Actinomycetes are prokaryotes that may look like fungi and were originally called ray fungi because they grow as filamentous mycelia and form spores. There are two important characteristics that distinguish actinomycetes from fungi: they are prokaryotic that have no cell nucleus and form hyphae that are from 0.5 to 1.0 μm in diameter, which are much smaller than fungal hyphae (which are 3 to 8 μm in diameter). Actinomycetes are not photosynthetic. Most actinomycetes are saprophytes, growing by decomposing organic matters. Some actinomycetes are human pathogens. Others actinomycetes are animal and plant pathogens. Like most other microorganisms, however, actinomycetes are usually harmless soil organisms. Some actinomycetes are particularly beneficial. Actinomycetes in the genus *Frankia* form associations with woody shrubs and trees and fix nitrogen. Actinomycetes compose 10% to 50% of the total microbial population in soil. They are found in soil (most commonly), composts, and sediment. These microorganisms, with some exception, are aerobic—requiring O_2 for growth. As a result, they do not grow well in wet soils. Actinomycetes are not tolerant of desiccation, but the spores they produced

can tolerant desiccation. Furthermore, they are isolated more commonly from hotter soils than colder soils. In all likehoods, this is due to spore recovery, since hotter soils are drier soils. Actinomycetes are tolerant of alkaline conditions. In alkaline soils, 95% of the microbial isolates may be actinomycetes. On the other hand, actinomycetes are acid intolerant for the most part, although acid-intolerant species exist. At a pH of less than 5, actinomycetes make up less than 1% of the microbial population (Coyne, 1999).

In 1997, Stackebrandt *et al*. proposed a new hierarchic classification structure for the taxa between the taxonomic levels of genus and class for the actinomycete line of descent as defined by analysis of small subunit (16S) rRNA and genes coding for this molecule (rDNA) (Scheme 1).

2. TAXONOMY OF *MICROMONOSPORA*

The genus *Micromonospora* belongs to the family *Micromonosporaceae*. The family *Micromonosporaceae* was first decribed by Krasil'nikov, in 1938. In 1997 Stackebrandt *et al.* described that this family contained seven genera, including *Micromonospora, Actinoplanes, Dactylosporangium, Catellatospora, Catenuloplanes, Couchioplanes,* and *Pilimelia*. Subsequently, the genera *Spirilliplanes* (Tamura *et al.*, 1997) and *Verrucosispora* (Rheims *et al.*, 1998) were found. Recently, microbiologists have described two new genera namely, *Virgisporangium* (Tamura *et al.,* 2001) and *Longispora* (Matsumoto *et al.,* 2003) and transfer *Catellatospora ferruginea* and *Catellatospora ishikariense* to *Asanoa* as *Asanoa ferruginea* and *Asanoa ishikariense* (Lee, Goodfellow, and Hah, 1999). These genera can be assigned to this family on the basis of their phylogenetic position. At present, the family *Micromonosporaceae* consists of twelve genera (Table 1).

The genus *Micromonospora* was first described by Ørskov (1923) for actinomycete strains producing spores singly borne on sporophores branched from substrate hyphae. In the *Approved Lists of Bacterial Names* (Skerman *et al*., 1980), twelve species and seven subspecies were cited as members of the genus *Micromonospora*, and *Micromonospora olivasterospora* (Kawamoto *et al*., 1983), *M. rosaria* (Horan & Brodsky, 1986), and *M. chersina* (Tomita *et al*., 1992), were validly

Class *Actinobacteria*

Subclass *Acidimicrobidae* **Order *Acidimicrobiales*** **Family *Acidimicrobiaceae***

Subcalss *Rubrobacteridae* **Order *Rubrobacterales*** **Family *Rubrobacteraceae***

Subclass *Coriobacteridae* **Order *Coriobacteriales*** **Family *Coriobacteriaceae***

Subclass *Sphaerobacteridae* **Order *Sphaerobacterales*** **Family *Sphaerobacteraceae***

Subclass *Actinobacteridae* **Order *Bifidobacteriales***

Family : *Bifidobacteriaceae*

Order *Actinomycetales*

Suborder : *Actinomycineae*

Family : *Actinomycetaceae*

Suborder : *Micrococcineae*

Family : *Micrococcaceae*

Brevibacteriaceae

Cellulomonadaceae

Dermabacteraceae

Dermatophilaceae

Intrasporangiaceae

Jonesiaceae

Microbacteriaceae

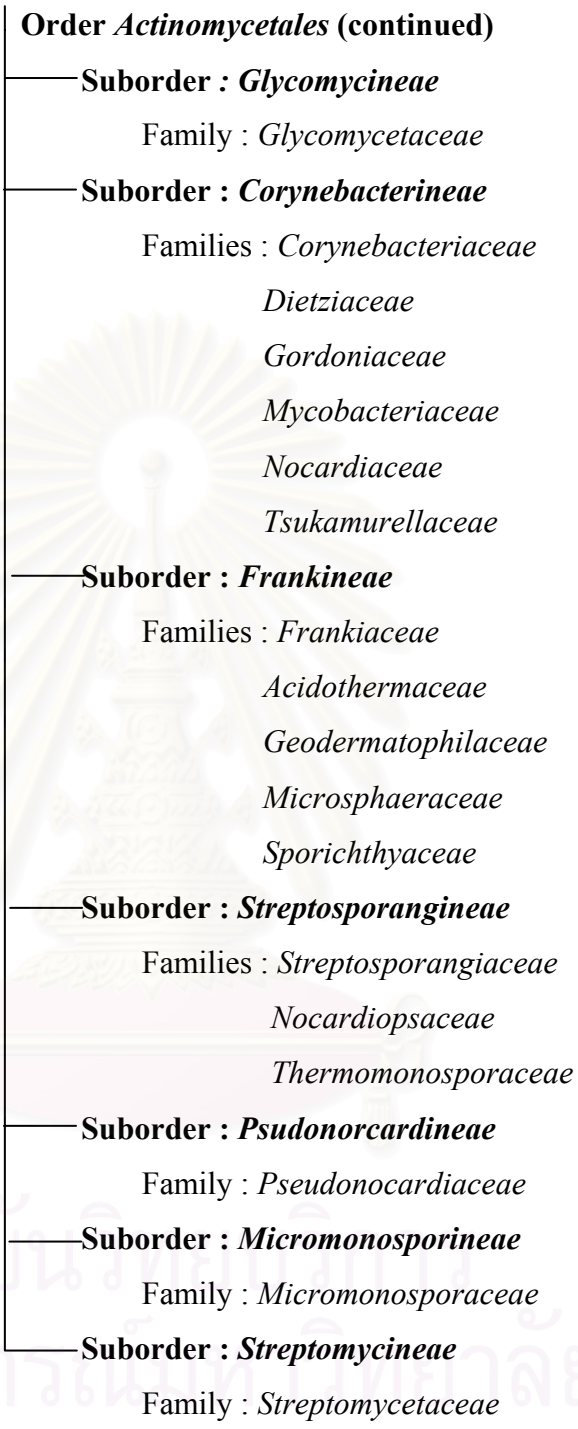
Promicromonosporaceae

Suborder : *Propionibacterineae*

Family : *Propionibacteriaceae*

Nocardioidaceae

Scheme 1. Proposed hierarchic classification system of the class Actinobacteria.



Scheme 1 (continued)

proposed as new species. Lee *et al.* (1999) transferred *Catellatospora matsumotoense* to the genus *Micromonospora* as *M. matsumotoense* on the basis of 16S rDNA analysis. In the meanwhile, this genus was well-defined from chemotaxonomic and phylogenetic points of view (Kroppenstedt, 1985; Kawamoto,

1989; Koch *et al*., 1996a, 1996b; Lee *et al.*, 1999) as well as morphological aspects. Reclassification at the species level of the genus *Micromonospora* was carried out by Kasai, Tamura, and Harayama (2000) on the basis of *gyrB* sequence analyses and DNA-DNA hybridization experiments. They elevated *M. echinospora* subsp. *pallida* and *M. halophytica* subsp. *nigra* to *M. pallida* and *M. nigra*, respectively, and considered *M. echinospora* subsp. *ferruginea*, *M. rhodorangea*, and *M. purpurea* as junior subjective synonyms of *M. echinospora.* *M. carbonacea* subsp. *carbonacea*, and *M. carbonacea* subsp. *aurantiaca*, were combined at the subspecies level as *M. carbonacea*, and the unification of *M. brunnea* into *M. purpureochromogenes* proposed by Szabó & Fernandez (1984) was supported. Consequently, the genus *Micromonospora* is considered to accommodate 15 species at present, i.e. *M. aurantiaca, M. carbonacea, M. chalcea, M. chersina, M. coerulea, M. echinospora, M. gallica, M. halophytica, M. inositola, M. matsumotoense, M. nigra, M. olivasterospora, M. pallida, M. purpureochromogenes*, and *M. rosaria.* According to Kawamoto (1989), the type strain (NTCT 4582^T) of *M. gallica* is not available any longer.

2.1 CHARACTERISTICS OF *MICROMONOSPORA*

Micromonospora is the large group of rare actinomycetes that can produce a large number of antibiotics. Approximately 6% of actinomycetes antibiotics came from *Micromonospora*. Although the actinomycetes posses cytologic and physiologic structures and filamentous properties which support a morphological basis for classification, colony characterization has been used extensively for describing *Micromonospora* species in terms of texture and color of mycelium and spores.

2.1.1 Phenotypic characteristics of *Micromonospora*

Micromonospora formed well-developed, branched, septate substrate mycelium and single conidia are produced. This microorganisms, normally lacking aerial mycelium, forming light yellow-orange to orange-red colonies (occasionally brown, maroon or blue-green) composed of tightly woven, fine hyphae (0.2-0.6 μm in diameter). The dark brown to black spores are formed within and at the surface of the colonies which darken as a result of sporulation and usually turn black and may become viscid or mucoid. The single spores are borne in dense clusters on repeatedly branched sporophores (cluster type) or are well dispersed throughout

Table 1. Differential characteristics of the genera belonged to family *Micromonosporaceae*.

Genus	G+C content of DNA (mol%)	Motility of spores	Spore-chains	Fatty acid type[b]	Cell wall type[c]	Major menaquinone(s)	Phospholipid type[d]	Characteristic sugar(s) in whole cell	Diamino acid
Longispora	70	-	+	2d	II	MK-10(H_4,H_6)	PII	Xyl, Ara, Gal	*Meso*-DAP
Virgosporangium	71	+	-	2d	II	MK-10(H_4,H_6)	PII	Xyl, Ara, Gal	*Meso*-DAP
Asanoa	71-72	-	+	2d	II	MK-10(H_6,H_8)	PII	Xyl, Ara, Gal	*Meso*-DAP
Actinoplanes	72-73	+	-	2d	II	MK-9(H_4), MK-10(H_4)	PII	Ara, Xyl	*Meso*-DAP
Spirilliplanes	69	+	+	2d	II	MK-10(H_4)	PII	Xyl, Gal	*Meso*-DAP
Micromonospora	71-73	-	-	3b	II	MK-10(H_4, H_6), MK-9(H_4, H_6)	PII	Ara, Xyl	*Meso*-DAP
Dactylosporangium	72-73	+	-	3b	II	MK-9(H_4, H_6, H_8)	PII	Ara, Xyl	*Meso*-DAP
Catellatospora	71-72	-	+	3b	II	MK-10(H_8, H_6), MK-9(H_4, H_6)	PII	Xyl, Ara, Gal/Xyl only	*Meso*-DAP
Catenuloplanes	71-73	+	+	2c	VI	MK-9(H_8), MK-10(H_8)	PIII	Xyl	L-Lys
Couchioplanes	70-72	+	+	2c	VI	MK-9(H_4)	PII	Xyl, Ara, Gal	L-Lys
Pilimelia	ND	+	-	2d	II	MK-9(H_2, H_4)	PII	Ara, Xyl	*Meso*-DAP
Verrucosispora	70	-	-	2b	II	MK-9(H_4)	PII	Man, Xyl	*Meso*-DAP

[a] +, present; -, absent; ND, not determined.

[b] According to the classification of Kroppenstedt (1985).

[c] According to the classification of Lechevalier and Lechevalier (1977)

[d] According to the classification of Lechevalier *et al.*, 1970.

Abbreviations : Xyl, xylose; Gal, galactose; Ara, arabinose.

the mycelium. Sporophore branching may be monopodial or sympodial (or obscured in the dense grape-like cluster) (Sykes and Skinner, 1973)

Mycelial pigment and pigments that are produced in the substrate mycelium and diffuse out into the medium have been used as criteria for descriptions of *Streptomycetes*. Pigments produced by the *Micromonospora* are numerous and often unpredictable but, in spite of their shortcomings, are helpful when present as ancially recognition units. Pigments are biochemical products and often are associated with certain species groups. The problem is to differentiate between strain-specific pigments and group-shared pigments. We may divide these pigments into the readily diffusible pigments and mycelium bound pigments, however, mycelial bound pigments in order culture may diffuse, possibly due to mycelial autolysis. The yellow-orange and orange-red mycelial pigments appear to differ little diagnostic value in strain or species recognition. Maroon mycelial pigments are often recognition units for the *Micromonospora echinospora* and *M. purprea* species group. These pigments are soluble in water and acid alcohol and act as acid-base indicators, being red in the acid range and blue-green and precipitable in the basic range. The most *Micromonospora* strains are sensitive to pH below 6.0. Growth occurs normally between 20˚C and 40 ˚C but not above 50 ˚C (Holt, 1989). This organism could grow in 1.5%-5% NaCl concentration, normally could grow in 3% NaCl. The temperature range for growth is 15-45 ˚C, and the optimal temperature is 25-30 ˚C. All strains of *micromonospora* showed positive results for gelatin liquefaction.

Sporulation appears almost as readily in submerged broth culture as on agar media. The formation of single spores on substrate mycelium is one of the well-defined criteria in the genus *Micromonospora*. Infrequently spores occur in longitudinal pairs and more infrequently as multiple longitudinal spores. Spore surface ornamentation of the strains and the species of *Micromonospora* have been characterized by terms “smooth”, “rough”, “warty” or “blunt spiny”. Sporogenesis occurs in two stages: sporulation septum formation and spore maturation. The process is initiated by swelling of the apical end of a hypha before its delimitation by a sporulation septum. In spore maturation, material is laid down centripetally to form much-thickened wall layers. The much-thickened walls probably account for their rather high refractility and their relatively high resistance to physical and chemical treatments. Mature spores are unaffected by ultrasonication but mycelia are quickly

killed. Spore survival of most strains is >50% after 20 min at 60 ˚C in phosphate buffer and <0.5% after 20 min at 80 ˚C. *Micromonospora* spores are more resistant to ketones than to alcohols and dioxin. The spore viability does not change between pH 6.0 and 8.0, but decreases outside this range, particularly at an acidic pH (Kawamoto, 1989).

2.1.2 Chemotaxonomic characteristics of *Micromonospora*

Chemotaxonomically, the genus *Micromonospora* is characterized by a cell wall type II (Lechevalier and Lechevalier, 1970) (Table 2), a whole cell sugar pattern D (Lechevalier and Lechevalier, 1970) (Table 3), and a phospholipids type II (Lechevalier, DeBievre, and Lechevalier, 1977) (Tables 4 and 5). The cell walls of *Micromonospora* have been found to contain glycine, glutamic acid, *meso*-diaminipimelic acid (*meso*-DAP) (including its 3-hydroxy derivative), and D-alanine in a molar ratio of 1:1:1:0.6-0.8. Based on this evidence, muramic acid does not occur as *N*-acetyl, but as the *N*-glycolyl derivative. The amino group in position 2 is not substituted by an acetyl group ($-COCH_3$) but by a glycolyl group ($-COCH_2OH$) and the L-diamino acid is replace by *meso*-DAP (Figure 1). The pentoses, xylose, and arabinose are always constituents of the cell wall, although the amounts vary to some extents. Hexoses, glucose, and galactose are detected more frequently than mannose and rhamnose. The phospholipids contained in the cells are diphosphatidylglycerol, phosphatidylinositol, phosphatidylinositolmannosides, and phosphatidylethanolamine, but phosphatidylcholine is not detected. This pattern corresponds to phospholipid type II as reported by Lechevalier *et al.* (1977). The predominant cellular fatty acids are *iso*- and *anteiso*-branched fatty acid. Unsaturated or 10-methyl fatty acids may be found in certain strain, that were iso-$C_{15:0}$, iso-$C_{16:0}$, iso-$C_{17:0}$, anteiso-$C_{15:0}$, $C_{17:0}$, and anteiso-$C_{17:0}$. This pattern corresponds to fatty acid type 3b of Kroppenstedt (1985), but mycolic acid and cyclic fatty acids are not presented. The genus *Micromonospora* currently consists of 15 validly described species, and they (except for *M. gallica*) can be divided into three groups based on the predominant menaquinone components, i.e. MK-9, MK-10, and MK-12 groups. Species containing hydrogenated MK-9 as major menaquinones (MK-9 group) are *M. carbonacea*, *M. halophytica*, *M. nigra*, and *M. chersina*, while the MK-12 group is found in only one species, *M. pallida*. The species, *M. chalcea, M. inositola, M. coerulea, M. purpureochromogenes, M. olivasterospora, M. echinospora, M. matsumotoense, M. rosaria*, and *M. aurantiaca*

contained MK-10 as a major menaquinone. The major menaquinones were MK-9, MK-10, and MK-12. The range of G+C contents of the DNA was 71-73 mol%.

Table 2. Cell wall chemotypes of the actinomycetes.

Major constituent	Chemotype							
	I	II	III	IV	V	VI	VII	VIII
L-Diaminopimelic acid	+							
meso-Diaminopimelic acid		+	+	+				
Diaminobutyric acid							+	
Aspartic acid						v		
Glycine	+	+					+	
Lysine					+		v	
Ornithine					+			+
Arabinose				+				
Galactose				+		v		

v, variable amounts.

Table 3. Whole-organism sugar patterns of the actinomycetes containing *meso*-diaminopimelic acid (chemotype II-IV).

Pattern	Sugar				
	Arabinose	Fucose	Galactose	Madurose*	Xylose
A	+		+		
B				+	
C	No diagnostic sugars				
D	+				+
E		+			

***Madurose is 3-O-methyl-D-galactose.**

Table 4. Phospholipid types in actinomycetes.

Phospholipid type	**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 = Variable

+ = Present

- = Absent

Table 5. Phospholipid types in actinomycetes.

Phospholipid type	Diagnostic phospholipids
I	No nitrogenous phospholipids
II	Phosphatidyl ethanolamine
III	Phosphatidyl choline
IV	GluNu (unknown glucosamine-containing phospholipid)
V	GluNu and phosphatidyl glycerol

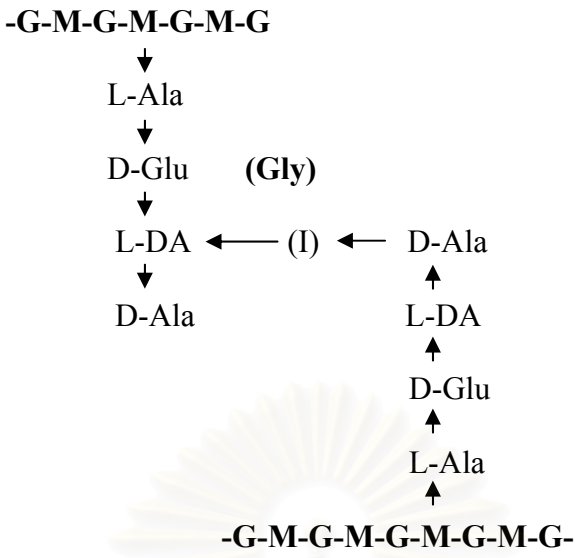
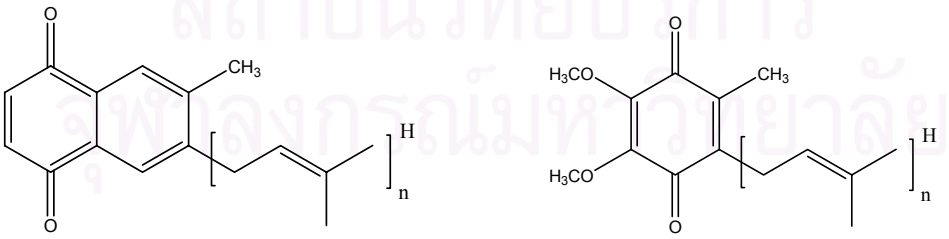


Figure 1. Fragment of the primary structure of a typical peptidoglycan.

Abbreviations: G, *N*-acetylglucosamine; M, *N*-acetyl(glycolyl)muramic acid; Ala, Alanine; Glu, Glutamic acid; Gly, glycerine; *L*-DA, *L*-diamino acid; *I*, interpeptide bridge.

2.1.2.1 Isoprenoid quinones

Isoprenoid quinones are important in the functioning of the electron transport system in respiration. Various kinds of quinones are found in bacterial cells (Figure 2), with most aerobic bacteria possessing isoprenoid menaquinone and/or isoprenoid ubiquinone (Komagata and Suzuki, 1987).



Menaquinone Ubiquinones

Figure 2. Isoprenoid quinones found in most aerobic bacteria cells.

Identification of the genus *Micromonospora* by using menaquinone system is characterized by the presence of three major menaquinones (hydrogenated MK-9, MK-10, and MK-12). The most members of genus *Micromonospora* presented hydrogenated MK-10. The four species of genus *Micromonospora* are *M. carbonacea*, *M. halophytica*, *M. nigra*, and *M. chersina*, presented hydrogenated MK-9 and the remaining one, *M. pallida*, presented hydrogenated MK-12.

2.1.2.2 DNA base composition

The determination of DNA base composition has become an important criterion in microbial taxonomy since Marmur and Doty (1962) reported a simplified method. Indeed, the guanine (G) plus cytosine (C) ratio of DNA is one of the few characters which can be used in the description of any cultivable microorganisms. DNA base composition has proved usedful for both classification and identification of prokaryotes. The base composition of bacterial chromosomal DNA ranges from 25% to 80% G+C. In general, the DNA base composition of strains of a species shows a narrow range of about 1 to 3 mole% G+C (Mandel, 1966; Tamaoka, 1984). Once the DNA base ratio of a strain has been determined, the number of species to be compared for identification of that strain decreases as microorganisms with base composition differences will have different chromosomal DNA and hence belong to separate species. The DNA base ratios of almost all prokaryotic species are available in texts such as Bergey's Manual of Systematic Bacteriology.

The determination of mean DNA base composition was the first unique feature of DNA that was seen to have taxonomic importance. The DNA base composition of a strain is constant and given the application of the HPLC method is highly reproducible (standard deviation <0.5 mole% G+C). Initially, DNA base composition studies provided an easy and useful way of distinguishing between phonetically similar but genetically different strains (Colwell and Mandel, 1964; Silvestri and Hill, 1965). It is, however, self-evident given the extensive nature of prokaryotic diversity that the mole % G+C contents of archaea and bacteria are not the preserve of single species.

The importance of the mole % G+C content of DNA in the systematics of prokaryotes is that it can be weighted as an excluding characteristic. Thus, if two organisms have DNAs with markedly different base composition values they must

belong to different taxa. It is, however, important to realize that two organisms with similar base compositions are not necessarily closely related as mole% G+C determinations do not make into account the linear arrangement of nucleotides in DNA.

DNA base composition studies are particularly important in highlighting taxa that are in need of taxonomic revision. This point is exemplified by the genus *Bacillus*, which currently encompasses organisms with DNA base compositions that range from 32 to 69 mole% G+C (Slepecky and Hemphill, 1991). Indeed, despite its limitations as a taxonomic criterion, DNA base composition is correctly seen as one of the required characteristics for the minimum descriptions of genera and species (Lévy-frébault and Portales, 1992). In the case of *Micromonospora* species, the G+C base composition of them ranges from 71 to 73 mol%.

2.1.3 Genetic analysis of *Micromonospora*

2.1.3.1 16S rDNA analysis

Ribosomal RNA represents only a small part (about 0.3-0.4%) of the genome and the citrons coding for it are highly conserved. They have evoled less rapidly than the rest of the chromosome. The three rRNAs in bacteria are classified by their sedimentation rates as 23S, 16S, and 5S, which have chain lengths of about 3,300, 1,600, and 120 nucleotides, respectively (Stent, 1981). Bacterial 16S rDNA sequences are attractive targets for developing identification methods because they represent conserved regions in all bacteria and species having 70% or greater DNA similarity usually have more than 97% sequence identity (Stackebrandt and Goebel, 1994, Stackebrandt *et al*., 2002). But this recommendation has been questioned (Fox, Wisotzkey, and Jurtshuk, 1992). Recently, it has been suggested that a difference rate of >0.5% could be considered indicative of a new species within a known genus (Palys, Nakamura, and Cohan, 1997). In the present study, in the absence of an accepted cut-off value, which 99% similarity as a suitable cutoff for identification at the species level and 97% similarity as a suitable cutoff for identification at the genus level (Drancourt *et al*., 2000).

Bacterial identification based on % similarity of 16S rDNA has been using PCR technique, DNA sequencing, and similarity analysis of rRNA genes. 16S

rDNA was amplified and sequenced using oligonucleotide primers complementary to highly conserved regions of bacterial rRNA gene.

2.1.3.1.1 Amplification of rDNA template by PCR

The development of PCR for amplifying specific regions of DNA has allowed investigators to generate rRNA genes from the DNA of most organisms. The following protocol works well for many rRNA gene (Johnson, 1994).

a. Symmetrical amplification Symmetrical amplification, which occurs when there is a large excess of both amplification primers, is the most efficient type of amplification. It should be used for testing DNA templates and when large amounts of double-stranded DNA are wanted, as, for example, when doing direct double-stranded DNA sequencing, using a biotin-labeled primer, or planning to generate single-strained sequencing template by T7 gene 6 exonuclease digestion.

b. Asymmetrical amplification The principle in asymmetrical amplification is that a lower concentration of one of the PCR primer results in less amplification of the corresponding DNA strand. The amount of single stranded DNA is not too as easy to evaluate the asymmetrical amplification, because the single-stranded DNA doses not stain as intensely with ethidium bromide. As a result, one just has to test the asymmetrical amplification product as a sequencing template.

An alternative approach is to do two-step amplification. First, a symmetrical amplification PCR is performed and then transfer part of that reaction mixture to a second PCR in which only one of the primers is included. The advantage of this is that the first amplification is improved and one can adjust the amount of material from the first PCR to add to the second PCR to get a consistent amount of single-strands DNA product.

2.1.3.1.2 Direct sequencing of PCR amplified DNA

The amplification of target DNA by PCR followed by the direct sequencing of amplification DNA has emerged as a powerful strategy for rapid molecular genetic analysis. Direct sequencing of PCR products consists of usually two steps.

a. Purification of templates The PCR amplification should be purified from the oligonucleotide primers and unused dNTPs.

b. Direct sequencing of PCR product Template DNA for DNA sequencing can be prepared by several methods.

1) Double strand DNA sequencing This method is simple and rapid method of sequencing PCR product directly (Johnson, 1994) and is ideal for sequencing PCR products that are less than 2 kb in length. The double stranded template is denatured in the presence of sequencing primer by boiling and then snaps chilling in ice/water. This prevents reassociation of the template, favouring primer-template annealing. And then proceed with the sequencing reaction.

2) Sequencing of asymmetrical PCR product The principle in asymmetrical amplification is that a lower concentration of one of the PCR primers results in less amplification of the corresponding DNA strand. The amount of single stranded DNA is difficult to estimate by standard ethidium bromide staining in agarose gels, because of a smaller amount of dye intercalation than with double stranded DNA. Consequently, one must assume that there will be sufficient template and must arbitrarily determine the volume of template needed (Johnson, 1994).

3) Digestion by bacteriophage exonuclease Bacteriophage T7 gene 6 exonuclease is specific for double stranded DNA. The enzyme degrades from 5' end, resulting in two half-length single strands, as illustrated in figure 3. The exonuclease is not a processive enzyme: it dissociates frequently from the substrate during the course of digestion and continued with the sequencing reaction. The disadvantages of the procedure are that one is limited to sequence the 5' end of the 16S rDNA with the forward sequencing primers and the 3' end with reverse primers, whereas the middle of the DNA fragment can not be sequenced well with either forward or reverse primer. One can be used other methods for sequencing the middle region of the fragment (Johnson, 1994).

4) Magnetic beads for separating DNA strands A recent approach for separating the strands of PCR-generated DNA has been to attach biotin at the 5' end of one of the PCR primers and then react the PCR-generated DNA with magnetic beads containing covalently couple streptavidin. The biotin will bind irreversibly with the streptavidin on the beads, and the complementary strand is then

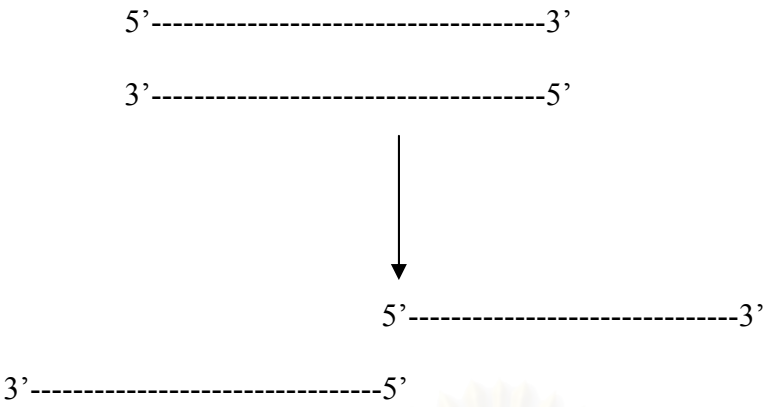


Figure 3. Digestion by bacteriophage T7 gene 6 exonuclease. This enzyme degrades from the 5' end, resulting in two half-length single strands.

denatured with alkali and washed away from the beads. DNA sequencing can be done directly on the DNA that is bound to the beads. The complementary DNA strand, after being precipitated, can also be used as a sequencing template (Johnson, 1994). The dideoxynucleotide-chain-terminating DNA sequencing method of Sanger *et al*., and automated DNA sequencing introduced the era of rapid DNA sequencing.

Dideoxynucleotide Method: Sanger and colleagues developed the dideoxynucleotide method in 1977. This technique involves the synthesis of a DNA strand from a single stranded template by DNA polymerase and chain terminating property of dideoxynucleotide molecule. Dideoxynucleotides are base analogues of the deoxynucleotide molecules, which lack 3'hydroxyl group (Figure 4). Normally, the chain elongation reaction in DNA synthesis requires DNA polymerase and four deoxyribonucleotide triphosphates (dATP, dCTP, dGTP, dTTP). DNA polymerase can incorporate dideoxynucleotides (ddNTPs) into the growing strand in the same way as the conventional deoxynucleotides. However, the absence of hydroxyl group prevents the formation of a phosphodiester bond with the succeeding deoxynucleotide when a ddNTP is incorporated into the new strand, therefore terminates chain elongation at that position.

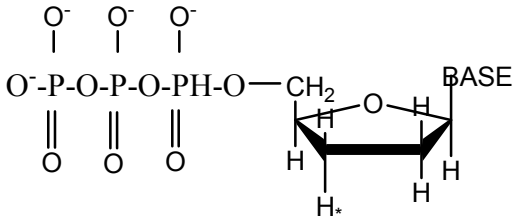


Figure 4. The structure of 2',3'-dideoxynucleotide-5'-triphosphate (ddNTP).

In a dideoxy sequencing experiment, four separate polymerization reactions are performed. Each of the four reaction mixtures contains a small amount of one of the dideoxynucleotide mixed with normal deoxynucleotides, target DNA, the primer, and DNA polymerase in an appropriate synthesis buffer. This generates different sized fragments, which vary at the 3' end according to the position at which a ddNTP is incorporated. The fragments are then electrophoretically size separated to generate a sequence ladder (Figure 5).

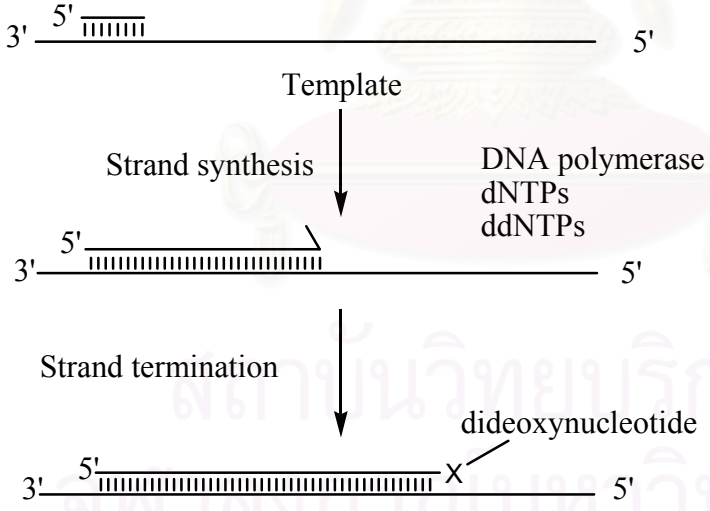


Figure 5. The dideoxynucleotide method.

Automated DNA Sequencing: one of the major advances in sequencing technology in recent years is the development of automated DNA sequencers. There are based on the chain termination method and use of fluorescent labels. The fluorescent dyes can be attached to the sequencing primer, to the dNTPs or the terminators and are incorporated into the DNA chain during strand synthesis

reaction mediated by DNA polymerase. During the electrophoresis of the newly generated DNA fragments on a polyacrylamide gel, a laser beam excites the fluorescent dyes. Detectors collect the emitted fluorescence and the information analyzed by the computer. The data are automatically converted to nucleotide sequence (Figure 6). Several automated DNA sequencers are now commercially available and are becoming increasingly popular.

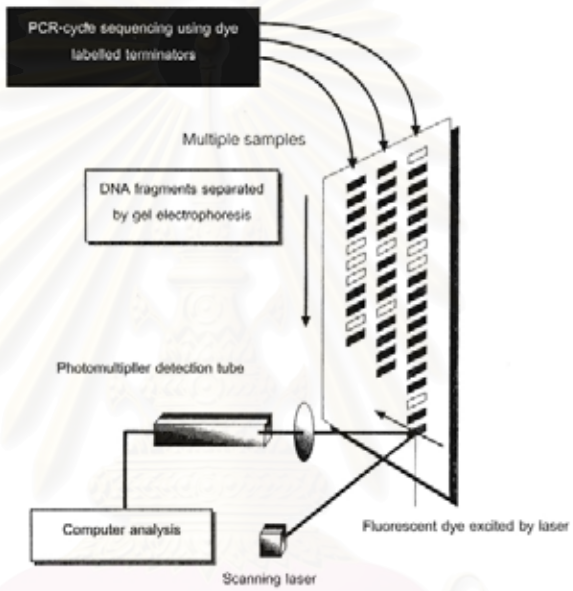


Figure 6. Automated DNA sequencer.

2.1.3.1.3 Analysis of nucleotide sequence and phylogenetic tree

2.1.3.1.3.1 Analysis of nucleotide sequence

a. Homology Search: analysis of nucleotide sequence is the last and may be the most important step in nucleotide sequencing. The goal is to determine the function of an obtained sequence. The most frequent analysis of an unknown sequence is to perform homology search, which is a search for sequence similarity with known sequence in the database. There are three international DNA databases available that provide sequence information as no cost over the Internet

(Table 6). All databases exchange their data daily to update the sequence information.

Table 6. Lists of international primary sequence databases.

Database	Sponsor	Location (URL)
DNA Data Bank of Japan	National Institute of Genetics	**http://www.ddbj.nig.ac.jp**
EMBL Data Library	European Molecular Biology Laboratory	**http://www.ebi.ac.uk/embl.html**
GenBank	National Center for Biotechnology Information	**http://www.ncbi.nlm.nih.gov**

A homology search can be carried out either with nucleotide sequence or amino acid sequence. However, homology search with nucleotide sequence rarely gives valuable information (Brown, 1994). Since, the DNA sequence information is so simple, just four different bases (A, T, G, and C). As a result, unrelated sequence can have high degrees of similarity and the identification for biologically significant homologies is very difficult. On the contrary, amino acid sequence information is relatively complex (20 different amino acids) and amino acid sequence similarity between proteins often indicates a corresponding similarity in the structure and function. Thus the determination for the structure and function of unknown proteins is easily achieved by comparing amino acid sequence of an unknown protein with previously characterized proteins. Two amino acid sequences with more than 50% positional identity is likely to be biological related. There are several programs for homology searching available free of charge from molecular biology databases on the Internet. Some sequence similarity search servers are listd in Table 7.

b. Phylogenetic analysis: phylogenetic analysis is the technique of methodically demonstrating a family relationship between species.

Table 7. Lists of sequence similarity search server on the Internet.

Program	Location (URL)
BLAST	**http://www.ncbi.nlm.nih.gov/BLAST**
FASTA	**http://www.ddbj.nig.ac.jp/E-mail/homology.html**
BLITZ	**http://www.ebi.ac.uk**
GenQuest	**http://avolon.epm.ornl.gov/grail-bin/**

This type of analysis is carried out on small section of aligned DNA taken from the same gene in the various species under consideration, rather than on protein sequences derived from them. DNA is used because the pattern of mutation, insertion, and deletion at the nucleotide level is definitive. Silent mutation, i.e. mutations at the DNA level that do not result in an amino acid substitution at the protein level are automatically incorporated into the analysis. Phylogenetic relationships are often represented graphically, either in the form of phylogenetic tree or dendrograms. The procedure of phylogenetic tree construction from nucleotide is shown in figure 7.

2.1.3.2 DNA-DNA hybridization

DNA hybridization is acknowledged as the superior method for the elucidation of relationships between closely related taxa, such as strains and species, in which a DNA homology value of about 70% plays a dominant role (Wayne *et al*., 1987). The methods usually used in this technique are either a free solution method in which S1 nuclease (Crosa, Brenner, and Falkow, 1973), spectrophometry or a method in which single-stranded DNA is fixed on a solid support, such as nitrocellulose filters or nylon membranes. However, in the former time, to carry out most of these hybridization experiments, DNA must be labeled with radioactive.

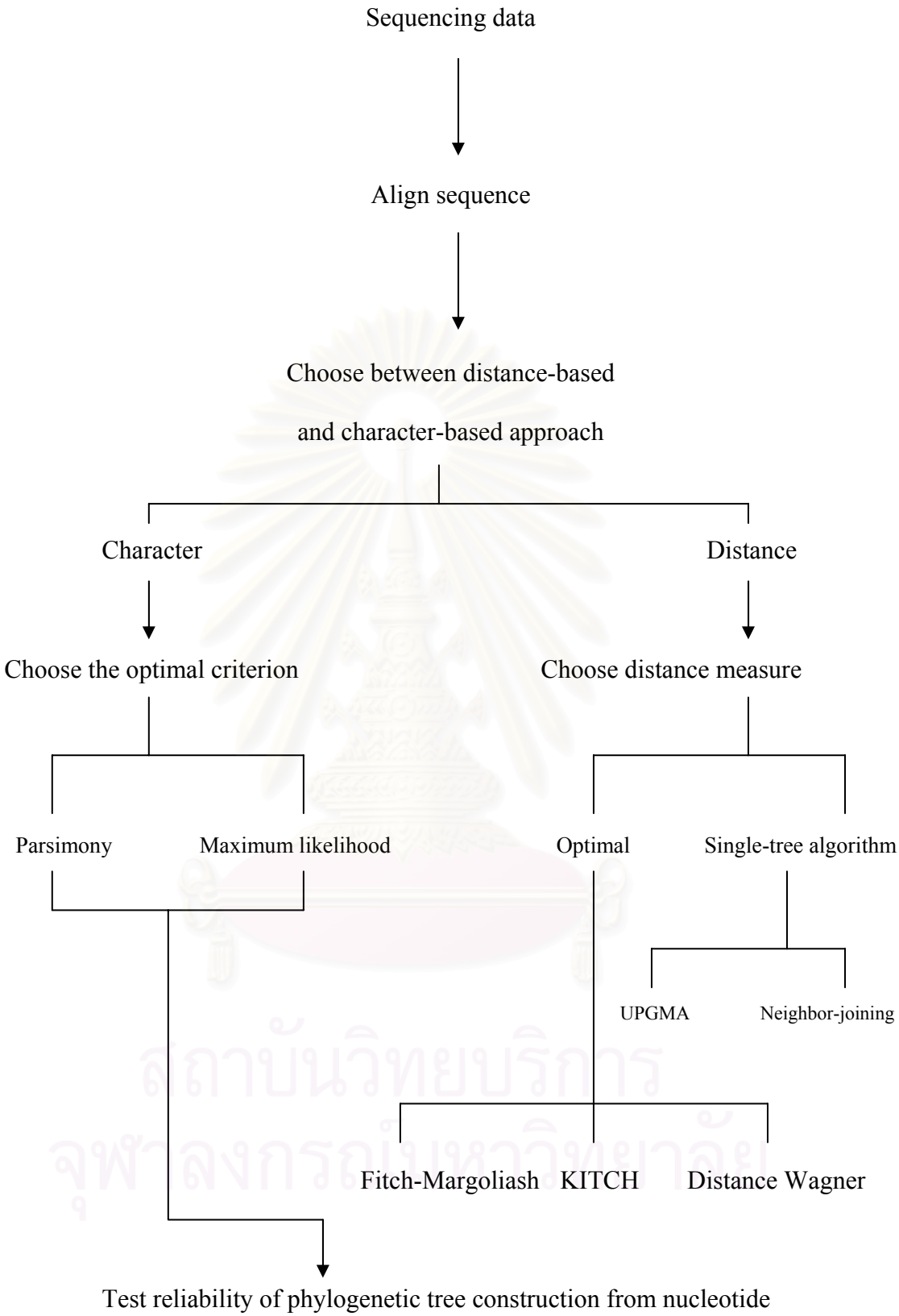


Figure 7. The procedure of phylogenetic tree construction from nucleotide.

2.1.4 Distribution of *Micromonospora*

The habitat of *Micromonospora* is general in soil and aquatic system such as lake mud and river sediments. Their occurrence in soil was first reported by Jensen in 1932 for Australian soils and later by Kriss in 1939 for Russian soil. Early work on lakes in Wisconsin showed that *Micromonospora* comprised 10-50% of the microbial population in the water mass, were the only actinomycetes in mud sample, but were rarely isolated from adjacent soils. *Micromonospora* have also been isolated from marine environments, such as beach sand, deep marine sediments, and sediment from White Sea and Black Sea. (Kawamoto, 1989). The distribution of the validly described *Micromonospora* species is shown in Table 8.

3. Antibiotics from *Micromonospora*

Alexander Fleming (1929) discovered that the fungus *Penicillium notatum* produced and excreted a compound which inhibited the growth of *Staphylococcus* species. Antibiotics is the word that used to describe a type of association in which one living creature was destroying another in order to sustain its own life. Waksman (1947) published the definition of the word "Antibiotic" that is a chemical substance, produced by microorganisms, which has the capacity to inhibit the growth and even to destroy bacteria and other microorganisms". Benedict and Langlyke modified this definition to comprise substances which act upon certain organisms at least in very dilute solutions. Abraham and Newton described the word "Antibiotics" as natural compounds derived from organisms which themselves or chemical modification are able, at low concentration, to inhibit or kill other microorganisms and abnormal cells in higher animals.

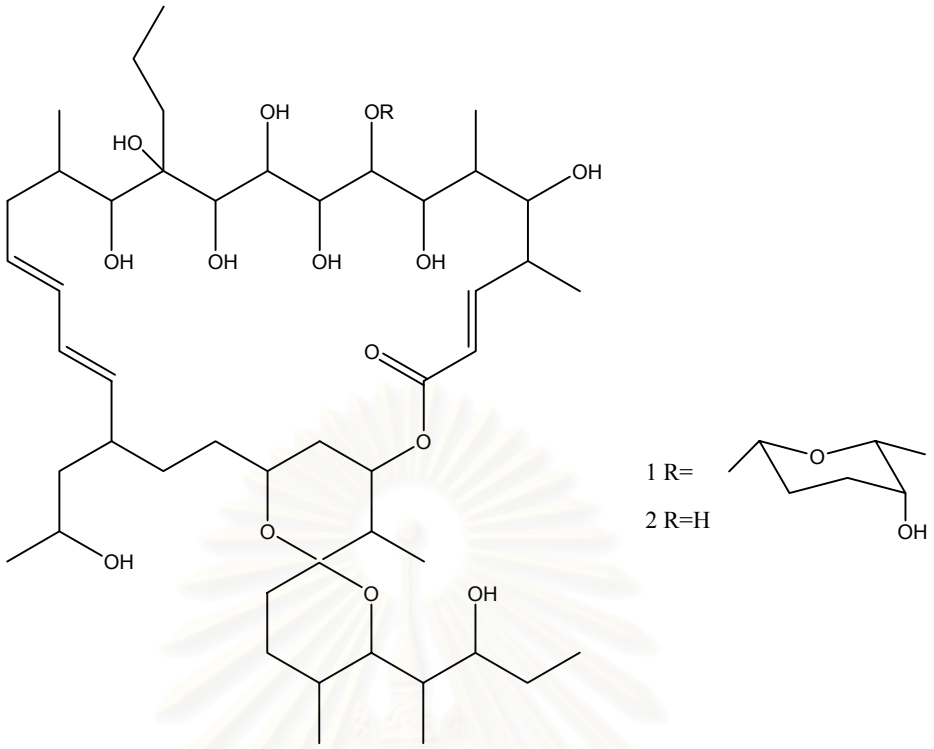
In the case of genus *Micromonospora* strains, there are many strains that could produce interesting bioactive compounds. Bioactive compounds produced from *Micromonospora* strains are summarized in Table 9.

Table 8. Sources of the validly described *Micromonospora* species.

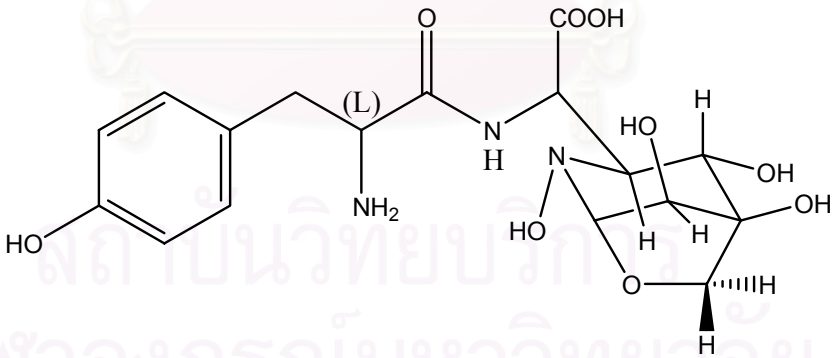
Strain	Source	Refference
M. chersina JCM 9459^T	Soil, Gujarat State, India	Tomita *et al.,* 1992
M. coerulea JCM 3175^T	Soil, Mt. Heleakala, Maui Island, USA	Luedemann., 1971
M. purpureochromogenes JCM 3156^T	Adobe soil, California, USA	Luedemann., 1971
M. echinospora JCM 3073^T	Soil, Jamesville, New York, USA	Luedemann and Brodsky., 1964
M. carbonacea JCM 3139^T	Soil	Luedemann and Brodsky., 1965
M. chalcea JCM 3031^T	Soil	Skerman *et al.*, 1980
M. inositola JCM 6239^T	Forest soil, Hokkaido Pref., Japan	Kawamoto *et al*., 1974
M. olivasterospora JCM 7348^T	Soil, Hiroshima Pref., Japan	Kawamoto *et al*., 1983
M. nigra JCM 8973^T	Mud, bottom of salt pool, New York, USA	Weinstein *et al*., 1968
M. halophytica JCM 3125^T	Mud, bottom of salt pool, New York, USA	Weinstein *et al*., 1968
M. aurantiaca JCM 10878^T	Soil, USSR	Sveshnikova, Maksimova, and Kudrina*,* 1969
M. rosaria JCM 3159^T	Soil, San Jacinto, Taxus, USA	Wagman *et al*., 1972
M. matsumotoense JCM 9104^T	Soil, Matsumoto, Nagano Pref., Japan	Asano, Masunaga, and Kawamoto, 1989
M. pallida JCM 3133^T	Soil, Jamesville, New York, USA	Luedemann and Brodsky., 1964

Table 9. Lists of bioactive compounds produced from *Micromonospora*.

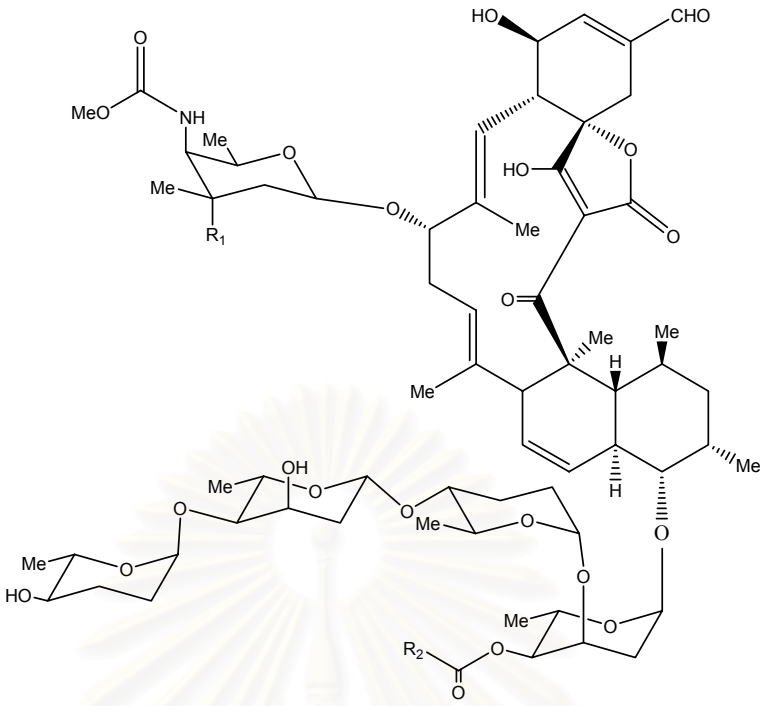
Compounds	Strains	Activity	References
IB-96212 [1]	*Micromonospora* sp. L-25-ES25-008	Cytotoxic acitivity	Chimeno *et al.,*2000
SB-219383 [2]	*Micromonospora* sp. SB-219383	Tyrosyl t RNA synthetase inhibitor	Stefanska *et al.,* 2000
Arisostatins A and B [3]	*Micromonospora* sp. TP-A0316	Antibacterial activity	Igarashi *et al*., 2000
4'-*N*-methyl-5'-hydroxystaurosporine, 5'-Hydroxystaurosporine [4]	*Micromonospora* sp. L-31-CLCO-002	Antitumor activities	Hernandez *et al*., 2000
GTRI-02 [5]	*Micromonospora* sp. SA246	Lipid peroxidation inhibitor	Yeo *et al.,*1998
YM-47515 [6]	*Micromonospora echinospora* subsp. *echinospora*	Antimicrobial activities	Sugawara *et al*., 1997
Thiocoraline [7]	*Micromonospora* sp. L-13-ACM2-092	Antimicrobial activities	Romero *et al*., 1997
1-Hydroxycrisamicin A [8]	*Micromonospora* sp. SA246	Antimicrobial activities	Yeo *et al.,*1998
Pyrrolosporin A [9]	*Micromonospora* sp. C39217-R109-7	Antimicrobial activities	Lam *et al.,* 1996
Antascomicins A, B, C, D and E [10]	*Micromonospora* sp.	Antagonize the immunosuppressive activity	Fehr *et al*., 1996
Macquarimicins A [11], B [12], C [13] and Cochleamycin A[14]	*Micromonospora chalcea*	Antileukemia cell line P-388	Hochlowski *et al*., 1995
Cororubicin [15]	*Micromonospora* sp. JY16	Cytotoxic acitivity	Ishigami, Hayakawa, and Seto, 1994
16-membered lactone compound and izenamicin B_2 and B_3 [16]	*Micromonospora* sp. YS-02930K	-	Yasumuro *et al*., 1994
Mycinamicins I and II [17]	*Micromonospora griseorubida* (FERM BP-705)	Antibacterial activity	Kinoshita *et al*., 1992
Sibanomicin [18]	*Micromonospora* sp. SF2364	Antitumor activities	Itoh *et al*., 1990
Dynemicins O [19], P [20], Q[21]	*Micromonospora chersina* M956-1	Antibacterial activity, antitumor activity	Saitoh *et al*., 1991
Citreamicin [22]	*Micromonospora citrea*	Antibacterial activity	Carter *et al.,* 1990
K-259-2 [23]	*Micromonospora olivasterospora* K-259	Inhibitor of Ca^{2+} and calmodulin-dependent cyclic nucleotide phosphodiesterase	Matsuda. Asano, and Kawamoto, 1987
K-13 [24]	*Micromonospora halophytica* subsp. *exilisia* K-13	Enzyme inhibitor	Kase, Kaneko, and Yamada, 1987
Antromicin [25]	*Micromonospora olivasterospora*	Antibacterial activity	Odakura *et al*., 1984
Sagamicin [26]	*Micromonospora sagamiensis*	Antibacterial activity	Odakura, Kase, and Nakayama, 1983
Echinosporamicin [27]	*Micromonospora echinospora*	Antibacterial activity	He *et al*., 2004



[1] Macrolide IB-96212

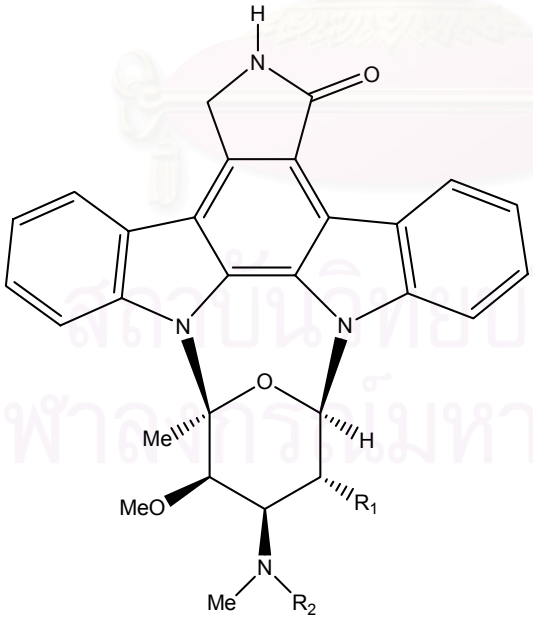


[2] SB-219383



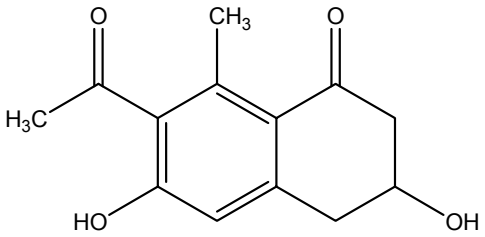
Arisostatin A:$R_1 = NO_2$, $R_2 = CH(CH_3)_2$
B:$R_1 = NH_2$, $R_2 = CH(CH_3)_2$

[3] Arisostatins A and B

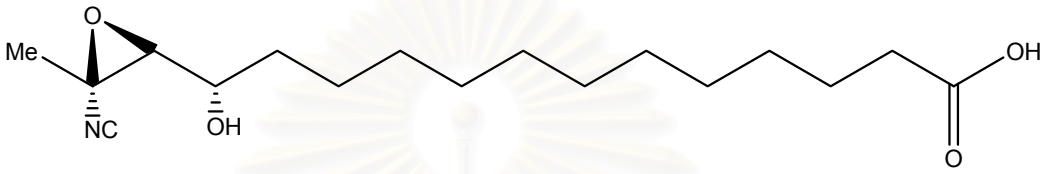


Staurosporine, R_1=H, R_2=H
Compound 2, R_1=OH, R_2=Me
Compound 3, R_1=OH, R_2=H

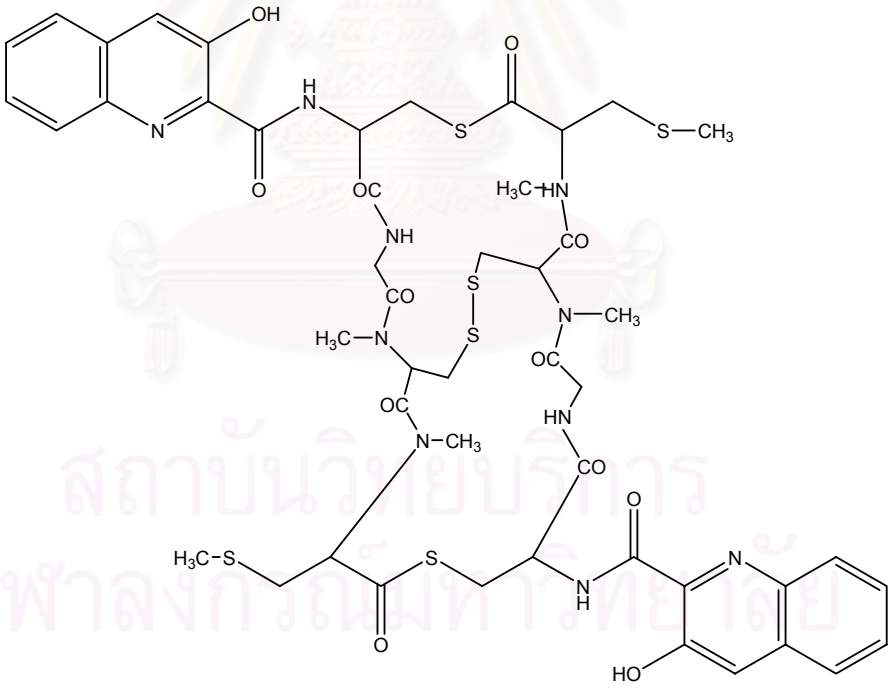
[4] 4'-*N*-Methyl-5'-hydroxystaurosporine (compound 2) and 5'-hydroxystaurosporine (compound 3)



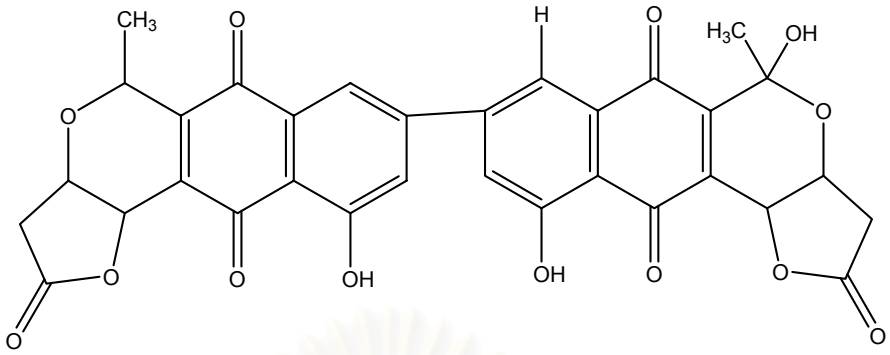
[5] Compound GTRI-02



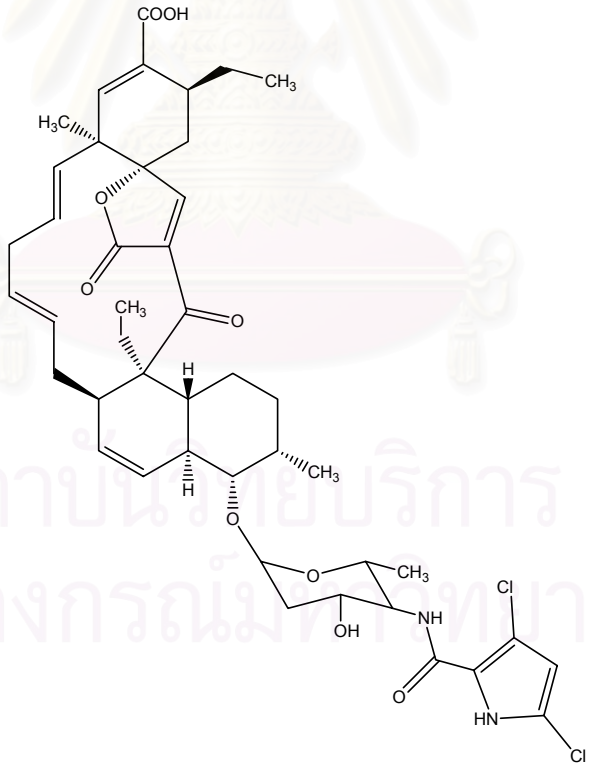
[6] Compound YM-47515



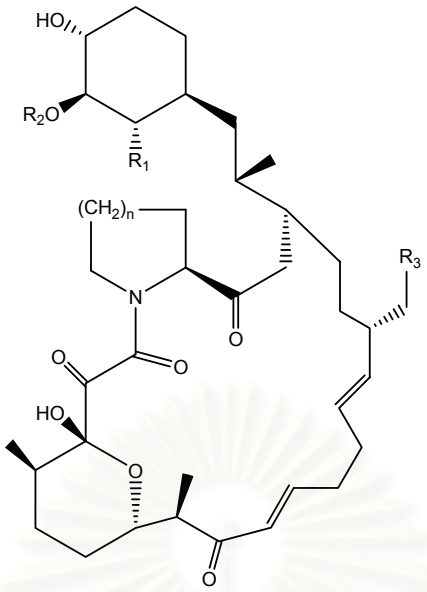
[7] Thiocoraline



[8] 1-Hydroxycrisamicin A

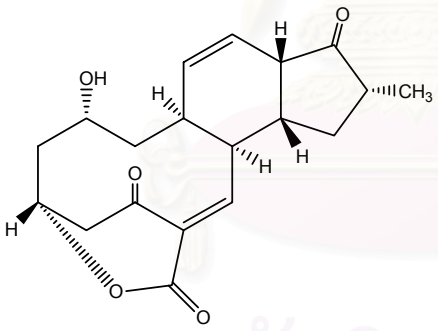


[9] Pyrrolosporin A

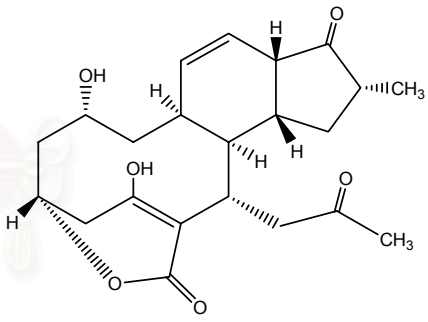


Antascomicin A R_1=H, R_2=H, R_3=H n=2
Antascomicin B R_1=OH, R_2=H, R_3=H n=2
Antascomicin C R_1=OH, R_2=CH_3, R_3=H n=2
Antascomicin D R_1=H, R_2=H, R_3=H n=1
Antascomicin E R_1=H, R_2=H, R_3=OH n=2

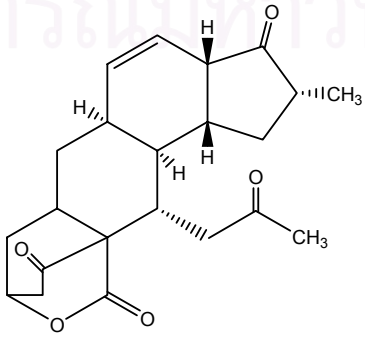
[10] Antascomicins



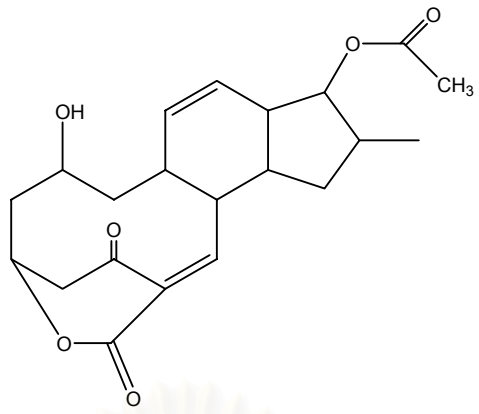
[11] Macquarimicin A



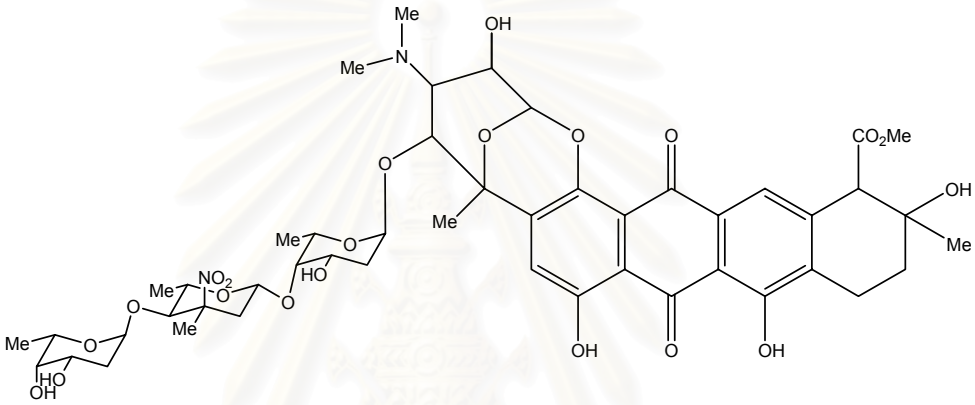
[12] Macquarimicin B



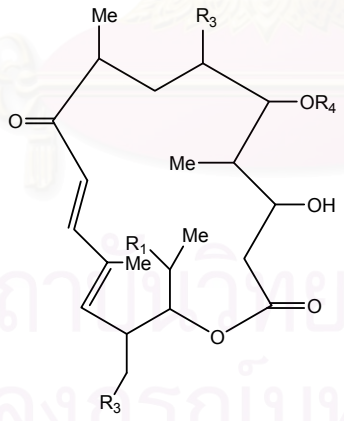
[13] Macquarimicin C



[14] Cochleamycin A

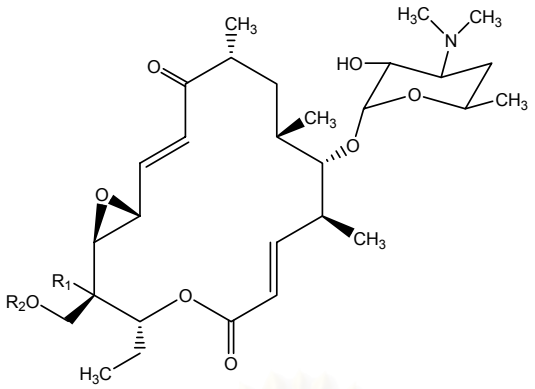


[15] Cororubicin



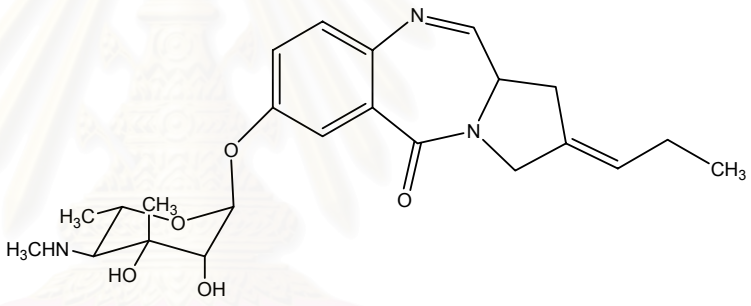
Compound 1 R_1=H, R_2=CH_2CH_3, R_3=OH, R_4=H
Compound 2 R_1=H, R_2=$CH(OH)CH_3$, R_3=OH, R_4=H
Compound 3 R_1=H, R_2=CH_3, R_3=OH, R_4=H
Compound 4 R_1=OH, R_2=CH_2CH_3, R_3=H, R_4=H
Compound 5 R_1=H, R_2=$CH(OH)CH_3$, R_3=OH, R_4=H
Compound 6 R_1=H, R_2=CH_2CHO, R_3=OH, R_4=Desosamine
Compound 7 R_1=H, R_2=CH_3, R_3=OH, R_4=Desosamine

[16] 16-Membered lactone compounds and izenamicins B_2(6) and B_3(7)

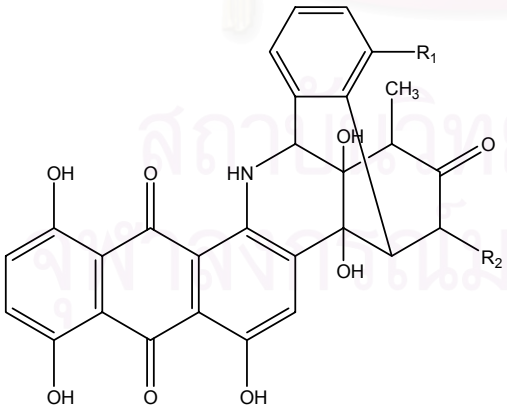


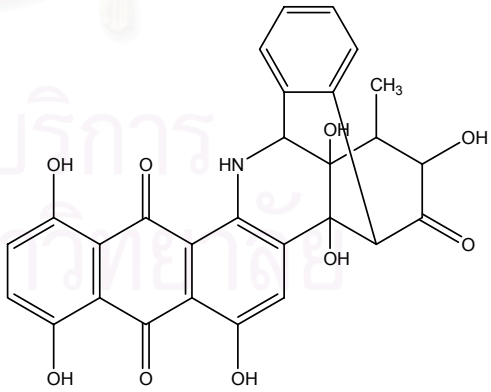
Mycinamicin I R_1=H, R_2=Mycinose
Mycinamicin II R_1=OH, R_2=Mycinose

[17] Mycinamicins I and II



[18] Sibanomicin

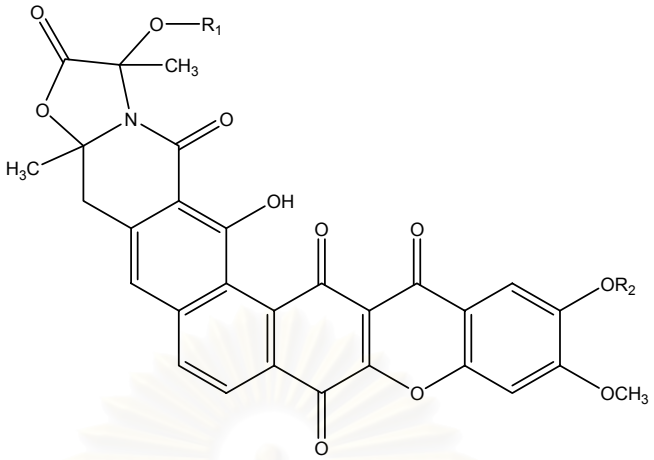




[19] Dynemicin O, R_1 = OH, R_2 = OCH_3

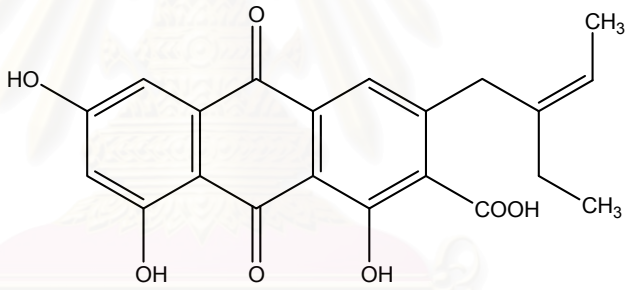
[20] Dynemicin P, R_1 = H, R_2 = OH

[21] Dynemicin Q(3)

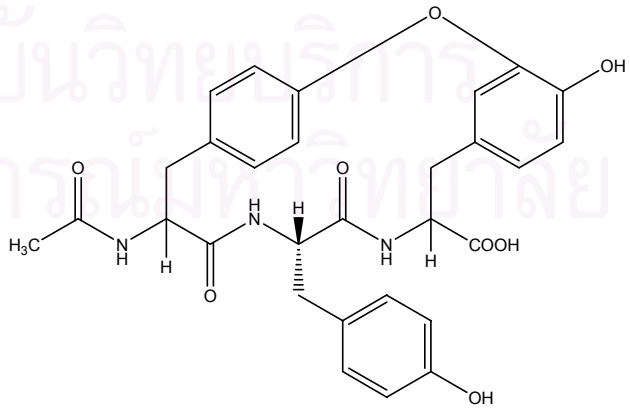


Citreamicin (1) R_1=$COCH_2CH(CH_3)_2$, R_2=CH_3
Citreamicin (2) R_1=$COCH(CH_3)_2$, R_2=CH_3
Citreamicin (3) R_1=$COCH_3$, R_2=CH_3
Citreamicin (4) R_1=$COCH_2CH(CH_3)_2$, R_2=H
Citreamicin (5) R_1=H , R_2=CH_3

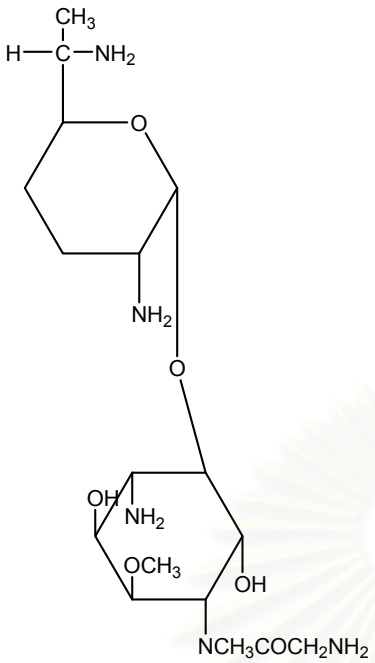
[22] Citreamicin α(1), β(2), γ(3),δ(4), η(5)



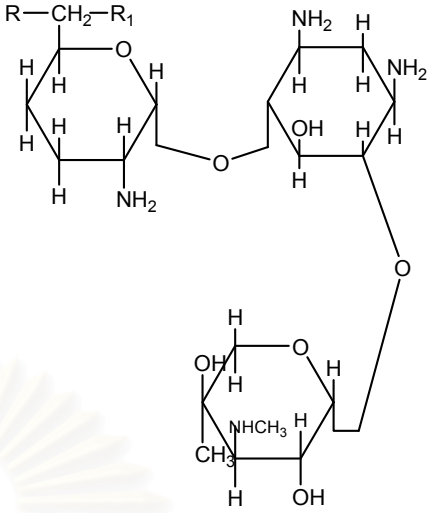
[23] Compound K-259-2



[24] Compound K-13

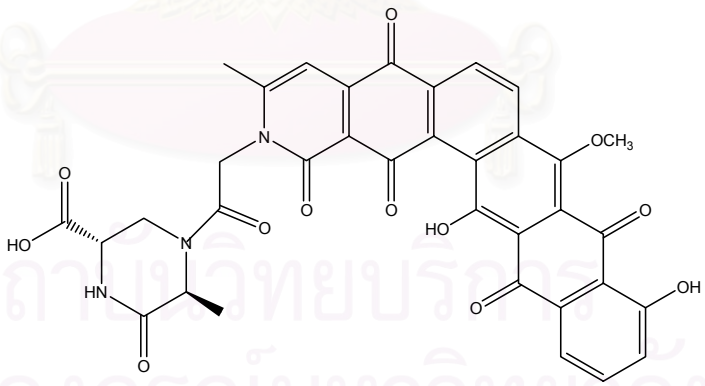


[25] Astromicin



Gentamicin C_{1a} R=H, R_1=NH_2
Gentamicin C_{1a} R=CH_3, R_1=NH_2
Gentamicin C_{1a} R=CH_3, R_1=$NHCH_3$
Sagamicin R=H, R_1=$NHCH_3$

[26] Sagamicin and Gentamicins C



[27] Echinospramicin

CHAPTER III

EXPERIMENTAL

1. Sample collection and isolation of *Micromonospora*

Soil samples were collected from peat swamp forest in the southern areas of Thailand, Trang Province, Patthalong Province, Yala Province and Narathiwat Province (Table 10). The soil sample (0.5 g) was suspended in a tested tube with 4.5 mL sterile distilled water and was heated in a water bath at 70 ^{o}C for 15 min. Then 0.5 mL of this suspension was transferred into 4.5 mL of sterile distilled water which was diluted to a tenfolds dilution series. Of the final dilution step (10^{-3}), aliquots of 100 μL were spread onto Starch-casein nitrate agar (SCA) plates (Appendix I) supplemented with antibiotics, nystatin, novobiocin, and tetracyclin (Brock *et al*., 1993), and the plates were incubated at 30 ^{o}C for 7-21 days. The moist, pale yellow, orange, red brown, brown, blue green, purple, and black colonies of *Micromonospora* species were picked up and streaked for purification on yeast extract-malt extract agar plates (YMA, ISP medium no. 2) (Appendix I) and were incubated at 30 ^{o}C for 7-21 days. A single colony was transferred into YMA slants and incubated at 30 ^{o}C for 14 days. The stock cultures were kept in cold room at 4 ^{o}C at the Department of Microbiology, Faculty of Pharmaceutical Sciences, Chulalongkorn University.

The pH of soil was measured by the following method: the soil samples were dried under room temperature for 1 week. Dried soil (1 g) was put into a test tube which then 2.5 mL of distilled water was added into and shaken for 30 sec. The soil solution was left for 30 min. Finally, the soil solution was gently shaken for 2 to 3 sec and measured pH value by pH glass electrode recorder (put the pH glass electrode in test tube at the 2/3 depth from the surface and hold it for more than 30 sec to stabilize electric potential).

2. Primary screening of isolates for antibiotic production

2.1 Primary screening

Micromonospora strains were streaked along the diameter of yeast extract-malt extract plate and grown for 14 day at 30 ^{o}C. The tested microorganisms were then

streaked out perpendicular to *Micromonospora* strains, as close as about 3 mm apart. Further incubation was at 30 ^{o}C for 1 day (Rhiems *et al*., 1998). The tested microorganisms were *Escherichia coli* ATCC 25922, *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 25923, *Micrococcus luteus* ATCC 9341, *Pseudomonas aeruginosa* ATCC 27853, and *Candida albicans* ATCC 10231.

3. Identification methods

Morphological, cultural, physiological, and biochemical properties of microorganisms were determined by the method of Shirling and Gottlieb (1966) and Arai (1975) along with several supplementary tests.

3.1 Morphological and cultural characteristics

The characteristics of *Micromonospora* were determined by using simple inclined coverslip technique (Williams and Cross, 1971). The simple inclined coverslip technique was used to determine the number of spores at the end of mature hyphae, the form of the spore chain and spore bearing hyphae and other special morphological observations. For the scanning electron microscopic examination, the selected strains of *Micromonospora* were grown on YMA plate (ISP medium no. 2) by crosshatch streak method (Shirling and Gottlieb, 1966) and incubated at 30 ^{o}C for 10-14 days. The culture was cut to some small cups (3-5 mm^3) and then were primarily fixed in 4 % solution of paraformaldehyde in 0.1 M phosphate buffer pH 7.2 at room temperature for 2 h. Subsequently, they were washed 3 times with buffer and were treated with secondary fixative, a 1 % solution of osmium tetroxide in buffer, and washed in the same process before drying. The specimens were dehydrated through a gradient ethanol series (35 %, 70 %, 95 %, and absolute ethanol). The specimens were fixed to a stub and coated with a thin film of gold by stutter coat. An electorn microscope was used to determine spore forming, spore surface, sporophore. The hyphae may be simple or complex, monopodial or sympodial. The microscopic structure of the substrate mycelium gives a clear picture of the morphology and reproductive structures of the organisms (Itoh *et al*., 1989).

Cultural characteristics were studied on the colors of mature substrate mycelium, spore, and diffusible pigment using crosshatch streak (Shirling and Gottlieb, 1966). The strains were cultivated on nine different agar media (Appendix I), yeast extract-malt extract agar (ISP medium no.2), Oatmeal agar (ISP medium no.3), Inorganic

salts-starch agar (ISP medium no.4), glycerol asparagine agar (ISPmedium no.5), peptone-yeast extract iron agar (ISPmedium no.6), tyrosine agar (ISP medium no.7), glucose asparagine agar, Czapek's sucrose agar, and nutrient agar and all were incubated at 30 ^{o}C for 7-14 days. The color of the reverse (under) side of the mass growth of substrate mycelium on nine media, the spore color, and the cultural characteristics were observed.

3.2 Biochemical and Physiological characteristics

3.2.1 Carbon utilization

Basal agar medium, ISP-9 (Shirling and Gottlieb, 1966) (Appendix I) supplement with 0.3 % Casamino acid was prepared and a carbon source was added to give concentration of approximately 1 %. After autoclave at 110 ^{o}C for 10 min, the mixture was agitated, and 25 mL of this mixture was poured into 9 cm petridish.

Carbon source and controls required for the test was repeated below :

No carbon source	(negative control)
D-glucose	(positive control)
D-mannitol	D-ribose
L-rhamnose	D-melibiose
Raffinose	Glycerol
Inositol	Salicin
Lactose	D-galactose
L-arabinose	Cellubiose
D-fructose	

To prepare the inoculum, cell culture was washed from yeast extract-malt extract agar slant with 5 mL of sterile distilled water, and then transferred into a sterile test tube. The suspension was centrifuged at 5000 rpm for 15 min. The supernatant was decanted, and the sediment was resuspended in sterile distilled water. The supernatant was washed twice. Sterile distilled water was added to wash sediment to restore the original volume, and the inoculum was used for carbon utilization tests.

The uninoculated plates were dried by leaving them at room temperature. A loopful of washed culture was inoculated on the agar surface by streaking straight across the dish. Plates were inoculated in duplicate and incubated at 28-30 ^{o}C for 10-14 days. Examination by comparing growth or a given carbon source with two controls, growth on basal medium alone, and growth on basal medium plus glucose was performed.

Results were recorded as follow:

1. Strongly positive utilization (++), when growth on tested carbon in basal medium is equal to or greater than growth on basal medium plus glucose.

2. Positive utilization (+), when growth on tested carbon is significantly better than on the basal medium without carbon, but somewhere less than on the basal medium plus glucose.

3. Utilization doubtful (+/-), when growth on tested carbon is only slightly better than on the basal medium without carbon and significantly less than on the basal medium plus glucose.

4. Utilization negative (-), when growth is similar to or less than growth on basal medium without carbon (Utilization is always recorded as negative if growth is not better than no carbon control).

3.2.2 Starch hydrolysis

All *Micromonospora* strains were streaked on the surface of inorganic salts-starch agar plate (ISP-4) (Shirling and Gottlieb, 1966, Appendix I) and incubated at 28-30 ^{o}C for 10 days. After inocubation was complete, Gram's iodine solution was poured on the surface of the agar plate. If starch hydrolysis was present, a dark blue color did not appear.

3.2.3 Gelatin liquefaction

All *Micromonospora* strains were inoculated into test tube of Bouillon gelatin broth (Arai, 1975, Appendix I) and incubated at 28-30 ^{o}C for 21 days. The inoculated tube was compared with uninoculate control when placed both tubes at 20 ^{o}C for 30 min. If the gelatin was hydrolyzed, it became liquid, not solidify.

3.2.4 Nitrate reduction

All *Micromonospora* strains were inoculated into Peptone KNO_3 broth (Arai, 1975, Appendix I) and incubated at 28-30 ^oC for 4-6 days. On the forth day, 1 mL of the culture was transferred into a test tube and two drops of the sulfanilic acid reagent, and then by three drops of *N,N*-dimethyl-1-naphthylamine solution were added. If nitrites were present, the mixture would become pink to red.

3.2.5 Milk coagulation and milk peptonization

Micromonospora strains were inoculated in tube of sterile 10 % skim milk (Appendix I) in distilled water and incubated at 28-30 ˚C for 7-14 days. If milk was peptonized, milk would be converted to clear solution. If milk was coagulated, milk would precipitate.

3.2.6 NaCl tolerance

All *Micromonospora* strains were streaked on YMA plates (ISP medium no. 2) to which NaCl was added to give concentrations of 0%, 1.5%, 3%, 4%, 5%, 6%, and 7%. The plates were incubated at 28-30 ^oC for 7-14 days. Observe maximum concentration for which the culture growth were recorded.

3.2.7 Temperature tolerance

All *Micromonospora* strains were streaked on YMA plates (ISP medium no. 2). The plates were incubated at 30, 40, and 45 ^oC, for 7-14 days. Observed maximum temperature for which the culture growth were recorded.

3.2.8 pH tolerance

All *Micromonospora* strains were streaked on YMA plates (ISP medium no. 2) that separately adjust the pH at 4, 4.5, 5, 6, 7, and 8. The plates were incubated at 30^oC for 7-14 days. Observed minimum pH for which the culture growth were recorded.

3.3 Chemotaxonomic studies

3.3.1 Cell wall acyl type

Dried cells (10 mg) were hydrolyzed with 100 µL of 6N HCL at 100 ˚C for 2 h. The hydrolyzed solution was then loaded into Dowex (CH_3COO^- form) column (5 cm in height). The column was eluted with 400 µL distilled water and twice of 1 mL

distilled water and 0.5N HCl. The final fraction was added with DON reagent (Appendix II) and 2N H_2SO_4 and measured O.D. at 530 nm. The sample that contained glycolylmuramic acid in the peptidoglycan, the value of $O.D._{.530}$ is higher than 10 nM.

3.3.2 Whole-cell sugar analysis

Dried cells (50 mg) were hydrolyzed with 1N H_2SO_4 at 100 ˚C for 2 h. The pH of hydrolyzed solution was adjusted with $Ba(OH)_2$ into pH 5.2-5.5. The solution was then centrifuged, and the supernatant was evaporated and added 400 µL of distilled water into the dried sample. The sample was deionized with Dowex 1 (OH^- form) and Dowex 50 (H^+ form) and filtered. Finally, the deionized sample was analized by HPLC.

3.3.3 Diaminopimelic acid analysis

Dried cells (10 mg) were hydrolyzed with 6N HCl at 100 ˚C for 18 h. The hydrolyzed solution was filtered and evaporated. The 400 µL of distilled water was added into dried sample. The solution was loaded into cellulose HPTLC plate no.5787 and developed with MeOH:H_2O:6N HCl:Pyridine (80:26:4:10). Finally, the cellulose HPTLC plate was sprayed with 0.5% ninhydrin in n-butanol for detection.

3.3.4 Amino acid composition of peptidoglycan

3.3.4.1 Purification of cell walls

Wet cells (2 g) were suspended with phosphate buffer (pH 7.2) and sonicated for 45 min. The unbroken cells were removed by centrifugation at 4,000 rpm for 10 min. The broken cells were collected by centrifugation of the supernatant at 10,000 rpm for 1 h and were treated with 25% SDS solution at 100 ˚C for 40 min. The cell wall samples were collected by centrifugation of the treated solution at 10,000 rpm for 1 h and treated with Pronase E solution for 2 h at 37 ˚C. The samples were then washed with phosphate buffer (pH 7.6) twice and treated with 5% TCA solution at 100 ˚C for 20 min. Finally, the samples were collected and washed with distilled water for three times, with ethanol and diethyl ether, and dried in the vacuum tray.

3.3.4.2 Hydrolysis and analysis of cell wall

Cell walls (1 mg) were hydrolyzed with 6N HCl at 100 ˚C for 18 h. The samples were filtered and dried by rotary evaporator. Finally, 100 µL of distilled water was added into the sample. For normal amino acid analysis: mix 10 µL of aqueous solution with 240 µL of 0.02 N HCL and detected with amino acid analyzer.

3.3.5 Cellular fatty acid analysis

3.3.5.1 Preparation of FAME sample

a. Saponification

Dried cells (40 mg) were put into screw-cap tube and added with 1 mL of reagent 1 (Appendix II), and this suspension was shaken well. The suspension was then heated at 100 ˚C for 30 min and cooled to room temperature in water.

b. Methylation

The reagent 2 (Appendix II) was added into the suspension and mixed for 5 to 10 sec with vortex mixer. The suspension was heated at 80 ˚C for 10 min and cooled to room temperature in water.

c. Extraction

The suspension was added with reagent 3 (Appendix II) and mixed for 10 min and then transferred the upper layer to another tube.

d. Base wash

The reagent 4 (Appendix II) was added into the suspention and mixed for 5 min, if it became to emulsion form, added the reagent 5 (Appendix II) into the suspension. The upper layer was transferred to vial for GC.

3.3.6 Polar lipid analysis

a. Extraction

Dried cells (150-300 mg) were added with 3 mL of MeOH:0.3%NaCl aq. (100:10) and 3 mL of petroleum ether and mixed them for 15 min. The lower layer was added with 1 mL of petroleum ether and mixed them for 2-5 min. 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 Chloroform:MeOH:water (90:100:30) and mixed for 1 h. The upper layer was transferred into another tube. The lower layer was extracted

again with Chloroform:MeOH:water (50:100:40) and the supernatant was transferred to the upper layer tube. The upper layer tube was added with 1.3 mL of chloroform and water. The final lower layer was dried with N_2 gas (<37 ˚C).

b. Analysis of polar lipid

The polar lipid fraction was dissolved with 60 µL of chloroform:MeOH (2:1) and applied to two-dimentional silica HPTLC no. 1.05633 developed with the follwing solvent systems.

The 1st solvent system:Chloroform:MeOH:Water (65:25:4)

The 2nd solvent system:Chloroform:Acetic acid:MeOH:Water (40:7.5:6:2)

c. Detection

1. Dittmer and Lester reagent (Appendix II). For all phospholipid (Blue spot)

2. Ninhydrin reagent (Appendix II). Heat at 110 ˚C for 10 min after spraying. For phosphatidylethanolamine (PE) and its derivatives (lyso-PE, OH-PE and methyl-PE)

3. Anisaldehyde reagent (Appendix II). Heat at 110 ˚C for 10 min after spraying. For glycolipids (green-yellow spot) and other lipid (blue spot)

4. Dragendroff's reagent (Appendix II). For choline-containing phospholipids (phosphatidyl choline)

3.3.7 Mycolic acid analysis

Dried cells (50-200 mg) were hydrolyzed with 10% KOH-MeOH at 100 ˚C for 2 h. The hydrolyzed suspension was added with 6 N HCl and extracted with n-hexane (or petroleum ether) twice. The upper layer was dried with N_2 gas.The dried sample was then added with benzene:MeOH:H_2SO_4 (10:20:1) and heated at 100 ˚C for 2 h (methylation step). After cooling, the suspension was added with water and n-hexane for the extraction. The upper layer was transferred to another tube and extracted again with n-hexane. The final upper layer was dried under N_2 gas and dissolved with a small with n-hexane and the applied to Silica gel TLC. The TLC plate was developed by n-hexane:diethylether (4:1) and detected the spot with I_2 vapor or H_2SO_4 reagen (heat at 110 ˚C for 10 min after spraying)

3.3.8 Menaquinone analysis

Dried cells (100-500 mg) were extracted with chloroform:MeOH (2:1) overnight. The suspension was then filtered and dried under rotary evaporator. The dried sample was dissolved with a small amount of acetone and applied onto a silica gel TLC (Merck no.1.05744). The applied TLC was then developed by 100% benzene and the band of menaquinone was detected by using a UV lamp (254 nm). The menaquinone band was scraped and dissolved with HPLC acetone. The suspension was filtered and dried it up with N_2 gas. The menaquinone sample was analyzed by HPLC.

3.3.9 Analysis of DNA base composition

a. Chromosomal DNA isolation and purification

Chromosomal DNA was isolated from cells grown in yeast extract-malt extract broth for 4-5 days according to the method of Tamaoka (1994) with minor modification. Cells were harvested and suspended in 10 mL of saline-EDTA buffer pH 8.0 (Appendix III). The cell suspension was inoculated with 20 mg of lysozyme at 37 ˚C for 30 min followed by the incubation period of 10 min at 50 ˚C with 1.0 mL of 10% SDS. The phenol extraction was then carried out by adding an equal volume of phenol:chloroform (1:1) (Appendix IV) to the sample for removal of protein and other debris. The upper layer of the mixture was collected after centrifugation at 10,000 rpm for 20 min. Chromosomal DNA was precipitated with two volumes of ice cold absolute ethanol. DNA was dissolved with 0.1x SSC (Appendix III) and treated with RNase A, RNase T_1 and proteinase K solution at 37 ˚C for 1 h for removal of RNA and protein, respectively. Chromosomal DNA was stored in 0.1x SSC at 4 ˚C.

b. DNA base composition analysis

The 10 µL of heated DNA (1 mg/mL) was hydrolyzed with 10 µL nuclease P_1 at 50 ˚C for 1 h and followed by the incubation period of 1 h at 37 ˚C with 10 µL of alkaline phosphatase. The hydrolyzed DNA was determined using the HPLC method of Tamaoka & Komagata (1984). An equimolar mixture of nucleotides (Yamasa Shoyu, Choshi, Japan) was used as the quantitative standard for analysis of DNA base composition.

3.4 DNA-DNA hybridization

Chromosomal DNA for DNA-DNA hybridization was purified by repeated phenol extraction to remove RNA and others. DNA was precipitated and dissolved in 0.1xSSC. Crude DNA was treated again with RNase A to crush completely RNA, and phenol:chloroform extraction was repeated twice. Then the DNA was precipitated by adding 2 volumes of cold absolute ethanol. The DNA was carefully washed with 70% and 95% ethanol, respectively and dried. DNA was dissolved with 0.1xSSC and stored at 4 ˚C.

The spectrophotometric method of DNA quantitative was used to determine both the concentration and relative purify of DNA in a solution. Two absorption spectra (A_{260} and A_{280}) were observed. The DNA was suitable for DNA-DNA hybridization if the ratio of A_{260}/A_{280} is 0.56-0.59.

3.4.1 DNA labeling probe with photobiotin

A 10 µL of DNA solution (1 mg/mL) and 15 µL of photobiotin solution (1 mg/mL) were mixed in an Eppendorf tube and then irradiated with sunlamp for 30 min on ice. After irradiation, free photobiotin was removed by 100 µL of 0.1 M Tris-HCl buffer pH 9.0, and 100 µL of n-butanol was added into biotinylated DNA solution. The solution was mixed and centrifuged at 12,000 rpm for 20 sec. The upper layer was removed. A 100 µL of n-butanol was added and mixed well. After centrifugation at 12,000 rpm for 20 sec, the upper layer was removed. The biotinylated DNA solution was boiled for 15 min and immediately cooled in ice. The solution was sonicated for 3 min and dissolved with 10 mL of hybridization solution (Appendix IV).

3.4.2 Photobiotin labeling DNA-DNA hybridization

The procedure of photobiotin labeling DNA-DNA hybridization was performed by the method described by Ezaki, Hashimoto, and Yabuuchi (1989). DNA (10 µg) of an unknown strain, type strain and reference DNA (calf thymus) were boiled for 10 min and immediately cooled in ice. Then, 500 µL of 2xPBS (Appendix III), 100 µL of 0.1 $MgCl_2$, and sterile distilled water were added to a total volume of 1 mL and mixed well. 100 µL of a heat denatured DNA solution was added to microdilution wells (Nunc-Immuno^{TM} Plate: MaxiSorp^{TM} surface) and fixed by incubation at 37 ˚C for 2 h. After incubation, the DNA solution was removed. 200

μL of a prehybridization solution (Appendix IV) was added to microdilution wells. The microdilution plate was incubated at 53-55 ˚C (hybridization temperature; Appendix V) for 1-2 h. The prehybridization solution was removed from the wells and replaced with 100 μL of a hybridization mixture containing biotinylated DNA. The microdilution plate was incubated at 53-55 ˚C (hybridization temperature) for 15-18 h. (For the fluorometric method: prehybridization solution (200 μL) was added to microdilution wells and incubated at 30 ˚C for 12-16 h. The prehybridization solution was removed from the wells and replaced with 100 μL of a hybridization mixture containing biotinylated DNA. The microdilution plate was incubated at 55 ˚C for 2 h).

3.4.3 Detection of biotin-containing hybrids

After hybridization, the microdilution wells were washed three times with 200 μL of 0.2xSSC buffer. Then 200 μL of solution I (Appendix IV) was added to microdilution wells and incubated at 30 ˚C for 10 min. Solution I was removed from the wells and replaced with 100 μL of solution II (Appendix IV). The microdilution plate was incubated at 37 ˚C for 30 min. After incubation, the microdilution plate was washed three times with 200 μL of PBS. 100 μL of solution III (Appendix IV) was added, and the plate was incubated at 37 ˚C for 10 min. The enzyme reaction was stopped with 100 μL of 2M H_2SO_4 (Appendix IV) (Verlander, 1992). The absorbance was measured at 450 nm with Microplate Reader (Microplate Manager^R 4.0 Bio-Rad Laboratories, Inc) and calculated for the value of percentage DNA homology (Appendix V).

3.5 16S rDNA analysis

3.5.1 16S rDNA amplification by PCR

The PCR was performed in a total volume of 50 μL containing 1 μL of DNA sample, 0.25 μL of *Taq* DNA polymerase, 5 μL of 10xpolymerase buffer, 4 μL of dNTP mixture, 2.5 μL of 10 μM forward and reverse primers (Appendix V) and 34.75 μL of Milliq water. A DNA Thermal Cycler (Gene Amp® PCR System 2400; Perkin Elmer) was used with a temperature profile of 3 min at 95 ˚C followed by 30 cycles of 30 sec at 95 ˚C (denaturing of DNA), 15 sec at 55 ˚C (primer annealimg), and 1 min at 72 ˚C (polymerization) and a final extension for 5 min at 72 ˚C. The PCR amplified products were analyzed by running 5 μL of the reaction mixture on a 1% agarose gel in Tris-acetate EDTA buffer (Appendix IV). Agarose gel was stained in

an ethidium bromide solution (0.5 mg/mL) and examined under UV-transilluminator (UVP Inc.) to visualize the amplified 16S rDNA band.

3.5.2 16S r DNA sequencing

The amplified 16S rDNA was used as templates for sequencing with big dye terminator sequencing Kit (Perkin Elmer) and analyzed by the ABI377 automated DNA sequencer (Perkin Elmer). The sequencing reaction for 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 (denaturing of DNA), 5 sec at 50 ˚C (primer annealimg), and 4 min at 60 ˚C (polymerization). Sequencing for each sample is carried out in both forward and reverse directions (Appendix V).

3.5.3 16S rDNA sequence analysis and phylogenetic tree construction

Homology search was performed using the standard BLAST sequence similarity searching program version 2.2.1 from the web server http://www.ncbi.nlm.nih.gov/BLAST/ against previously reported sequences at the GenBank/EMBL/DDBJ databases. The sequence was multiply aligned with selected sequences obtained from the three main databases by using the CLUSTAL W version 1.81. The alignment was manually verified and adjusted prior to the construction of a phylogenetic tree. The phylogenetic tree was constructed by using the neighbor-joining (Saitou and Nei, 1987) and maximum parsimony methods (Kluge and Farris, 1969) in the MEGA program version 2.1 (Kumar *et al.*; 2001). The confidence values of branches of the phylogenetic tree were determined using the bootstrap analyses (Felsenstein, 1985) based on 1000 resamplings. The values for sequence similarity among the closest strains were calculated manually after pairwise alignments obtained using the CLUSTAL X program (Thompson *et al.*, 1997). Gaps and ambiguous nucleotides were eliminated from the calculations.

4. Fermentation of the selected strain for antibiotic production

4.1 Seed medium

A loopful of *Micromonospora* sp. TT1-11 was inoculated into 50 mL of seed medium consisting of glucose (0.4 %), yeast extract (0.4 %), malt extract (1.0 %), pH 7.3 (in a 250 mL Erlenmeyer flask). The flask was incubated on a rotary shaker at 200 rpm at room temperature for 4 days.

4.2 Production medium

The vegetative seed (2 mL) from the Section 4.1 was transferred into a 500 mL of Erlenmeyer flask containing 200 mL of production medium which comprised glucose (0.4 %), yeast extract (0.4 %), malt extract (1.0 %), and $CaCO_3$ (0.1%), pH 7.3. The flask was incubated on a rotary shaker at 200 rpm at room temperature for 10 days.

5. Chromatographic techniques

5.1 Analytical thin-layer chromatography

Technique	:	One dimension ascending
Absorbent	:	Silica gel GF_{254} coated on an aluminium sheet (E. Merck)
Layer thickness	:	250 μm
Distance	:	5 cm
Temperature	:	Laboratory temperature (30-35 ^{o}C)
Detection	:	1. Visual detection under daylight 2. Visual detection under ultraviolet light at wavelengths of 254 and 365 nm. 3. Spraying with anisaldehyde reagent and heated until colors developed. 4. Visual detection in an iodine vapor

5.2 Column chromatography

5.2.1 Flash column chromatography

Adsorbent	:	Silica gel 60 (No. 7734), particle size 0.063-0.200 nm (70-230 mesh ASTM) (E. Merck)

Packing method : The adsorbent was suspended in an eluant. The adsorbent slurry was poured into a column and allowed to settle overnight.

Sample loading : The sample was dissolved in a small volume of the eluant and loaded on the top of a column.

Detection : Fractions were examined by TLC technique in the same manner as described in the Section 5.1.

5.2.2 Gel filtration chromatography

Adsorbent : Sephadex LH-20 (Amersham Biosciences)

Packing method : Sephadex LH-20 gel was suspended in an eluant and left standing overnight to swell prior to use, then poured into the column and allowed to settle.

Sample loading : The sample was dissolved in a small volume of an eluant and loaded on the top of a column.

Detection : Fractions were examined by TLC technique in the same manner as described in the Section 5.1.

6. Spectroscopy

6.1 Ultraviolet (UV) absorption spectroscopy

UV spectra (in MeOH) were obtained on a Milton Roy Spectronic 3000 Array spectrophotometer (Pharmaceutical Research Instrument Center, Faculty of Pharmaceutical Sciences, Chulalongkorn University).

6.2 Infrared (IR) absorption spectroscopy

IR spectra (KBr disc and film) were recorded on a Perkin Elmer FT-IR 1760X spectrometer (Scientific and Technological Research Equipment Center, Chulalongkorn University).

6.3 Mass spectrometry

Electrospray ionization-time of flight mass spectra (ESI-TOF MS) were recorded on a Micromass LCT mass spectrometer (The National Center for Genetic Engineering and Biotechnology (BIOTEC)). The mixture of MeCN:H_2O (50:50) containing 0.02% of formic acid was used as a solvent.

6.4 Proton and carbon nuclear magnetic resonance (^{1}H and ^{13}C NMR) spectroscopy

^{1}H and ^{13}C NMR, DEPT 90 and 135, HMQC, HMBC,NOESY, TOCSY, and ^{1}H-^{1}H COSY spectra were obtained from a Bruker AVANCE DPX-300 FT-NMR spectrometer, operated at 400 MHz for protons and 100 MHz for carbons. The chemical shifts (ppm) of the residual undeuterated solvents ($CDCl_3$ or DMSO-d_6) were used as reference. Proton detected heteronuclear correlations were measured using HMQC (optimized for $^1J_{HC}$ = 145 Hz) and HMBC (optimized for $^nJ_{HC}$ = 3, 4 and 8 Hz) pulse sequences.

6.5 Optical Rotations

Optical Rotations were measured on a Perkin Elmer Polarimeter 341 (Pharmaceutical Research Instrument Center, Faculty of Pharmaceutical Sciences, Chulalongkorn University).

7. Solvents

Throughout this work, all organic solvents were of commercial grade and were redistilled prior to use.

8. Biological activity

8.1 Antimicrobial activity

The antimicrobial activity of the isolated fractions and pure compounds was examined by the agar disc diffusion method (Lorian, 1980) against *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 25922, *Micrococcus luteus* ATCC 9341, *Pseudomonas aeruginosa* ATCC 27853, and *Candida albicans* ATCC 10231. All tested microorganisms were cultivated on Mueller-Hinton agar (MHA, Difco®) slant at 37 ^{o}C for 24 h, but the yeast strain was cultivated on Sabouraud's dextrose agar (SDA, Difco®) slant at 30 ^{o}C for 24 h. The cell cultures were washed from an agar surface and suspended with sterile normal

saline solution (NSS), and standardized to match a 0.5 turbidity standard of MacFarland No.1, providing approximately 1 x 10^8 CFU. Each of molten (25 mL) MHA and SDA was separated and poured into 9 cm diameter petri dish and allowed to solidify to form base layer. A loopful of each tested microorganisms was swabbed on the surface of MHA and SDA plates. All tested samples (1 mg/disc) were dissolved in the suitable solvent and then applied on a sterile paper dish for disc diffusion assay. These paper discs were left in a sterile petri dish until the solvent was completely dried. The dried paper discs were placed on the surface of the swabbed plates and incubated at 37 ^oC and 30 ^oC for bacterial strains and yeast strains, respectively, for 24 h. The diameters of inhibition zones were subsequently measured.

8.2 Antimalarial activity

Plasmodium falciparum (K1, multidrug resistant strain) was 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 which causes 50% reduction in parasite growth as indicated by the *in vitro* uptake of [^{3}H]-hypoxanthine by *P. falciparum*. An IC_{50} value of 1-3 ng/mL was observed for the standard sample, dihydroartemisinin, in the same test system.

8.3 Cytotoxic activity

Cytotoxic activity against a breast cancer cell line (BC), a human epidermoid carcinoma cell line of the nasopharynx (KB), and a Vero cell line (African monkey kidney cell line) was performed by the colorimetric method (Skehan *et al*., 1990).

8.4 Antimycobacterial activity

The antimycobacterial activity was assessed against *Mycobacterium tuberculosis* H37Ra with the Microplate Alamar Blue Assay (MABA) (Collins and Franzblau, 1997). The standard drugs, isoniazid, and kanamycin sulfate, used as reference compounds for the antimycobacterial assay, showed *MIC* values of 0.04-0.09 and 2.0-5.0 μg/mL, respectively in the test system.

9. Extraction and isolation of micromonosporin A from *Micromonospora* sp. TT1-11

The YM fermentation broth (20 L) of *Micromonospora* sp. TT1-11 was filtered through a Buchner funnel packed with Kieselguhr (diatomaceous earth or diatomite or bacillarieae earth). The filtrate was partitioned with ethyl acetate (30 L x 3). The ethyl acetate layer was collected and concentrated under reduced pressure at 45 ˚C to yield 3.9 g of the ethyl acetate extract (dark brown oily liquid) as shown in Scheme 2.

The ethyl acetate extract of *Micromonospora* sp. TT1-11 was examined for antimicrobial activity using the method described in the Section 8.1. The crude ethyl acetate extract of *Micromonospora* sp. TT1-11 showed antibacterial activity against *S. aureus*, and *B. subtilis* with the inhibition zones of 17 and 19 mm (1mg/6 mm diameter paper disc), respectively.

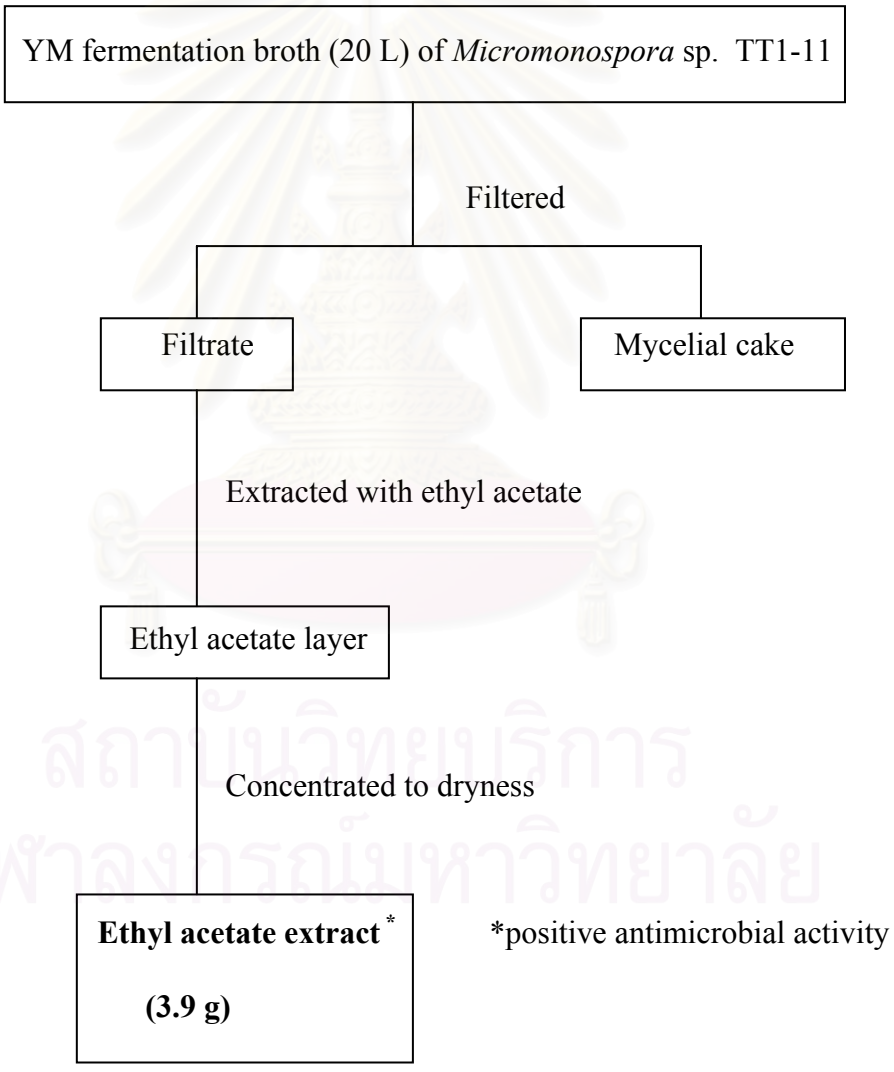
The crude ethyl acetate extract (3.9 g) of *Micromonospora* sp. TT1-11 was fractionated by quick column chromatography using silica gel as an adsorbent as shown in Scheme 3. The gradient of MeOH in $CHCl_3$ was used as a mobile phase to give five fractions (TT01-TT05) after combination of fractions which showed similar thin-layer chromatographic patterns (Si Gel GF, 10% MeOH in $CHCl_3$). Fractions (20 mL) were collected. Directed by bioassay using antimicrobial activity against *S. aureus* and *B. subtilis*, the fraction TT04 (1.5 g) was further purified by a silica gel flash column (3 cm inner diameter and 16 cm long), using an isocratic elution of $CHCl_3$:MeOH (9:1) to yield six fractions (TT041-TT046).

The TT043 fraction (328.4 mg) showed an antimicrobial activity and several black spots under UV light (254 nm), as well as small orange spots on the TLC (Si Gel, 10% MeOH in $CHCl_3$). The spot at R_f = 0.27 was the major product, and the fraction containing this compound was purified by a silica gel flash column (3 cm inner diameter and 16 cm long), using an isocratic elution of $CHCl_3$:MeOH (9:1) to yield micromonosporin A and fraction TT0431.

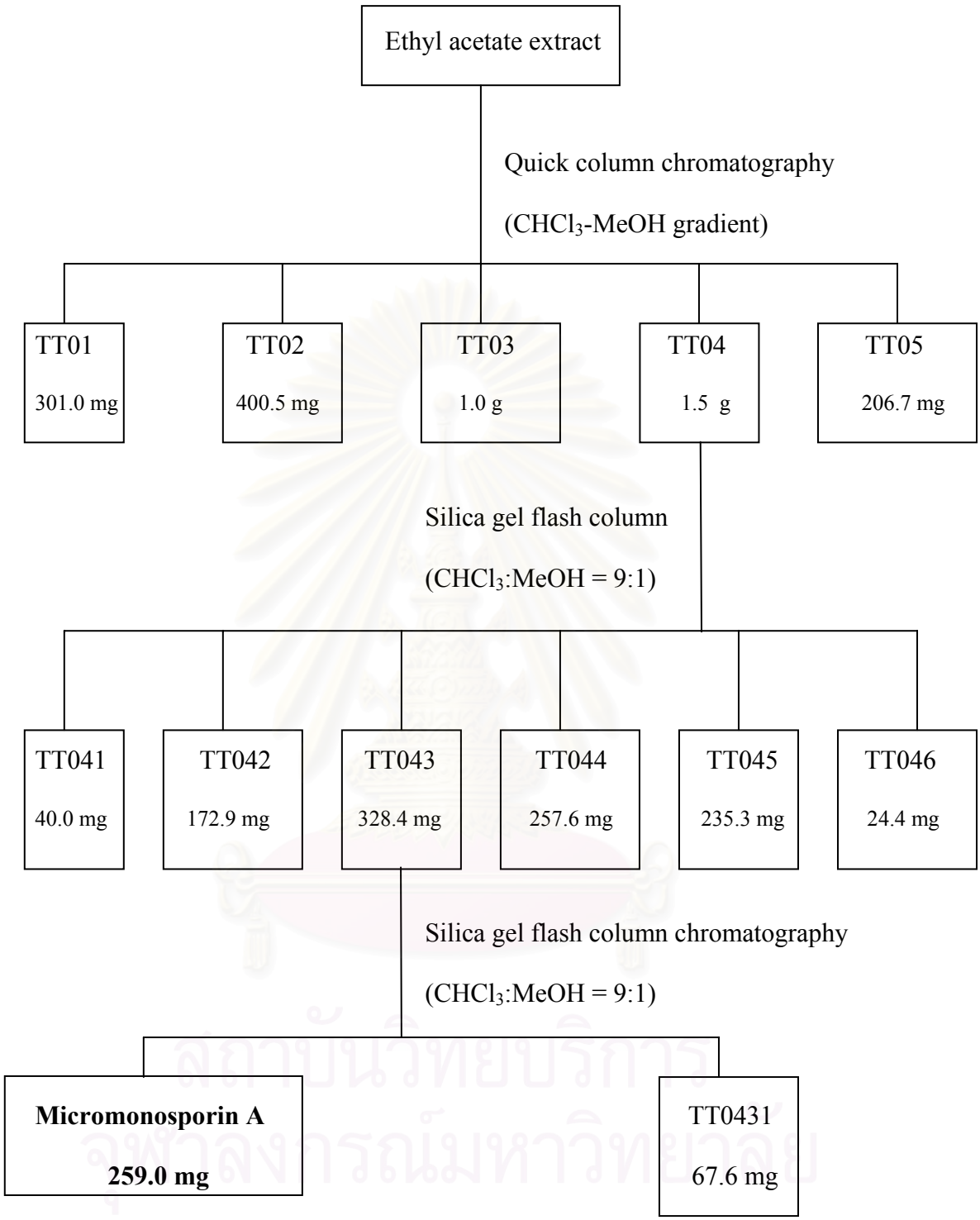
Micromonosporin A (259.0 mg) was obtained as an off-white powder. The compound showed R_f = 0.27 (Si Gel GF, $CHCl_3$:MeOH = 9:1) under UV light (254 nm) detection. Micromonosporin A was a major product in fermentation broth. Unfortunately, micromonosporin A was very unstable and spontaneously degraded into many unidentified products.

10. Pd/C Hydrogenation of micromonosporin A

Micromonosporin A (20 mg) was put into a 5-mL round bottom flask with a magnetic bar inside. Dimethyl formamide (DMF) (2 mL) and 10% Pd/C (1 mg) were added. The flask was sealed with a rubber septum and the air inside was replaced by hydrogen gas using a balloon. The mixture was stirred at room temperature for 2 h. The mixture was extracted with ethyl acetate and then purified by Sephadex LH-20 column chromatograhphy (eluted with MeOH) to yield 12 mg of compound 2 (9,11,13-trihydroxy-14,19,24-trimethyl-1-azacyclotetracosan-2-one).



Scheme 2. Extraction of the YM fermentation broth of *Micromonospora* sp.TT1-11.



Scheme 3. Isolation of the ethyl acetate extract of *Micromonospora* sp.TT1-11.

11. Physical and spectral data of isolated compounds

11.1 Micromonosporin A

Micromonosporin A was obtained as an off-white powder from ethyl acetate, soluble in dimethylsulphoxide (259 mg, 6.71% based on the ethyl acetate extract).

Melting point : 146 ˚C

$[\alpha]^{25}{}_{D}$: -248.4 (*c* = 1.0, MeOH)

ESI-TOFMS : $[M+Na]^+$ *m/z* (positive ion mode); Figure 47

UV : λ_{max} nm (log ε), in MeOH; Figure 45

291 (4.77)

IR : ν_{max} cm^{-1}, HBr disc; Figure 46

3430, 2930, 1631, 1555, 1436,1391, 1002

^{1}H NMR : δ ppm, 400 MHz, in DMSO-d_6; Figure 48-50, Table 23

^{13}C NMR : δ ppm, 100 MHz, in DMSO-d_6; Figure 51-52, Table 23

11.2 Compound 2

Compound 2 was obtained as colorless viscous liquid, soluble in chloroform.

$[\alpha]^{25}{}_{D}$: -7.73 (*c* = 0.1, MeOH)

ESI-TOFMS : $[M+Na]^+$ *m/z* (positive ion mode); Figure 69

UV : λ_{max} nm (log ε), in MeOH; Figure 67

203 (3.73)

IR : ν_{max} cm^{-1}, HBr disc; Figure 68

3298, 2928, 2856, 2353, 1642,1547, 1373

^{1}H NMR : δ ppm, 400 MHz, in $CDCl_3$; Figure 70

^{13}C NMR : δ ppm, 100 MHz, in $CDCl_3$; Figure 71

CHAPTER IV

RESULTS AND DISCUSSION

1. Bacterial strains and sources of isolation

A total of 52 actinomycete strains, which produced single non-motile spores were isolated from 17 samples of peat swamp forest soils in Trang, Pattaloong, Yala, and Narathiwat provinces, Thailand (Table 10). The pH of the soil samples ranges from 3.26–6.37. The isolates were cultivated on YMA for working and stock culture. The type strains of all validly described species of *Micromonospora* except for *M. gallica* were obtained from the Japan Collection of Microorganisms (JCM) as shown in Table 8.

2. Morphological and cultural characteristics of the isolates

The morphological characteristics (colonial appearance and scanning electron microscope pictures) of representative strains are shown in Figures 8-18. They produced well-developed and branched substrate hyphae on yeast extract-malt extract medium, but no aerial hyphae. Spores of them were borne singly on the substrate hyphae having approximately diameter of 0.5-0.6 μm. The spores were rough, nodular, and smooth on the surface and non-motile. The colors of the substrate mycelium were yellowish white to vivid orange and turned to brownish black to black after sporulation. The morphological characteristics of these isolates were consistent with their classification in the genus *Micromonospora* (Kawamoto, 1989). The cultural characteristics of all strains are summarized in Table 11.

3. Biochemical and Physiological Characteristics

On the basis of phenotypic characteristics (Table 12 and 13) including the results of intra-grouping by DNA-DNA hybridization experiment (Tables 14), fifty-two *Micromonospora* isolates could be classified into 11 groups. The members in each group showed the high values of DNA similarity (>70%) and shared many phenotypic properties. All isolates liquefied gelatin. They grew in 2-6% NaCl but almost strains could grow at 4-5% NaCl. Fifty isolates could hydrolyze skim milk and

Table 10. Sources, Locations, pH, and isolate numbers of strains from peat swamp forest soils.

Sources	Locations	pH	Isolate no.
Peat	Baan Toong Kong, Narathiwat	3.44	BTG1-1, BTG1-2, BTG1-4
Peat	Baan Toong Kong, Narathiwat	3.39	BTG2-3
Muck	Baan Toong Kong, Narathiwat	4.25	BTG3-2, BTG3-4, BTG3-6
Muck	Baan Toong Kong, Narathiwat	4.01	BTG4-1, BTG4-2
Muck	Baan Toong Kong, Narathiwat	4.45	BTG6-2
Soil	Baan Toong Kong, Narathiwat	4.05	BTG7-2, BTG7-3
Soil	Baan Toong Kong, Narathiwat	4.29	BTG10-2, BTG10-14
Soil	Laan Khway, Yhala	6.37	LK2-3, LK2-4, LK2-5, LK2-6, LK2-10, LK2-12, LK2-15
Muck	Laan Khway, Yhala	4.57	LK3-1
Muck	Laan Khway, Yhala	4.36	LK5-4, LK5-7, LK5-9
Peat	Laan Khway, Yhala	4.64	LK6-1, LK6-9, LK6-12
Muck	Kao Mhak, Patthaloong	4.92	KM1-2, KM1-3, KM1-5, KM1-6, KM1-7, KM1-9
Soil	Kao Mhak, Patthaloong	4.16	KM3-1, KM3-2, KM3-3, KM3-4, KM3-7, KM3-10 KM3-14, KM3-37
Peat	Kao Mhak, Patthaloong	3.41	KM4-4, KM4-7, KM4-11, KM4-24, KM4-25, KM4-29, KM4-33
Soil	Toong kai, Trang	5.53	TK2-2
Soil	Thatien, Patthaloong	4.30	TT2-4
Peat	Thatien, Patthaloong	3.26	TT1-11

Table 11. Cultural characteristics of the isolates.

Isolate no.	Media	Growth	Color of upper surface	Color of reverse surface	Soluble pigment
LK3-1	Y.M.	abundant	black	deep orange	-
	O.M.	abundant	dark brown	deep orange	-
	I.S.	abundant	brownish black	deep orange	-
	T.A.	poor	dark yellowish brown	brownish gold	-
	Gly.A.	poor	brownish gold	brownish gold	-
	Glu.A.	moderate	brownish black	light yellowish brown	-
	Cz.sucrose	abundant	dark brown	deep orange	-
	N.A.	abundant	dark grayish brown to black	light yellowish brown	-
	P.I.A.	abundant	brownish black	brownish gold	-
LK5-4	Y.M.	abundant	black	deep orange	-
	O.M.	abundant	dark grayish brown	deep orange	-
	I.S.	abundant	brownish black	deep orange	-
	T.A.	poor	brownish gold	brownish gold	-
	Gly.A.	poor	brownish gold	light yellowish brown	-
	Glu.A.	good	dark grayish brown	light yellowish brown	-
	Cz.sucrose	abundant	dark yellowish brown to black	deep orange	-
	N.A.	good	dark yellowish brown to black	light yellowish brown	-
	P.I.A.	abundant	brownish black	deep orange	-
KM1-3	Y.M.	abundant	brownish black	deep orange	-
	O.M.	abundant	dark brown to dark grayish brown	deep orange	-
	I.S.	abundant	black	deep orange	-
	T.A.	moderate	dark yellowish brown to black	yellowish brown	-
	Gly.A.	moderate	dark yellowish brown to black	vivid orange	-
	Glu.A.	abundant	dark yellowish brown to black	reddish brown	-
	Cz.sucrose	abundant	brownish black	vivid orange	-
	N.A.	good	dark brown to dark reddish brown	reddish brown	-
	P.I.A.	abundant	brownish black	deep orange	-

Table 11 (continued)

Isolate no.	Media	Growth	Color of upper surface	Color of reverse surface	Soluble pigment
	Y.M.	abundant	black	deep orange	-
	O.M.	abundant	brownish black	deep orange	-
	I.S.	abundant	brownish black	deep orange	-
	T.A.	moderate	yellowish brown	yellowish brown	-
LK5-9	Gly.A.	poor	brownish gold	light yellowish brown	-
	Glu.A.	good	dark yellowish brown to black	brownish gold	-
	Cz.sucrose	abundant	brownish black	deep orange	-
	N.A.	abundant	brownish black	yellowish brown	-
	P.I.A.	abundant	brownish black	deep orange	-
	Y.M.	abundant	brownish black	deep orange	-
	O.M.	abundant	dark brownish black	deep orange	-
	I.S.	abundant	brownish black	deep orange	-
	T.A.	moderate	dark yellowish brown	brownish gold	-
LK5-7	Gly.A.	poor	light yellowish brown	light yellowish brown	-
	Glu.A.	good	brownish black	brownish gold	-
	Cz.sucrose	abundant	black	vivid orange	-
	N.A.	moderate	brownish black to black	deep reddish orange	-
	P.I.A.	good	dark yellowish brown to black	deep orange	-
	Y.M.	abundant	dark brown to brownish black	deep orange	yellow
	O.M.	abundant	dark grayish brown	vivid orange	-
	I.S.	abundant	brownish black	vivid orange	-
	T.A.	poor	dark yellowish brown	yellowish brown	-
KM3-1	Gly.A.	moderate	brownish black	light yellowish brown	-
	Glu.A.	abundant	dark grayish brown	deep orange	-
	Cz.sucrose	abundant	dark grayish brown	deep orange	-
	N.A.	moderate	vivid orange	vivid orange	-
	P.I.A.	moderate	deep orange	deep orange	-

Table 11 (continued)

Isolate no.	Media	Growth	Color of upper surface	Color of reverse surface	Soluble pigment
KM3-2	Y.M.	abundant	dark brown to brownish black	deep orange	yellow
	O.M.	abundant	dark grayish brown	vivid orange	-
	I.S.	abundant	brownish black	vivid orange	-
	T.A.	poor	dark yellowish brown	yellowish brown	-
	Gly.A.	moderate	brownish black	light yellowish brown	-
	Glu.A.	good	dark brown	deep orange	-
	Cz.sucrose	abundant	brownish black	deep orange	-
	N.A.	moderate	yellowish brown	vivid orange	-
	P.I.A.	moderate	deep orange	deep orange	-
KM3-3	Y.M.	abundant	brownish black	deep orange	-
	O.M.	abundant	dark brownish black	deep orange	-
	I.S.	abundant	brownish black	deep orange	-
	T.A.	moderate	dark yellowish brown	brownish gold	-
	Gly.A.	moderate	light yellowish brown	light yellowish brown	-
	Glu.A.	poor	brownish black	brownish gold	-
	Cz.sucrose	abundant	black	vivid orange	-
	N.A.	abundant	brownish black to black	deep reddish orange	-
	P.I.A.	good	dark yellowish brown to black	deep orange	-
KM4-33	Y.M.	abundant	vivid orange	vivid orange	pale yellow
	O.M.	abundant	vivid orange	vivid orange	-
	I.S.	poor	vivid orange	vivid orange	-
	T.A.	moderate	pale yellow pink	pale yellow pink	-
	Gly.A.	poor	pale yellow pink	pale yellow pink	-
	Glu.A.	moderate	vivid orange	vivid orange	-
	Cz.sucrose	poor	vivid orange	vivid orange	-
	N.A.	moderate	vivid orange	vivid orange	-
	P.I.A.	good	vivid orange	vivid orange	-

Table 11 (continued)

Isolate no.	Media	Growth	Color of upper surface	Color of reverse surface	Soluble pigment
KM4-29	Y.M.	abundant	vivid orange	vivid orange	pale yellow
	O.M.	moderate	vivid orange	vivid orange	-
	I.S.	moderate	vivid orange	vivid orange	-
	T.A.	moderate	dull orange	dull orange	-
	Gly.A.	poor	pale yellow pink	pale yellow pink	-
	Glu.A.	moderate	vivid orange	vivid orange	-
	Cz.sucrose	moderate	vivid orange	vivid orange	-
	N.A.	moderate	vivid orange	vivid orange	-
	P.I.A.	good	vivid orange	vivid orange	-
KM4-24	Y.M.	abundant	deep orange	deep orange	pale yellow
	O.M.	good	vivid orange	vivid orange	-
	I.S.	moderate	vivid orange	vivid orange	-
	T.A.	poor	vivid orange	vivid orange	-
	Gly.A.	poor	vivid orange	vivid orange	-
	Glu.A.	moderate	strong reddish orange	strong reddish orange	-
	Cz.sucrose	moderate	vivid orange	vivid orange	-
	N.A.	moderate	vivid orange	vivid orange	-
	P.I.A.	abundant	vivid orange	vivid orange	-
KM4-25	Y.M.	abundant	deep orange	deep orange	pale yellow
	O.M.	good	vivid orange	vivid orange	-
	I.S.	moderate	vivid orange	vivid orange	-
	T.A.	poor	vivid orange	vivid orange	-
	Gly.A.	poor	vivid orange	vivid orange	-
	Glu.A.	moderate	strong reddish orange	strong reddish orange	-
	Cz.sucrose	moderate	vivid orange	vivid orange	-
	N.A.	moderate	vivid orange	vivid orange	-
	P.I.A.	abundant	vivid orange	vivid orange	-

Table 11 (continued)

Isolate no.	Media	Growth	Color of upper surface	Color of reverse surface	Soluble pigment
KM3-4	Y.M.	abundant	dark yellowish brown	light yellowish brown	-
	O.M.	abundant	brownish black	deep orange	-
	I.S.	abundant	brownish black	vivid orange	-
	T.A.	poor	dark yellowish brown	yellowish brown	-
	Gly.A.	moderate	yellowish brown	light yellowish brown	-
	Glu.A.	good	brownish black	yellowish brown	-
	Cz.sucrose	moderate	dark yellowish brown to black	deep orange	-
	N.A.	good	brownish black to black	deep orange	-
	P.I.A.	abundant	deep orange	deep orange	-
KM3-7	Y.M.	abundant	dark yellowish brown	light yellowish brown	-
	O.M.	good	brownish black to black	deep orange	-
	I.S.	abundant	brownish black to black	vivid orange	-
	T.A.	poor	dark yellowish brown	yellowish brown	-
	Gly.A.	moderate	yellowish brown	light yellowish brown	-
	Glu.A.	good	brownish black	yellowish brown	-
	Cz.sucrose	moderate	dark yellowish brown to black	deep orange	-
	N.A.	good	brownish black to black	deep orange	-
	P.I.A.	abundant	deep orange	deep orange	-
KM3-10	Y.M.	abundant	dark yellowish brown	light yellowish brown	-
	O.M.	abundant	brownish black to black	deep orange	-
	I.S.	abundant	brownish black	vivid orange	-
	T.A.	poor	dark yellowish brown	yellowish brown	-
	Gly.A.	moderate	yellowish brown	light yellowish brown	-
	Glu.A.	good	brownish black	yellowish brown	-
	Cz.sucrose	moderate	dark yellowish brown to black	vivid orange	-
	N.A.	good	brownish black to black	deep orange	-
	P.I.A.	good	deep orange	deep orange	-

Table 11 (continued)

Isolate no.	Media	Growth	Color of upper surface	Color of reverse surface	Soluble pigment
KM3-14	Y.M.	abundant	dark yellowish brown	light yellowish brown	-
	O.M.	good	brownish black to black	deep orange	-
	I.S.	good	brownish black to black	vivid orange	-
	T.A.	poor	dark yellowish brown	yellowish brown	-
	Gly.A.	moderate	yellowish brown	light yellowish brown	-
	Glu.A.	good	brownish black	yellowish brown	-
	Cz.sucrose	moderate	dark yellowish brown to black	deep orange	-
	N.A.	good	brownish black to black	deep orange	-
	P.I.A.	abundant	deep orange	deep orange	-
KM3-37	Y.M.	abundant	dark yellowish brown	light yellowish brown	-
	O.M.	good	brownish black to black	deep orange	-
	I.S.	abundant	brownish black	vivid orange	-
	T.A.	poor	dark yellowish brown	yellowish brown	-
	Gly.A.	moderate	yellowish brown	light yellowish brown	-
	Glu.A.	good	brownish black	yellowish brown	-
	Cz.sucrose	moderate	dark yellowish brown to black	vivid orange	-
	N.A.	good	brownish black to black	deep orange	-
	P.I.A.	abundant	deep orange	deep orange	-
BTG7-2	Y.M.	abundant	black	deep orange	-
	O.M.	abundant	vivid orange	vivid orange	-
	I.S.	moderate	strong yellowish orange	strong yellowish orange	-
	T.A.	moderate	dull orange	dull orange	-
	Gly.A.	moderate	dull orange	dull orange	-
	Glu.A.	moderate	strong yellowish orange	strong yellowish orange	-
	Cz.sucrose	moderate	pale orange	pale orange	-
	N.A.	good	dark brown	deep orange	-
	P.I.A.	abundant	dark brown to brownish black	deep orange	-

Table 11 (continued)

Isolate no.	Media	Growth	Color of upper surface	Color of reverse surface	Soluble pigment
BTG3-2	Y.M.	abundant	brownish black to black	deep orange	-
	O.M.	good	vivid orange	vivid orange	-
	I.S.	moderate	strong yellowish orange	strong yellowish orange	-
	T.A.	moderate	dull orange	dull orange	-
	Gly.A.	moderate	dull orange	dull orange	-
	Glu.A.	moderate	strong yellowish orange	strong yellowish orange	-
	Cz.sucrose	moderate	pale orange	pale orange	-
	N.A.	good	dark brown	deep orange	-
	P.I.A.	abundant	dark brown to brownish black	deep orange	-
BTG10-2	Y.M.	moderate	vivid orange	vivid orange	pale yellow
	O.M.	good	vivid orange	vivid orange	-
	I.S.	poor	brownish black	yellowish pink	-
	T.A.	moderate	black	dull orange	-
	Gly.A.	moderate	dark yellowish brown	pale orange	-
	Glu.A.	abundant	dark grayish brown	deep orange	-
	Cz.sucrose	moderate	vivid orange	vivid orange	-
	N.A.	poor	vivid orange	vivid orange	pale yellow
	P.I.A.	abundant	vivid orange	vivid orange	-
BTG10-14	Y.M.	good	dark grayish brown	vivid orange	pale yellow
	O.M.	good	deep orange	deep orange	-
	I.S.	moderate	vivid orange	vivid orange	-
	T.A.	moderate	dark yellowish brown	dull orange	-
	Gly.A.	moderate	yellowish brown	yellowish pink	-
	Glu.A.	good	dark brown	deep orange	-
	Cz.sucrose	poor	vivid orange	vivid orange	-
	N.A.	moderate	vivid orange	vivid orange	pale yellow
	P.I.A.	moderate	vivid orange	vivid orange	-

Table 11 (continued)

Isolate no.	Media	Growth	Color of upper surface	Color of reverse surface	Soluble pigment
BTG7-3	Y.M.	abundant	dark grayish brown	vivid orange	pale yellow
	O.M.	good	vivid orange	vivid orange	-
	I.S.	moderate	vivid orange	vivid orange	-
	T.A.	moderate	dark brown	strong yellowish orange	-
	Gly.A.	moderate	dull orange	dull orange	-
	Glu.A.	moderate	black	deep orange	-
	Cz.sucrose	moderate	gold	gold	-
	N.A.	moderate	vivid orange	vivid orange	pale yellow
	P.I.A.	moderate	vivid orange	vivid orange	-
LK2-4	Y.M.	abundant	vivid orange	vivid orange	pale yellow
	O.M.	abundant	vivid reddish orange	vivid reddish orange	-
	I.S.	moderate	vivid orange	vivid orange	-
	T.A.	moderate	gold	gold	-
	Gly.A.	moderate	strong yellowish orange	strong yellowish orange	-
	Glu.A.	moderate	vivid orange	vivid orange	-
	Cz.sucrose	poor	vivid orange	vivid orange	-
	N.A.	moderate	vivid orange	vivid orange	pale yellow
	P.I.A.	moderate	vivid orange	vivid orange	-
TT2-4	Y.M.	abundant	vivid orange	vivid orange	yellow
	O.M.	abundant	vivid orange	vivid orange	-
	I.S.	abundant	dark brown	vivid orange	-
	T.A.	moderate	yellowish brown to dark brown	yellowish brown to dark brown	-
	Gly.A.	poor	light yellowish brown	light yellowish brown	-
	Glu.A.	moderate	light yellowish brown	light yellowish brown	-
	Cz.sucrose	abundant	vivid orange	vivid orange	-
	N.A.	good	vivid orange	vivid orange	-
	P.I.A.	abundant	deep orange	deep orange	-

Table 11 (continued)

Isolate no.	Media	Growth	Color of upper surface	Color of reverse surface	Soluble pigment
KM4-4	Y.M.	abundant	yellowish brown to dark brown	vivid orange	yellow
	O.M.	good	yellowish brown to dark brown	vivid orange	-
	I.S.	good	dark brown	vivid orange	-
	T.A.	moderate	yellowish brown to dark brown	yellowish brown to dark brown	-
	Gly.A.	poor	light yellowish brown	light yellowish brown	-
	Glu.A.	moderate	light yellowish brown	light yellowish brown	-
	Cz.sucrose	abundant	vivid orange	vivid orange	-
	N.A.	good	vivid orange	vivid orange	-
	P.I.A.	abundant	deep orange	deep orange	-
KM4-11	Y.M.	abundant	yellowish brown to dark brown	vivid orange	yellow
	O.M.	abundant	vivid orange	vivid orange	-
	I.S.	abundant	dark brown	vivid orange	-
	T.A.	moderate	yellowish brown to dark brown	yellowish brown to dark brown	-
	Gly.A.	poor	light yellowish brown	light yellowish brown	-
	Glu.A.	moderate	light yellowish brown	light yellowish brown	-
	Cz.sucrose	abundant	vivid orange	vivid orange	-
	N.A.	good	yellowish brown to dark brown	vivid orange	-
	P.I.A.	abundant	deep orange	deep orange	-
BTG1-4	Y.M.	abundant	grayish black	grayish yellow	pale yellow
	O.M.	abundant	gold	gold	-
	I.S.	poor	yellowish white	yellowish white	-
	T.A.	poor	yellowish white	yellowish white	-
	Gly.A.	poor	yellowish white	yellowish white	-
	Glu.A.	poor	yellowish white	yellowish white	-
	Cz.sucrose	poor	yellowish white	yellowish white	-
	N.A.	good	dark yellowish brown	gold	pale yellow
	P.I.A.	abundant	light yellowish brown	pale yellowish orange	-

Table 11 (continued)

Isolate no.	Media	Growth	Color of upper surface	Color of reverse surface	Soluble pigment
BTG4-1	Y.M.	abundant	black	grayish yellow	pale yellow
	O.M.	abundant	dull orange	dull orange	-
	I.S.	poor	yellowish white	yellowish white	-
	T.A.	poor	yellowish white	yellowish white	-
	Gly.A.	poor	yellowish white	yellowish white	-
	Glu.A.	poor	yellowish white	yellowish white	-
	Cz.sucrose	poor	yellowish white	yellowish white	-
	N.A.	good	dark yellowish brown	yellowish white	pale yellow
	P.I.A.	abundant	light yellowish brown	pale yellowish orange	-
LK2-12	Y.M.	abundant	light yellowish brown	light yellowish brown	pale yellow
	O.M.	abundant	pale orange	pale orange	-
	I.S.	poor	yellowish white	yellowish white	-
	T.A.	poor	yellowish white	yellowish white	-
	Gly.A.	poor	yellowish white	yellowish white	-
	Glu.A.	poor	yellowish white	yellowish white	-
	Cz.sucrose	poor	yellowish white	yellowish white	-
	N.A.	good	pale orange	pale orange	pale yellow
	P.I.A.	abundant	light yellowish brown	pale yellowish orange	-
KM1-6	Y.M.	abundant	grayish black	grayish yellow	pale yellow
	O.M.	abundant	vivid orange	vivid orange	-
	I.S.	poor	yellowish white	yellowish white	-
	T.A.	poor	yellowish white	yellowish white	-
	Gly.A.	poor	yellowish white	yellowish white	-
	Glu.A.	poor	yellowish white	yellowish white	-
	Cz.sucrose	poor	yellowish white	yellowish white	-
	N.A.	good	dark yellowish brown	gold	pale yellow
	P.I.A.	abundant	light yellowish brown	pale yellowish orange	-

Table 11 (continued)

Isolate no.	Media	Growth	Color of upper surface	Color of reverse surface	Soluble pigment
KM1-5	Y.M.	abundant	dull orange	dull orange	pale yellow
	O.M.	abundant	gold	gold	-
	I.S.	poor	yellowish white	yellowish white	-
	T.A.	poor	yellowish white	yellowish white	-
	Gly.A.	poor	yellowish white	yellowish white	-
	Glu.A.	poor	yellowish white	yellowish white	-
	Cz.sucrose	poor	yellowish white	yellowish white	-
	N.A.	good	vivid yellow orange	vivid yellow orange	pale yellow
	P.I.A.	good	strong yellowish orange	strong yellowish orange	-
KM1-2	Y.M.	abundant	brownish black	dull orange	pale yellow
	O.M.	good	vivid orange	vivid orange	-
	I.S.	poor	yellowish white	yellowish white	-
	T.A.	poor	yellowish white	yellowish white	-
	Gly.A.	poor	yellowish white	yellowish white	-
	Glu.A.	poor	yellowish white	yellowish white	-
	Cz.sucrose	poor	yellowish white	yellowish white	-
	N.A.	moderate	vivid yellow orange	vivid yellow orange	pale yellow
	P.I.A.	good	strong yellowish orange	strong yellowish orange	-
KM1-9	Y.M.	good	dull orange	dull orange	pale yellow
	O.M.	good	vivid orange	vivid orange	-
	I.S.	poor	yellowish white	yellowish white	-
	T.A.	poor	yellowish white	yellowish white	-
	Gly.A.	poor	yellowish white	yellowish white	-
	Glu.A.	poor	yellowish white	yellowish white	-
	Cz.sucrose	poor	yellowish white	yellowish white	-
	N.A.	good	vivid yellow orange	vivid yellow orange	pale yellow
	P.I.A.	good	strong yellowish orange	strong yellowish orange	-

Table 11 (continued)

Isolate no.	Media	Growth	Color of upper surface	Color of reverse surface	Soluble pigment
BTG1-1	Y.M.	abundant	black	dull orange	pale yellow
	O.M.	abundant	gold	gold	-
	I.S.	poor	yellowish white	yellowish white	-
	T.A.	poor	yellowish white	yellowish white	-
	Gly.A.	poor	yellowish white	yellowish white	-
	Glu.A.	poor	yellowish white	yellowish white	-
	Cz.sucrose	poor	yellowish white	yellowish white	-
	N.A.	moderate	pale vivid orange	pale vivid orange	pale yellow
	P.I.A.	abundant	dark grayish brown	brownish gold	-
LK2-3	Y.M.	abundant	black	dull orange	pale yellow
	O.M.	good	gold	gold	-
	I.S.	poor	yellowish white	yellowish white	-
	T.A.	poor	yellowish white	yellowish white	-
	Gly.A.	poor	yellowish white	yellowish white	-
	Glu.A.	poor	yellowish white	yellowish white	-
	Cz.sucrose	poor	pinkish white	pinkish white	pale yellow
	N.A.	moderate	vivid orange	vivid orange	-
	P.I.A.	abundant	dark grayish brown	brownish gold	-
KM4-7	Y.M.	good	black	dull orange	pale yellow
	O.M.	good	vivid orange	vivid orange	-
	I.S.	poor	yellowish white	yellowish white	-
	T.A.	poor	yellowish white	yellowish white	-
	Gly.A.	poor	yellowish white	yellowish white	-
	Glu.A.	poor	yellowish white	yellowish white	-
	Cz.sucrose	poor	yellowish white	yellowish white	-
	N.A.	moderate	pale vivid orange	pale vivid orange	pale yellow
	P.I.A.	abundant	dark grayish brown	brownish gold	-

Table 11 (continued)

Isolate no.	Media	Growth	Color of upper surface	Color of reverse surface	Soluble pigment
LK2-5	Y.M.	abundant	grayish black	light yellowish brown	pale yellow
	O.M.	abundant	dull orange	dull orange	-
	I.S.	poor	yellowish white	yellowish white	-
	T.A.	moderate	yellowish white	yellowish white	-
	Gly.A.	poor	yellowish white	yellowish white	-
	Glu.A.	moderate	yellowish white	yellowish white	-
	Cz.sucrose	poor	yellowish white	yellowish white	-
	N.A.	good	dark yellowish brown	light yellowish brown	-
	P.I.A.	good	grayish black	strong yellowish orange	-
LK2-10	Y.M.	good	dark grayish yellow	grayish yellow	pale yellow
	O.M.	abundant	dull orange	dull orange	-
	I.S.	poor	yellowish white	yellowish white	-
	T.A.	poor	yellowish white	yellowish white	-
	Gly.A.	poor	yellowish white	yellowish white	-
	Glu.A.	poor	yellowish white	yellowish white	-
	Cz.sucrose	poor	yellowish white	yellowish white	-
	N.A.	moderate	dark yellowish brown	light yellowish brown	-
	P.I.A.	good	dark yellowish brown	light yellowish brown	-
LK6-1	Y.M.	abundant	dark grayish brown	deep orange	pale yellow
	O.M.	abundant	vivid orange	vivid orange	-
	I.S.	poor	gold	gold	-
	T.A.	poor	yellowish white	yellowish white	-
	Gly.A.	moderate	dull orange	dull orange	-
	Glu.A.	poor	orange	orange	-
	Cz.sucrose	poor	pale orange	pale orange	-
	N.A.	moderate	vivid orange	vivid orange	-
	P.I.A.	good	vivid orange	vivid orange	-

Table 11 (continued)

Isolate no.	Media	Growth	Color of upper surface	Color of reverse surface	Soluble pigment
LK6-12	Y.M.	good	dark grayish brown	deep orange	pale yellow
	O.M.	good	vivid orange	vivid orange	-
	I.S.	poor	pale orange	pale orange	-
	T.A.	moderate	light yellowish brown	light yellowish brown	-
	Gly.A.	poor	yellowish white	yellowish white	-
	Glu.A.	poor	pale orange	pale orange	-
	Cz.sucrose	poor	pale orange	pale orange	-
	N.A.	moderate	brownish black	deep reddish orange	-
	P.I.A.	good	vivid orange	vivid orange	-
TK2-2	Y.M.	good	vivid orange	vivid orange	yellowish orange
	O.M.	good	vivid orange	vivid orange	-
	I.S.	poor	pale orange	pale orange	-
	T.A.	poor	vivid orange	vivid orange	-
	Gly.A.	poor	pale orange	pale orange	-
	Glu.A.	poor	strong yellowish orange	strong yellowish orange	-
	Cz.sucrose	poor	pale orange	pale orange	-
	N.A.	moderate	strong reddish orange	strong reddish orange	-
	P.I.A.	good	vivid orange	vivid orange	-
KM1-7	Y.M.	moderate	strong reddish orange	strong reddish orange	yellowish orange
	O.M.	moderate	vivid orange	vivid orange	-
	I.S.	poor	pale orange	pale orange	-
	T.A.	poor	vivid orange	vivid orange	-
	Gly.A.	poor	pale orange	pale orange	-
	Glu.A.	moderate	strong yellowish orange	strong yellowish orange	-
	Cz.sucrose	poor	pale orange	pale orange	-
	N.A.	good	strong reddish orange	strong reddish orange	-
	P.I.A.	good	vivid orange	vivid orange	-

Table 11 (continued)

Isolate no.	Media	Growth	Color of upper surface	Color of reverse surface	Soluble pigment
BTG3-4	Y.M.	abundant	black	grayish yellow	pale yellow
	O.M.	abundant	gold	gold	-
	I.S.	poor	yellowish white	yellowish white	-
	T.A.	poor	yellowish white	yellowish white	-
	Gly.A.	poor	yellowish white	yellowish white	-
	Glu.A.	moderate	yellowish white	yellowish white	-
	Cz.sucrose	poor	yellowish white	yellowish white	-
	N.A.	good	pale orange	pale orange	-
	P.I.A.	abundant	light yellowish brown	light yellowish brown	-
LK6-9	Y.M.	abundant	grayish black	dull orange	pale yellow
	O.M.	abundant	dull orange	dull orange	-
	I.S.	poor	yellowish white	yellowish white	-
	T.A.	poor	yellowish white	yellowish white	-
	Gly.A.	poor	yellowish white	yellowish white	-
	Glu.A.	poor	yellowish white	yellowish white	-
	Cz.sucrose	poor	yellowish white	yellowish white	-
	N.A.	moderate	light yellowish brown	strong yellowish orange	-
	P.I.A.	abundant	yellowish brown	dull orange	-
BTG6-2	Y.M.	abundant	dark yellowish brown	yellowish brown	pale yellow
	O.M.	abundant	strong yellowish orange	strong yellowish orange	-
	I.S.	poor	yellowish white	yellowish white	-
	T.A.	poor	yellowish white	yellowish white	-
	Gly.A.	poor	yellowish white	yellowish white	-
	Glu.A.	moderate	pinkish white	pinkish white	-
	Cz.sucrose	poor	yellowish white	yellowish white	-
	N.A.	good	black	light yellowish brown	-
	P.I.A.	good	grayish brown	yellowish brown	-

Table 11 (continued)

Isolate no.	Media	Growth	Color of upper surface	Color of reverse surface	Soluble pigment
BTG1-2	Y.M.	abundant	dark yellowish brown	yellowish brown	pale yellow
	O.M.	abundant	gold	gold	-
	I.S.	poor	yellowish white	yellowish white	-
	T.A.	poor	yellowish white	yellowish white	-
	Gly.A.	poor	yellowish white	yellowish white	-
	Glu.A.	poor	pinkish white	pinkish white	-
	Cz.sucrose	poor	yellowish white	yellowish white	-
	N.A.	good	black	light yellowish brown	-
	P.I.A.	abundant	grayish brown	yellowish brown	-
BTG2-3	Y.M.	abundant	black	grayish yellow	pale yellow
	O.M.	abundant	strong yellowish orange	strong yellowish orange	-
	I.S.	poor	yellowish white	yellowish white	-
	T.A.	poor	yellowish white	yellowish white	-
	Gly.A.	poor	yellowish white	yellowish white	-
	Glu.A.	moderate	pinkish white	pinkish white	-
	Cz.sucrose	poor	yellowish white	yellowish white	-
	N.A.	moderate	black	light yellowish brown	-
	P.I.A.	good	grayish brown	yellowish brown	-
BTG4-2	Y.M.	abundant	black	grayish yellow	pale yellow
	O.M.	abundant	strong yellowish orange	strong yellowish orange	-
	I.S.	poor	yellowish white	yellowish white	-
	T.A.	moderate	yellowish white	yellowish white	-
	Gly.A.	poor	yellowish white	yellowish white	-
	Glu.A.	poor	pinkish white	pinkish white	-
	Cz.sucrose	poor	yellowish white	yellowish white	-
	N.A.	good	dark yellowish brown	vivid orange	-
	P.I.A.	abundant	grayish brown	yellowish brown	-

Table 11 (continued)

Isolate no.	Media	Growth	Color of upper surface	Color of reverse surface	Soluble pigment
BTG3-6	Y.M.	abundant	dark yellowish brown	yellowish brown	pale yellow
	O.M.	good	strong yellowish orange	strong yellowish orange	-
	I.S.	poor	yellowish white	yellowish white	-
	T.A.	poor	yellowish white	yellowish white	-
	Gly.A.	poor	yellowish white	yellowish white	-
	Glu.A.	poor	pinkish white	pinkish white	-
	Cz.sucrose	poor	yellowish white	yellowish white	-
	N.A.	abundant	yellowish black	light yellowish brown	-
	P.I.A.	good	grayish brown	yellowish brown	-
LK2-6	Y.M.	good	yellowish brown	pale yellowish pink	pale yellow
	O.M.	abundant	yellowish pink	yellowish pink	-
	I.S.	poor	yellowish white	yellowish white	-
	T.A.	poor	pale yellowish pink	pale yellowish pink	pale brown
	Gly.A.	poor	yellowish white	yellowish white	-
	Glu.A.	poor	pale yellowish pink	pale yellowish pink	-
	Cz.sucrose	poor	yellowish white	yellowish white	-
	N.A.	moderate	dark yellowish brown	deep orange	-
	P.I.A.	abundant	dark yellowish brown	light yellowish brown	-
LK2-15	Y.M.	abundant	grayish black	light yellowish brown	pale yellow
	O.M.	abundant	pale orange	pale orange	-
	I.S.	poor	yellowish white	yellowish white	-
	T.A.	moderate	light yellowish brown	light yellowish brown	pale brown
	Gly.A.	poor	yellowish white	yellowish white	-
	Glu.A.	poor	yellowish white	yellowish white	-
	Cz.sucrose	poor	yellowish white	yellowish white	-
	N.A.	moderate	dark yellowish brown	vivid orange	-
	P.I.A.	good	strong yellowish orange	strong yellowish orange	-

Table 11 (continued)

Isolate no.	Media	Growth	Color of upper surface	Color of reverse surface	Soluble pigment
TT1-11	Y.M.	abundant	brownish black to black	vivid orange	brown
	O.M.	abundant	brownish black	deep orange	-
	I.S.	good	brownish black to black	vivid orange	-
	T.A.	moderate	black	grayish white	brown
	Gly.A.	moderate	black	grayish white	-
	Glu.A.	moderate	pale orange	pale orange	-
	Cz.sucrose	abundant	brownish black to black	vivid orange	-
	N.A.	moderate	black	vivid orange	-
	P.I.A.	good	black	dark yellowish brown	-

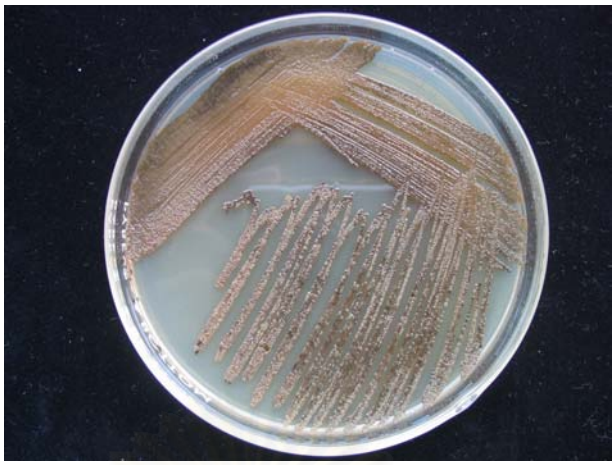
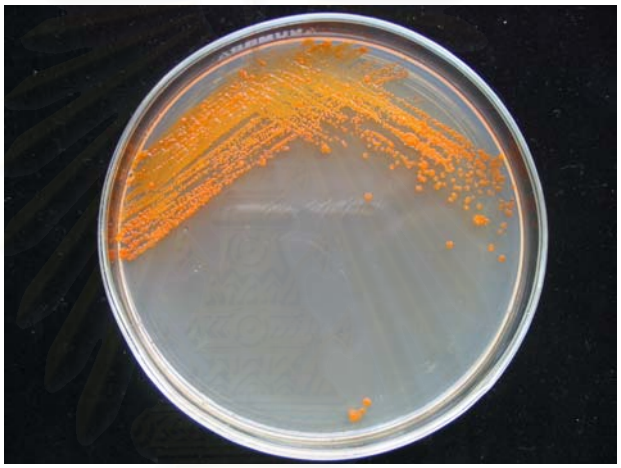


Figure 8. The colonial appearance of *Micromonospora* sp. LK5-4 on YMA medium (21 days).



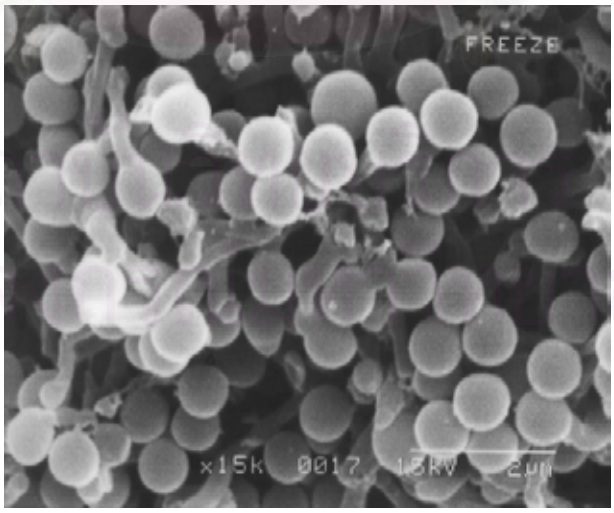


Figure 9. The colonial appearance and scaning electron micrograph of *Micromonospora* sp. KM4-29 on YMA medium (21 days).

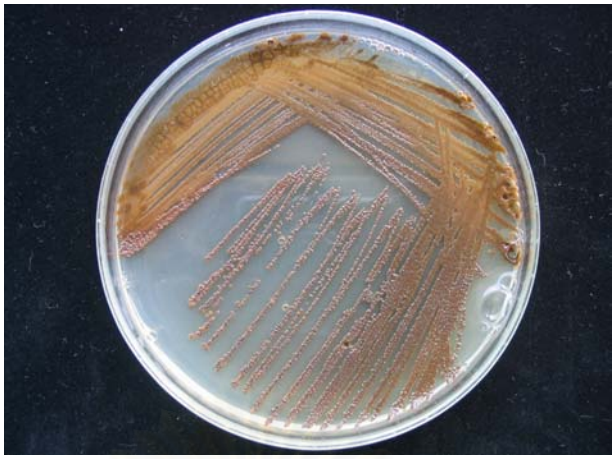
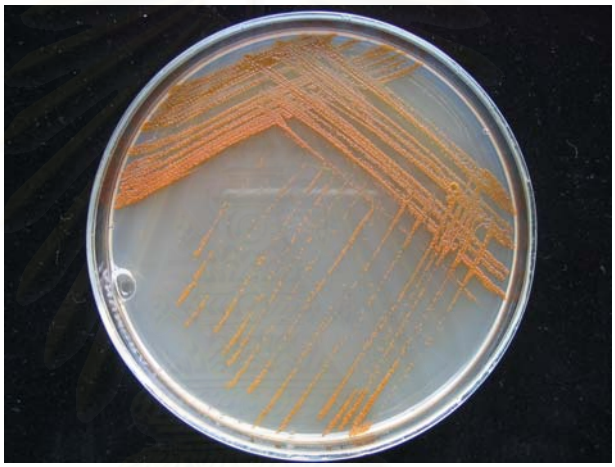


Figure 10. The colonial appearance of *Micromonospora* sp. KM3-14 on YMA medium (21 days).



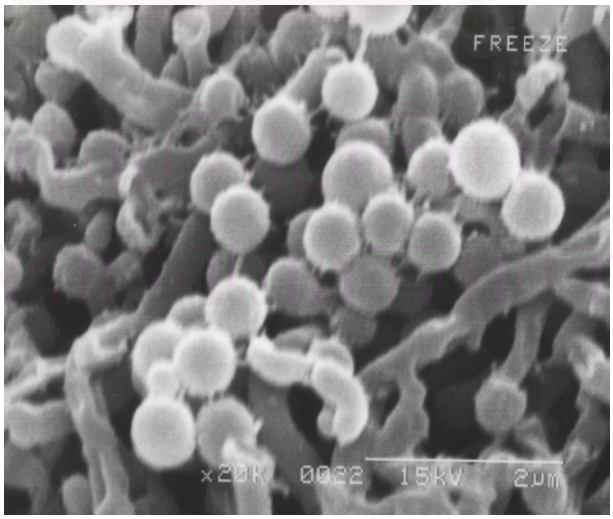


Figure 11. The colonial appearance and scaning electron micrograph of *Micromonospora* sp. BTG10-2 on YMA medium (21 days).

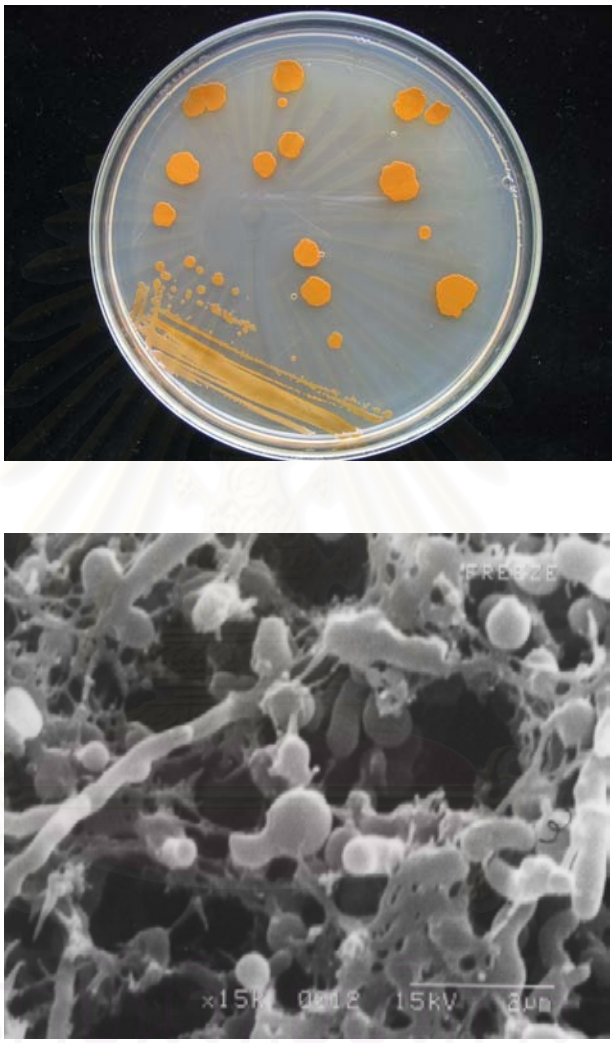


Figure 12. The colonial appearance and scaning electron micrograph of *Micromonospora* sp. TT2-4 on YMA medium (21 days).

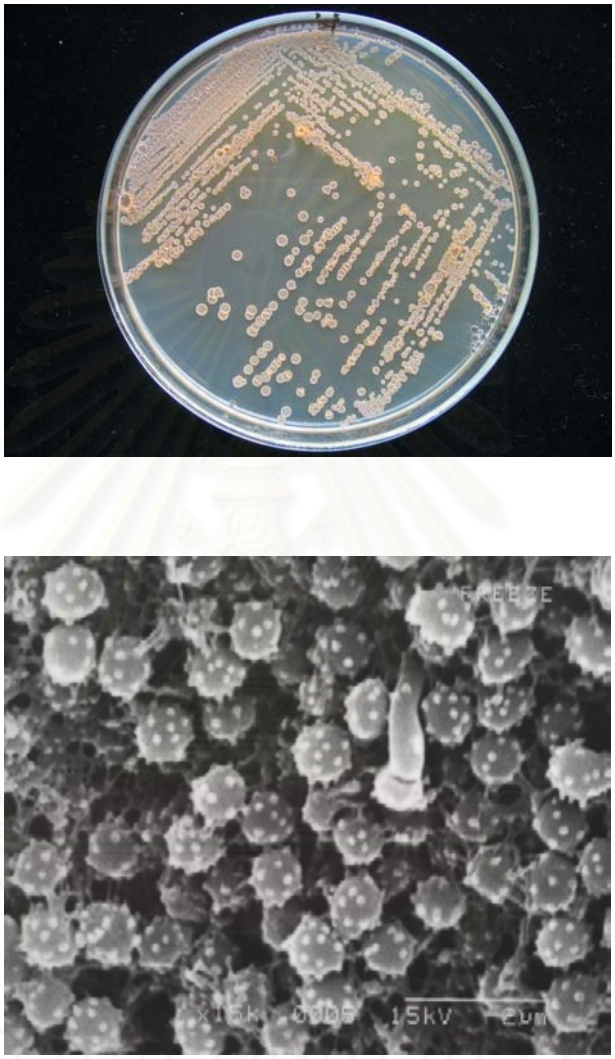


Figure 13. The colonial appearance and scaning electron micrograph of *Micromonospora* sp. BTG4-1 on YMA medium (21 days).

Figure 14. The colonial appearance and scaning electron micrograph of *Micromonospora* sp. LK2-10 on YMA medium (21 days).

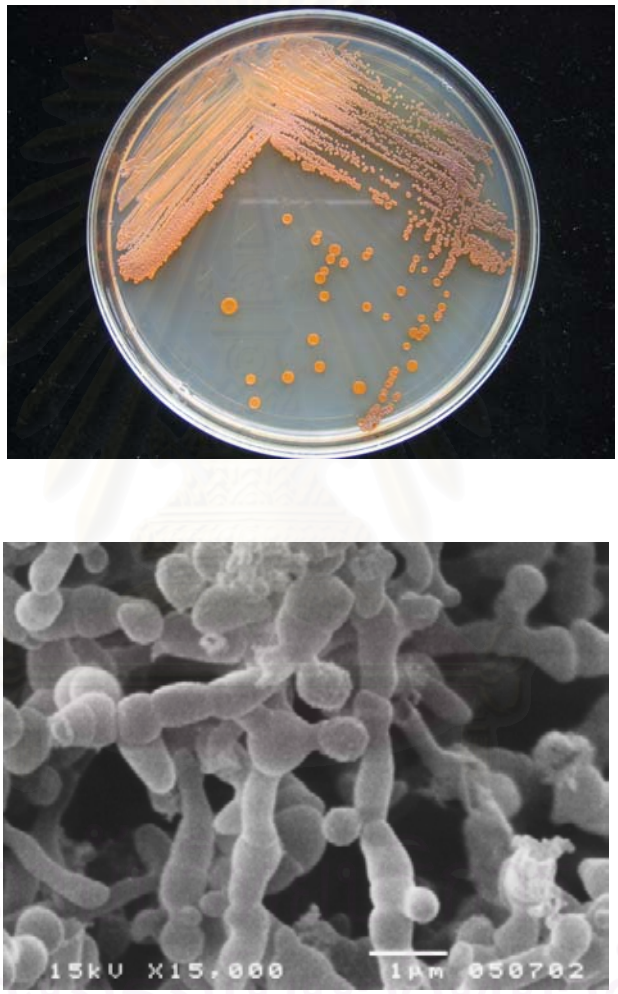


Figure 15. The colonial appearance and scaning electron micrograph of *Micromonospora* sp. LK6-12 on YMA medium (21 days).

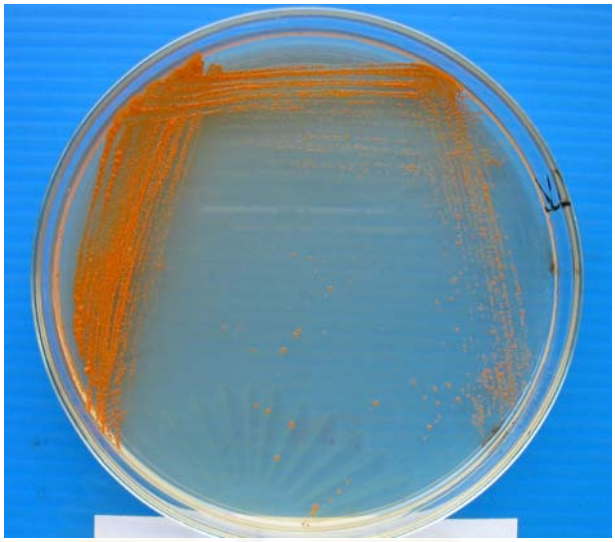


Figure 16. The colonial appearance of *Micromonospora* sp. KM1-7 on YMA medium (21 days)

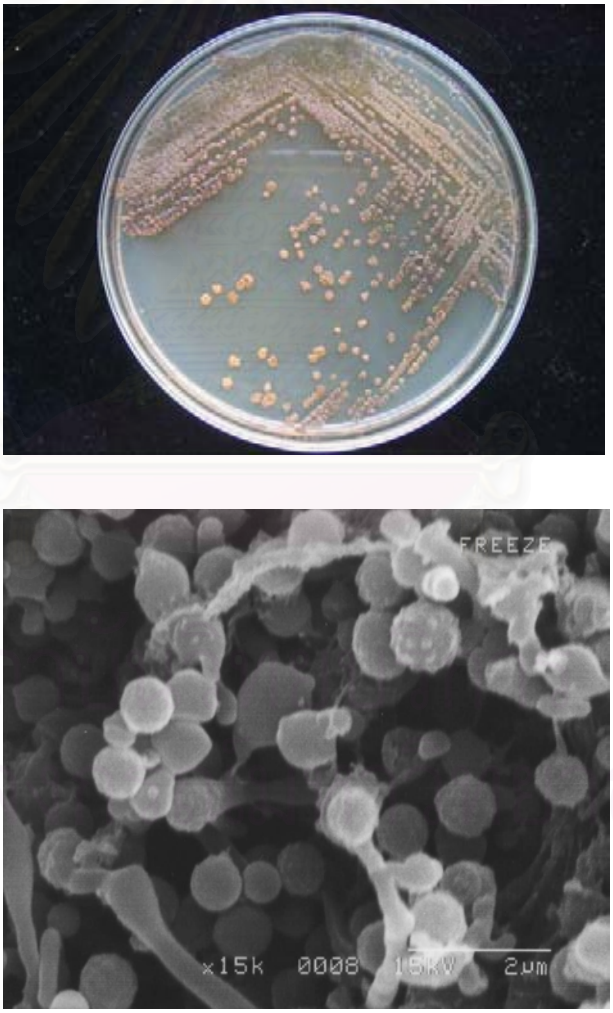


Figure 17. The colonial appearance and scaning electron micrograph of *Micromonospora* sp. BTG2-3 on YMA medium (21 days).

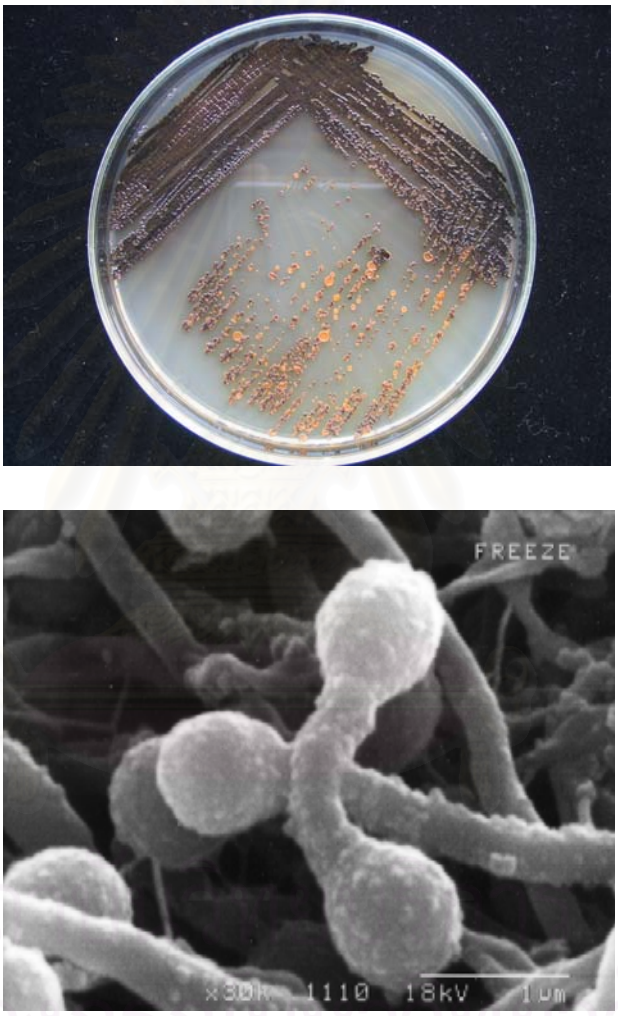


Figure 18. The colonial appearance and scaning electron micrograph of *Micromonospora* sp. TT1-11 on YMA medium (21 days).

starch and could grow at 40 ˚C, whereas the remaining (2 isolates) could grow at 45 ˚C. The minimum pH for growth of all isolates was 5. Among fifty-two isolates, seven isolates could inhibit growth of *B. subtilis*, *S. aureus*, and *M. luteus*. Variable characteristics and utilization of carbon sources are shown in Tables 12 and 13.

Micromonospora group I contained eight isolates, including LK3-1, LK5-4, KM1-3, LK5-9, LK5-7, KM3-1, KM3-2, KM3-3. They were positive for hydrolysis of starch, peptonization and coagulation of milk, and nitrate reduction. Well growth was observed between 25-30 ^{o}C. No growth was observed above 40 ^{o}C. The minimum pH for growth was 5 and they grew on 5-6% NaCl.

Micromonospora group II comprised of four isolates, including KM4-33, KM4-29, KM4-24, and KM4-25. They were positive for hydrolysis of starch and peptonization and coagulation of milk, and negative for reduction of nitrate. Well growth was observed between 25-30 ^{o}C. The maximum temperature for growth was 40 ^{o}C. The minimum pH for growth was 5. The range of NaCl concentration for growth was 5-6%.

Micromonospora group III consisted seven isolates. They were BTG3-2, BTG7-2, KM3-4, KM3-14, KM3-10, KM3-37, and KM3-7. They showed positive results of starch hydrolysis, peptonization and coagulation of milk, reduction of nitrate. Well growth was observed between 25-30 ^{o}C. The maximum temperature for growth of these strains were at 40 ^{o}C. The minimum pH for growth was 5. The range of NaCl concentration for growth was 4-5%.

Micromonospora group IV contained four isolates, including BTG10-2, BTG10-14, BTG7-3, and LK2-4. They were positive for hydrolysis of starch, peptonization and coagulation of milk, but negative for reduction of nitrate. Well growth was observed between 25-30 ^{o}C. The maximum temperature for growth of strains BTG10-2 and BTG10-14 were at 40 ^{o}C whereas strains BTG7-3 and LK2-4 were at 37 ^{o}C. The minimum pH for growth was 5. They grew on 4-5% NaCl. Their secondary metabolites inhibited growth of *Bacillus subtilis* ATCC 6633 and *Micrococcus luteus* ATCC 9341.

Micromonospora group V contained three isolates. They were TT2-4, KM4-11, and KM4-4. They showed positive results for hydrolysis of starch, peptonization and coagulation of milk, but negative for reduction of nitrate. Well growth was observed

between 25-30 °C. No growth was observed above 40 °C. The minimum pH for growth was 5. The maximum NaCl concentration for growth was 5%.

Micromonospora group VI comprised ten isolates, including BTG1-4, BTG4-1, LK2-12, KM1-6, KM1-9, KM1-2, KM1-5, BTG1-1, KM4-7, and LK2-3. They were positive for hydrolysis of starch, peptonization of milk, and reduction of nitrate. Well growth was observed between 25-30 °C. No growth was observed above 40 °C. The minimum pH for growth was 5. They grew on 0-4% NaCl.

Micromonospora group VII consisted of two isolates, including LK2-10 and LK2-5. They were positive for hydrolysis of starch, peptonization and coagulation of milk, and reduction of nitrate. Well growth was observed between 25-30 °C. No growth was observed above 45 °C. The minimum pH for growth was 5. The range of NaCl concentration for growth was 0-4%. They produced secondary metabolites that could inhibit growth of *Bacillus subtilis* ATCC 6633 and *Micrococcus luteus* ATCC 9341.

Micromonospora group VIII contained two isolates, including LK6-1 and LK6-12. They were positive for hydrolysis of starch and peptonization of milk, but negative for reduction of nitrate. Well growth was observed between 25-30 °C. No growth was observed above 40 °C. The minimum pH for growth was 5. The maximum NaCl concentration for growth was 4%.

Micromonospora group IX comprised of two isolates. They were strains KM1-7 and TK2-2. They showed negative results for hydrolysis of starch, reduction of nitrate, but weakly positive for peptonization of milk and gelatin liquefaction. Well growth was observed between 25-30 °C. No growth was observed above 40 °C. The minimum pH for growth was 5. The range of NaCl concentration for growth was 0-4%.

Micromonospora group X contained nine isolates, including LK6-9, BTG3-4, BTG1-2, BTG2-3, BTG6-2, BTG4-2, BTG3-6, LK2-6, and LK2-15. They were positive for hydrolysis of starch, peptonization and coagulation of milk, but negative for reduction of nitrate. Well growth was observed between 25-30 °C. No growth was observed above 40 °C. The minimum pH for growth was 5. The range of NaCl concentration for growth was 3-4%.

Micromonospora group XI comprised of one isolate, strain TT1-11 which was positive for hydrolysis of starch, peptonization and coagulation of milk, but negative for reduction of nitrate. Well growth was observed between 25-30 ^{o}C. No growth was observed above 40 ^{o}C. The minimum pH for growth was 5. The maximum NaCl concentration for growth was 2%. This strain produced secondary metabolites that could inhibit growth of *Bacillus subtilis* ATCC 6633 and *Staphylococcus aureus* ATCC 25923.

Table 12. The physiological and biochemical characteristics of the isolates.

Group	Isolate no.	NaCl tolerance							pH tolerance					Growth at (°C):					Skim milk		Gelatin liquefaction	Nitrate reduction	Starch hydrolysis	Antimicrobial activities
		1.5	2	3	4	5	6	7	4	4.5	5	6	8	20	37	40	45	50	Peptonization	Coagulation				
I	LK3-1	+++	+++	+++	++	+	+	-	-	-	+	++	+	++	+++	+	-	-	+	++	+	+	+	-
	LK5-4	+++	+++	+++	++	+	+	-	-	-	+	++	++	++	+++	+	-	-	++	++	+	+	+	-
	KM1-3	+++	+++	++	+	+	+	-	-	-	+	++	++	+	+++	+	-	-	+	++	+	+	+	-
	LK5-9	+++	+++	++	++	+	+	-	-	-	+	++	++	+	+++	+	-	-	+	++	+	+	+	-
	LK5-7	+++	+++	++	++	+	-	-	-	-	+	++	++	+	+++	+	-	-	+	++	+	w	+	-
	KM3-1	++	+++	++	+	+	-	-	-	-	+	++	++	++	+++	+	-	-	+	++	+	w	+	-
	KM3-2	++	+++	++	+	+	-	-	-	-	+	++	++	+	+++	+	-	-	++	+	+	w	+	-
	KM3-3	++	+++	++	+	+	-	-	-	-	+	++	++	++	+++	+	-	-	+	++	+	w	+	-
II	KM4-33	+++	+++	+++	++	+	w	-	-	-	+	++	+	+	++	+	-	-	+	++	w	-	+	-
	KM4-29	+++	+++	+++	+++	++	+	-	-	-	+	++	+	+	++	+	-	-	+	++	w	-	+	-
	KM4-24	++	+++	++	++	++	-	-	-	-	+	++	+	++	++	+	-	-	++	++	+	-	+	-
	KM4-25	++	+++	++	++	++	-	-	-	-	+	++	+	++	++	+	-	-	++	++	+	-	+	-
III	KM3-4	++	++	++	+	+	-	-	-	-	+	+++	+	+	+++	+	-	-	+	++	+	+	w	-
	KM3-7	++	++	++	+	+	-	-	-	-	+	++	++	+	++	+	-	-	+	++	+	+	+	-
	KM3-10	++	++	++	+	+	-	-	-	-	+	+++	++	+	+++	+	-	-	+	++	+	+	+	-
	KM3-14	++	++	++	+	+	-	-	-	-	+	+++	++	+	+++	+	-	-	+	++	+	+	w	-
	KM3-37	++	++	++	+	+	-	-	-	-	+	++	++	+	++	+	-	-	+	++	+	+	w	-
	BTG7-2	++	++	+	+	-	-	-	-	-	+	+++	++	+	+++	+	-	-	+	++	+	+	+	-
	BTG3-2	++	++	+	+	-	-	-	-	-	+	++	++	+	++	+	-	-	+	++	+	+	w	-

Table 12 (continued)

Group	Isolation No.	NaCl tolerance							pH tolerance					Growth at (˚C):					Skim milk		Gelatin liquefaction	Nitrate reduction	Starch hydrolysis	Antimicrobial activities
		1.5	2	3	4	5	6	7	4	4.5	5	6	8	20	37	40	45	50	Peptonization	Coagulation				
IV	BTG10-2	++	++	+	+	w	-	-	-	-	+	++	+	++	+++	+	-	-	+	++	+	-	+	*B. subtilis, M. luteus*
	BTG10-14	++	++	+	+	w	-	-	-	-	+	++	+	++	+++	+	-	-	+	++	+	-	+	*B. subtilis, M. luteus*
	BTG7-3	++	++	+	+	-	-	-	-	-	+	++	+	++	+++	-	-	-	++	+	w	-	+	*M. luteus, B. subtilis*
	LK2-4	++	++	+	+	-	-	-	-	-	+	++	+	++	+++	-	-	-	+	++	+	-	+	*M. luteus, B. subtilis*
V	TT2-4	++	++	+	+	+	-	-	-	-	+	++	+	+	++	+	-	-	+	++	+	-	+	-
	KM4-4	+++	+++	++	+	+	-	-	-	-	+	++	++	+	++	+	-	-	+	++	+	-	+	-
	KM4-11	+++	+++	++	+	w	-	-	-	-	+	++	+	+	++	+	-	-	+	++	w	-	+	-
VI	BTG1-4	+++	+++	++	+	-	-	-	-	-	+	+++	+	+	+++	+	-	-	++	w	+	w	+	-
	BTG4-1	+++	+++	+	+	-	-	-	-	-	+	+++	+	+	+++	+	-	-	+	++	+	w	+	-
	LK2-12	++	++	+	+	-	-	-	-	-	+	++	+	+	+++	+	-	-	++	+	+	w	+	-
	KM1-6	+++	++	+	+	-	-	-	-	-	+	+++	++	+	+++	+	-	-	++	+	+	w	+	-
	KM1-5	++	++	++	+	-	-	-	-	-	+	+++	+	+	+++	+	-	-	w	-	+	w	+	-
	KM1-2	+++	++	+	+	-	-	-	-	-	+	++	+	+	+++	+	-	-	w	-	+	w	+	-
	KM1-9	++	++	++	+	-	-	-	-	-	+	+++	+	+	+++	+	-	-	w	-	+	w	+	-
	BTG1-1	+++	+++	++	w	-	-	-	-	-	+	+++	+	+	+++	+	-	-	w	++	+	w	+	-
	LK2-3	+++	++	++	+	-	-	-	-	-	+	+++	++	+	+++	+	-	-	w	++	+	w	+	-
	KM4-7	++	++	+	+	-	-	-	-	-	+	++	+	+	+++	+	-	-	+	++	w	w	+	-

Table 12 (continued)

Group	Isolation No.	NaCl tolerance							pH tolerance					Growth at (˚C):					Skim milk		Gelatin liquefaction	Nitrate reduction	Starch hydrolysis	Antimicrobial activities
		1.5	2	3	4	5	6	7	4	4.5	5	6	8	20	37	40	45	50	Peptonization	Coagulation				
VII	LK2-5	++	++	+	+	-	-	-	-	-	+	++	++	++	+++	+	+	-	+	++	+	+	+	*M. luteus, B. subtilis*
	LK2-10	++	++	+	+	-	-	-	-	-	+	++	++	++	+++	+	+	-	+	++	+	+	+	*M. luteus, B. subtilis*
VIII	LK6-1	+++	++	+	+	-	-	-	-	-	+	+++	++	++	+++	+	-	-	w	-	w	-	+	-
	LK6-12	+++	++	+	+	-	-	-	-	-	+	+++	++	+	+++	+	-	-	w	-	w	-	+	-
IX	TK2-2	+	+	w	w	-	-	-	-	-	+	++	++	+	++	+	-	-	w	-	w	-	-	-
	KM1-7	+	+	w	w	-	-	-	-	-	+	++	++	+	++	+	-	-	w	-	w	-	-	-
X	BTG3-4	+++	+++	+	+	-	-	-	-	-	+	+++	++	++	++	+	-	-	+	++	+	-	+	-
	LK6-9	+++	++	++	+	-	-	-	-	-	+	++	++	+	+++	+	-	-	++	+	+	-	+	-
	BTG6-2	+++	++	++	-	-	-	-	-	-	+	+++	++	+	++	+	-	-	w	++	+	-	+	-
	BTG1-2	++	++	+	-	-	-	-	-	-	+	+++	++	++	++	+	-	-	++	+	+	-	+	-
	BTG2-3	++	++	+	-	-	-	-	-	-	+	++	++	++	++	+	-	-	w	++	+	-	+	-
	BTG4-2	++	++	+	-	-	-	-	-	-	+	+++	+++	++	+++	+	-	-	++	+	+	-	+	-
	BTG3-6	++	++	++	-	-	-	-	-	-	+	+++	++	++	+++	+	-	-	w	++	+	-	+	-
	LK2-6	++	++	+	+	-	-	-	-	-	+	+++	++	+	++	+	-	-	+	++	w	-	+	-
	LK2-15	++	++	+	+	-	-	-	-	-	+	+++	++	+	+++	+	-	-	+	++	w	-	+	-
XI	TT1-11	++	+	-	-	-	-	-	-	-	+	+++	++	++	++	+	-	-	w	++	+	-	+	*B. subtilis, S. aureus*

+++ = abundant, ++ = good, + = fair, w = weak

Table 13. Carbon utilization of the isolates.

Carbon sources	Isolates																									
	LK3-1	LK5-4	KM1-3	LK5-9	LK5-7	KM3-1	KM3-2	KM3-3	KM4-33	KM4-29	KM4-24	KM4-25	KM3-4	KM3-7	KM3-10	KM3-14	KM3-37	BTG7-2	BTG3-2	BTG10-2	BTG10-14	BTG7-3	LK2-4	TT2-4	KM4-4	KM4-11
Utilization of :																										
D-Mannitol	w	w	w	w	w	w	w	w	-	-	-	-	-	-	-	-	-	-	-	+	+	w	w	-	-	-
D-Ribose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
L-Rhamnose	w	w	+	w	w	+	+	+	+	+	w	+	-	-	-	-	-	-	-	+	+	w	w	w	w	w
D-Melibiose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	w	+	w	+	+	+
D-Raffinose	+	+	+	+	+	w	w	w	+	+	+	+	w	w	w	w	+	+	+	+	+	+	+	+	w	+
Glycerol	w	w	+	w	w	+	+	+	+	+	w	+	-	-	-	-	-	-	-	+	+	w	w	-	-	-
Salicin	-	-	-	-	-	w	w	w	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	+	+	+
Lactose	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	+	+	+	+	+	w	+
D-Galactose	+	+	+	+	+	+	+	+	-	-	-	-	+	+	+	+	+	+	+	+	w	+	+	+	+	+
L-Arabinose	-	-	-	-	-	-	-	-	-	-	-	-	w	w	w	w	w	w	w	-	-	-	-	+	+	w
Cellobiose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	w	w	+	+	+	+	+
D-Fructose	w	w	w	+	w	w	w	w	-	-	-	-	+	+	+	+	+	+	+	-	-	-	-	w	w	w
D-Xylose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Table 13 (continued)

Carbon sources	Isolates																									
	BTG1-4	BTG4-1	LK2-12	KM1-6	KM1-5	KM1-2	KM1-9	BTG1-1	LK2-3	KM4-7	LK2-5	LK2-10	LK6-1	LK6-12	TK2-2	KM1-7	BTG3-4	LK6-9	BTG6-2	BTG1-2	BTG2-3	BTG4-2	BTG3-6	LK2-6	LK2-15	TT1-11
Utilization of :																										
D-Mannitol	+	+	w	w	w	+	w	w	w	w	w	w	+	+	-	-	-	-	-	-	-	-	-	w	w	-
D-Ribose	+	+	w	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
L-Rhamnose	-	-	-	-	-	-	-	-	-	-	+	+	w	w	w	w	+	+	+	+	+	+	+	-	-	-
D-Melibiose	w	+	+	+	+	+	+	+	+	+	+	+	-	-	+	w	+	+	+	+	w	w	w	-	-	+
D-Raffinose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+
Glycerol	w	+	+	w	w	w	w	w	w	w	+	+	+	+	-	-	-	-	-	-	-	-	-	w	w	-
Salicin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	w	+	+	w	w	w	w	+	+	+
Lactose	w	+	+	w	w	w	w	w	+	w	+	+	+	w	+	+	-	-	-	-	-	-	-	w	+	+
D-Galactose	+	+	+	+	+	+	+	+	+	+	+	w	-	-	+	+	-	-	-	-	-	-	-	-	-	+
L-Arabinose	w	w	w	-	-	-	-	w	w	w	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
Cellobiose	+	+	+	+	+	+	+	+	+	+	+	+	+	w	+	+	-	-	-	-	-	-	-	-	-	+
D-Fructose	-	-	-	w	w	w	w	-	-	-	-	-	-	-	w	w	-	-	-	-	-	-	-	-	-	+
D-Xylose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Table 14. DNA-DNA similarity among the isolates and the representative *Micromonospora* species in each groups.

Isolates no.	Percentage DNA similarity with labeled DNA from:										
	LK2-10	LK5-4	KM4-29	KM3-14	BTG10-2	TT2-4	BTG4-1	LK6-12	KM1-7	BTG2-3	TT1-11
LK2-10	100										
LK2-5	89										
LK5-4		100									
LK3-1		93									
KM1-3		86									
LK5-9		89									
LK5-7		92									
KM3-1		80									
KM3-2		101									
KM3-3		99									
KM4-29			100								
KM4-33			87								
KM4-24			95								
KM4-25			98								

Table 14 (continued)

Isolate no.	Percentage DNA similarity with labeled DNA from:										
	LK2-10	LK5-4	KM4-29	KM3-14	BTG10-2	TT2-4	BTG4-1	LK6-12	KM1-7	BTG2-3	TT1-11
KM3-14				100							
KM3-4				88							
KM3-10				83							
KM3-37				89							
KM3-7				78							
BTG3-2				82							
BTG7-2				90							
BTG10-2					100						
BTG10-14					102						
BTG7-3					98						
LK2-4					86						
TT2-4						100					
KM4-11						84					
KM4-4						79					

Table 14 (continued)

Isoolate no.	Percentage DNA similarity with labeled DNA from:										
	LK2-10	LK5-4	KM4-29	KM3-14	BTG10-2	TT2-4	BTG4-1	LK6-12	KM1-7	BTG2-3	TT1-11
BTG4-1							100				
BTG1-4							103				
LK2-12							91				
KM1-6							87				
KM1-9							82				
KM1-2							89				
KM1-5							79				
BTG1-1							88				
KM4-7							79				
LK2-3							94				
LK6-12								100			
LK6-1								85			
KM1-7									100		
TK2-2									96		

Table 14 (continued)

Isolate no.	Percentage DNA similarity with labeled DNA from:										
	LK2-10	LK5-4	KM4-29	KM3-14	BTG10-2	TT2-4	BTG4-1	LK6-12	KM1-7	BTG2-3	TT1-11
BTG2-3										100	
LK6-9										83	
BTG3-4										91	
BTG1-2										95	
BTG6-2										81	
BTG4-2										98	
BTG3-6										82	
LK2-6										80	
LK2-15										79	
TT1-11											100

4. Chemotaxonomic characteristics of *Micromonospora* strains

The representative strains in each group (nineteen isolates from the total of fifty-two isolates) were selected for chemotaxonomic characterization. They showed the same pattern of chemotaxonomic characteristics which were similar to those of members of the genus *Micromonospora* (Tables 15 to 19). Cell wall hydrolysates of them contained glutamic acid, glycine, alanine, and diaminopimelic acid in a molar ratio of 1.0:0.9:0.4:1.1 (calculated by defining the amount of glutamic acid as 1.0), and the isomer of diaminopimelic acid was *meso*, indicating that these strains have wall chemotype II as described by Lechevalier & Lechevalier (1970) and peptidoglycan type A1γ' as described by Schleifer & Kandler (1972). The acyl type of cell wall muramic acid was glycolyl type. The strains contained glucose, xylose, arabinose, galactose, mannose, and ribose as whole-cell sugars, but rhamnose was not detected (whole-cell sugar pattern D as described by Lechevalier & Lechevalier, 1970). Characteristic phospholipids were diphosphatidylglycerol, phosphatidylinositol, phosphatidylinositolmannosides, and phosphatidylethanolamine, but not phosphatidylcholine. This pattern corresponds to phospholipid type II as described by Lechevalier *et al.* (1977). The cellular fatty acid compositions are shown in Table 19. Their major fatty acids were iso-$C_{15:0}$, iso-$C_{16:0}$, iso-$C_{17:0}$, anteiso-$C_{15:0}$, $C_{17:0}$, and anteiso-$C_{17:0}$. This pattern corresponds to fatty acid type 3b of Kroppenstedt (1985). Mycolic acids were absent. The predominant menaquinones were MK-9(H_4), MK-10(H_4), MK-9(H_6), MK-10(H_6), MK-10(H_8), and small amounts of MK-9(H_8), MK-10(H_0), MK-10(H_2), MK-9(H_2), and MK-11(H_4) were also present. Their G+C contents of the DNA were 71.4 and 73.0 mol%, respectively.

Table 15. Diaminopimelic acid types of the representative *Micromonospora* strains.

Isolate no.	Diaminopemilic acid type		
	meso-DAP	LL-DAP	OH-DAP
LK5-4	+	Trace amount	-
KM3-1	+	Trace amount	-
KM4-33	+	Trace amount	-
KM4-24	+	Trace amount	-
KM3-14	+	Trace amount	-
BTG3-2	+	Trace amount	-
BTG10-2	+	Trace amount	-
BTG7-3	+	Trace amount	-
TT2-4	+	Trace amount	-
BTG4-1	+	Trace amount	-
KM1-9	+	Trace amount	-
BTG1-1	+	Trace amount	-
LK2-10	+	Trace amount	-
LK6-12	+	Trace amount	-
KM1-7	+	Trace amount	-
BTG3-4	+	Trace amount	-
BTG2-3	+	Trace amount	-
LK2-6	+	Trace amount	-
TT1-11	+	Trace amount	+

Table 16. Polar lipid composition and glycolic analyses of the representative *Micromonospora* strains.

Isolate no.	Polar lipid type						Glycolic acid
	DPG	PIMs	PI	PE	Methyl-PE	OH-PE	(nM)
LK5-4	+	+	+	+	-	-	63.5
KM3-1	+	+	+	+	-	-	33.2
KM4-29	+	+	+	+	-	-	24.7
KM4-24	+	+	+	+	-	-	29.6
KM3-14	+	+	+	+	-	-	227.4
BTG3-2	+	+	+	+	-	-	98.0
BTG10-2	+	+	+	+	-	-	65.9
BTG7-3	+	+	+	+	-	-	83.9
TT2-4	+	+	+	+	-	-	26.5
BTG4-1	+	+	+	+	-	-	77.9
KM1-9	+	+	+	+	-	-	47.9
BTG1-1	+	+	+	+	-	-	59.5
LK2-10	+	+	+	+	-	-	78.9
LK6-12	+	+	+	+	-	-	54.0
KM1-7	+	+	+	+	-	-	31.4
BTG3-4	+	+	+	+	-	-	77.5
BTG2-3	+	+	+	+	-	-	31.4
LK2-6	+	+	+	+	-	-	64.4
TT1-11	+	+	+	+	-	-	45.6

Abbreviations:

DPG; Diphosphatidylglycerol PIMs; Phosphatidylinositolmannosides

PI; Phosphatidylinositol PE; Phosphatidylethanolamine

Methyl-PE; Methylphosphatidylethanolamine

OH-PE; Hydroxyphosphatidylethanolamine

Table 17. Menaquinone types of the representative *Micromonospora* strains.

Isolate no.	% of Menaquinone Type									
	MK-9(H_2)	MK-9(H_4)	MK-9(H_6)	MK-9(H_8)	MK-10(H_0)	MK-10(H_2)	MK-10(H_4)	MK-10(H_6)	MK-10(H_8)	MK-11(H_4)
LK5-4	Trace	23.2	6.7	1.3	Trace	-	**28.1**	14.4	4.3	-
KM3-1	Trace	22.1	6.8	1	Trace	-	**29**	12	6.8	-
KM4-29	1.3	8	4.9	1.1	Trace	1.2	**35.2**	23.5	7.2	Trace
KM4-24	1.2	5.7	3.6	1.2	Trace	2.3	**41.5**	25.7	9.3	-
KM3-14	4.3	14.9	1.5	-	Trace	6.4	**49.9**	7.7	Trace	-
BTG3-2	1.4	16	7.3	3.8	Trace	2	**34.05**	14	8	-
BTG10-2	Trace	6.6	2.6	Trace	Trace	1.5	**52.9**	26.9	5.3	Trace
BTG7-3	1.1	1.3	1.9	-	Trace	1.3	27.6	**33.4**	23.8	-
TT2-4	1.1	7.2	Trace	-	Trace	2.9	**63.9**	8.7	1	-
BTG4-1	1.2	3.8	8.8	13.1	-	-	10.9	19.5	**34.6**	-
KM1-9	-	10.4	12.5	6.2	Trace	-	24.3	**28.4**	17.6	-
BTG1-1	-	11.8	3.5	-	-	1.3	**51.2**	20.3	3.1	-
LK2-10	Trace	**19.3**	17.4	11.1	Trace	-	13.5	13.8	10.2	-
LK6-12	1.1	6.6	-	-	Trace	4.5	**66**	5.4	-	1.2
KM1-7	Trace	1.3	**41.7**	9.4	-	-	Trace	15.9	5.4	-
BTG3-4	-	5.3	10.8	10.5	-	-	12.5	24.7	**30.6**	-
BTG2-3	-	11.1	9.7	3	-	-	29.1	**31.7**	13	-
LK2-6	Trace	12.6	4.4	Trace	Trace	-	**45.5**	20.6	3.4	-
TT1-11	1.9	15.5	3.5	Trace	Trace	3.9	**54.8**	10.8	1.1	Trace

Table 18. Whole-cell sugar of the representative *Micromonospora* strains.

Isolate no.	Whole cell sugar type						
	Rhamnose	Ribose	Mannose	Arabinose	Galactose	Xylose	Glucose
LK5-4	+	+	+	+	+	+	+
KM3-1	Trace amount	+	+	+	+	+	+
KM4-29	-	+	+	+	+	+	+
KM4-24	-	+	+	+	+	+	+
KM3-14	Trace amount	+	+	+	+	+	+
BTG3-2	-	+	+	+	+	+	+
BTG10-2	-	+	+	+	+	+	+
BTG7-3	-	+	+	+	+	+	+
TT2-4	-	+	+	+	+	+	+
BTG4-1	Trace amount	+	+	+	+	+	+
KM1-9	Trace amount	+	+	+	+	+	+
BTG1-1	-	+	+	+	+	+	+
LK2-10	-	+	+	+	+	+	+
LK6-12	-	+	+	+	+	+	+
KM1-7	-	-	+	-	+	+	+
BTG3-4	-	+	+	+	+	+	+
BTG2-3	Trace amount	+	+	+	+	+	+
LK2-6	-	+	+	+	+	+	+
TT1-11	-	+	+	+	+	+	+

Table 19. Fatty acid compositions of the representative *Micromonospora* strains.

Fatty acid	% fatty acid of representative strains																		
	LK5-4	KM3-1	KM4-29	KM4-24	KM3-14	BTG3-2	BTG10-2	BTG7-3	TT2-4	BTG4-1	KM1-9	BTG1-1	LK2-10	LK6-12	KM1-7	BTG3-4	BTG2-3	LK2-6	TT1-11
Saturated fatty acid																			
C9:0	-	-	-	-	0.2	0.1	0.1	-	-	-	-	-	-	-	-	-	-	-	0.1
C10:0	-	-	0.2	0.1	0.1	-	0.1	0.2	-	-		-	-	-	-	-	-	-	0.1
C11:0	-	-	0.1	0.1	-	-	0.1	0.1	-	-	0.1	-	-	-	-	-	-	-	-
C12:0	-	-	0.4	0.1	0.3	-	0.3	0.6	-	-	0.1	-	-	-	-	0.1	0.1		0.3
C13:0	-	-	0.3	0.4		0.1	0.3	0.4	0.1	0.1	0.3	0.2	0.1	-	-	0.1	0.1	0.1	0.1
C14:0	0.1	0.1	0.7	0.5	0.1	0.1	0.2	0.6	0.1	0.1	0.3	0.2	0.2	0.3	0.1	0.2	0.2	0.2	0.2
C15:0	0.1	0.3	4	9.7	0.2	0.4	3.3	5.1	1	2	7.9	3.5	1.6	1.9	0.7	6.2	7.3	2.8	0.5
C16:0	0.4	0.9	2	1.9	0.5	0.7	1.4	1.1	0.9	1.4	2.4	1.5	1.1	2.4	0.4	2.2	1.9	1.7	0.7
3OH-C16:0	0.2	0.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C17:0	0.8	1.7	9.6	9.8	1	2.8	9.2	10.2	5.2	8.3	20.4	9.5	7.9	4	4	16.4	16.3	7.7	1.4
3OH-C17:0	-	-	-	-	-	-	-	-	-	-	-	-	-	0.2	-	-	-	-	-
C18:0	1.6	1.6	1.3	0.5	1.1	1.2	0.7	0.4	1.6	1.7	1.4	1.1	2.5	1.8	1.5	1.4	1.2	1	1.5
C19:0	0.2	0.2	0.2	0.1		0.3	0.2	0.1	1.1	0.5	0.4	0.3	1.4	0.4	1	0.4	0.4	0.3	-
C20:0	0.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Unsaturated fatty acid																			
C15:1(ω6c)	-	0.1	0.3	1.3	0.1	0.2	0.6	0.3	0.2	-	0.4	0.1	0.1	0.1	0.3	0.3	0.6	0.3	0.1
C15:1(ω8c)	-	0.1	0.1	0.1		0.2	0.1	0.1	-	-	0.2	0.1	-	0.1	-	-	-	-	-
2OH-C16:1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.5	-	-	0.6
C17:1(ω5c)	-	-	0.1	0.1	-	-	0.1	0.2	0.2	-	-	-	-	-	0.2		-	-	-
C17:1(ω8c)	2.7	6.4	12.7	14	2.9	8.7	11.4	13.3	6.2	3.3	8.5	4.2	5.2	9.5	2.6	11.3	11.7	6.9	1.5
C18:1(ω5c)	-	0.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C18:1(ω7c)	0.1	0.1		0.1	0.1	0.1	0.1	0.1	0.2	0.1	0.1	0.1	0.1	0.7		0.1	0.1	0.1	0.1
C18:1(ω9c)	4.5	4.9	0.8	0.7	3.6	4.7	0.7	0.5	2.3	0.6	0.9	0.6	1.9	4.3	0.7	0.9	0.8	0.7	1.1
C20:1(ω9c)	-	-	-	-	-	-	-	-	0.1	-	-	-	-	-	-	-	-	-	-

Table 19 (continued)

Fatty acid	% fatty acid of representative strains																		
	LK5-4	KM3-1	KM4-29	KM4-24	KM3-14	BTG3-2	BTG10-2	BTG7-3	TT2-4	BTG4-1	KM1-9	BTG1-1	LK2-10	LK6-12	KM1-7	BTG3-4	BTG2-3	LK2-6	TT1-11
Branched fatty acids																			
i-C10:0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.3
i-C11:0	-	-	-	-	-	0.1	0.1	0.1	-	-	-	-	-	-	-	-	-	-	0.1
a-C11:0	-	-	-	-	-	0.1	-	-	-	-	-	-	-	-	-	-	-	-	0.6
3OH-i-C11:0	-	-	-	-	-	0.1	-	-	-	-	-	-	-	-	-	-	-	-	-
i-C12:0	0.1		0.1	0.1	0.2		0.1	0.2	-	-	-	-	-	-	-	-	-	-	0.8
i-C13:0	0.1	0.2	0.1	0.1	0.2	0.3	0.3	0.5	0.1	0.1	0.2	0.1	-	-	0.1	0.1	0.1	0.1	0.3
a-C13:0	0.1	0.1	0.1	0.1	0.1	0.3	0.3	0.8	0.1		0.1	0.1	-	-	-	-	-	-	0.2
i-C14:0	1.6	0.5	5.2	7.7	2	0.5	2.5	1.7	0.8	0.4	1.3	0.7	0.8	0.9	2.2	1.2	1.1	1	3.5
3OH-i-C14:0	-	0.1	-	-	-	-	-	-	-	0.1	-	-	-	-	-	-	-	-	0.1
i-C15:0	5.6	25.4	10.5	11.6	10.4	18.8	20.4	22.6	17.6	39.6	-	37.7	24.1	18.6	22.4	-	-	42.4	11.5
a-C15:0	4.3	6.4	8.7	9.3	5.1	10.3	16.1	14.4	4.2	6.4	14.9	10.3	8.5	6	11.7	13.8	14.6	7.5	4.6
i-C15:1	-	0.1	-	0.1	0.1	0.2	-	-	0.1	-	0.4	0.2	0.1	-	0.1	0.2	0.1	0.1	1
2OH-C16:1	0.2	-	-	-	0.3	-	-	-	-	0.9	-	-	-	-	0.9	-	-	-	-
i-C16:0	45.4	14.7	33	23.1	37.2	13.3	17.9	16.3	23.8	9.6	12	8.4	17.9	18.2	17.9	17.2	12.6	11.5	39.1
3OH-i-C16:0	0.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.2
i-C16:1	1.5	0.4	1.3	1.3	1.6	0.7	0.5	0.2	0.9	0.2	0.5	0.3	0.3	0.9	0.9	0.6	0.8	0.5	9.5
i-C17:0	2.5	10.4	1.2	0.6	4.7	8	2.3	1.6	7.7	10.8	9.6	7.8	7.7	5.2	9.3	8.2	9.2	5.3	4.7
a-C17:0	9.6	10.8	4	1.7	8.9	14.2	6	4.4	10.4	7.2	10.6	8.2	10.1	7.7	14.3	9.3	10.1	4.6	5.8
a-C17:1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.4
i-C17:1 ω9c	3.8	8.9	0.6		5.5	8.3	1.2	1.7	7.9	2.5	3.4	2.6	1.6	11.1	2.9	3.7	4.2	2.7	2.7
a-C17:1 ω9c	1.3	0.9	0.4	0.2	1.1	1.8	0.3	0.2	1.2	0.4	0.7	0.5	0.3	1.6	0.3	0.8	1.1	0.4	
i-C18:1	0.2	-	0.1	-	0.4	-	-	-	0.2	-	-	-	-	0.1	-	-	-	-	0.1
i-C18:0	2.9	0.5	0.8	0.2	1.5	0.3	0.2	0.2	0.5	0.2	0.1	0.1	0.7	0.2	0.9	0.2	0.2	0.1	1.9
i-C19:0	-	-	-	-	0.1	-	-	-	0.1	0.1	-	-	-	-	0.2	-	-	-	0.1
i-C19:1	-	-	-	-	0.1	0.1	-	-	0.1	-	-	-	-	-	-	-	-	-	-

Table 19 (continued)

Fatty acid	% fatty acid of representative strains																		
	LK5-4	KM3-1	KM4-29	KM4-24	KM3-14	BTG3-2	BTG10-2	BTG7-3	TT2-4	BTG4-1	KM1-9	BTG1-1	LK2-10	LK6-12	KM1-7	BTG3-4	BTG2-3	LK2-6	TT1-11
10-Methyl fatty acids																			
C16:0	-	-	-	0.7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C17:0	7.4	1.4	0.7	2.8	8.1	1.2	2.1	2.3	3.1	2.9	2.1	1.1	4.2	1.4	3.5	3.1	3.6	1.3	2.4
C18:0 TBSA	1.5	0.5		0.1	1.3	0.4	0.1	0.1	0.8	0.5	0.2	0.2	0.9	0.2	0.3	0.3	0.3	0.1	0.9
Summed feature 3	0.5	1.2	0.7	0.7	0.4	0.9	0.8	0.6	0.4	0.1	0.5	0.4	0.3	1.6	0.2	0.8	0.7	0.9	0.3
Summed feature 6	0.3	0.4	0.1	0.1	0.2	0.5	0.1	0.1	1.1	0.2	0.2	0.12	0.5	0.4	-	0.3	0.2	0.1	0.1

a Values are percentages of total cellular fatty acids

b Summed features represent groups of one or two fatty acids which could not be separated by GLC with the MIDI system. Summed feature 3 contained one or more of the following fatty acids: 2-OH-i-C15:0 and/or C16:1(ω7c). Summed feature 6 contained one or more of the following fatty acids : C19:1 ω11c/C19:1 ω9c**.**

5. Comparison of DNA-DNA hybridization of the representative strains and all of validly described *Micromonospora* species

The representative strains of *Micromonospora* group I and group III showed DNA-DNA similarity values of 91.0% and 79.2% similar to those of *M. chalcea* JCM 3031^T and *M. aurantiaca* JCM 10878^T, respectively. The value are well higher than the 70% cut-off point recommended by Wayne *et al*. (1987) for the delineation of genomic species. These results indicated that they should be identified as *M. chalcea* and *M. aurantiaca*, respectively, whereas *Micromonospora* groups II, IV, V, VI, VII, VIII, IX, X, and XI, have DNA-DNA similarity value (< 53.1%) that less than the 70% cut-off point. So, they could not be classified as any known species of the genus *Micromonospora* (Table 20).

6. 16S rDNA Amplification and Nucleotide Sequence Analysis

6.1 16S rDNA amplification by PCR

The chromosomal DNAs of the representative strains were used as the DNA templates for 16S rDNA amplification by PCR with forward and reverse primer (20F: 5'-AGTTTGATCCTGGCTC-3' and 1541R: 5'-AAGGAGGTGATCCAGCC-3'). The PCR products of each representative strains were analyzed by 0.8% agarose gel electrophoresis. All of them showed clearly yield ~1.5 kb PCR product which their sizes corresponded well with the size of 16S rDNA.

6.2 16S rDNA sequencing

The PCR products of all of the representative strains were determined for their 16S rDNA nucleotide sequences. Their nucleotide sequences were illustrated in Appendix VI. The 16S rDNA sequence alignment results between the representative strains and those of all of validly described *Micromonospora* species are shown in Figure 20.

6.3 16S rDNA sequence and phylogenetic tree analysis

The almost complete 16S rDNA sequences consisting of 1,440-1,510 nucleotides were determined for all of representative strains; a 1,368 nt fragment was used for phylogenetic analysis, similarity percentage calculation, and compared with 16S rDNA database sequences of members of the family *Micromonosporaceae*. The

Table 20. DNA-DNA similarity among the representative *Micromonospora* strains.

Species and Strain no.	G+C content (mol%)	Percentage DNA similarity with labeled DNA from:										
		LK2-10	LK5-4	KM4-29	KM3-14	BTG10-2	TT2-4	BTG4-1	LK6-12	KM1-7	BTG2-3	TT1-11
LK2-10	71.5	100.0										
LK5-4	72.3	23.6	100.0									
KM4-29	72.4	19.5	31.2	100.0								
KM3-14	72.4	20.9	42.1	38.2	100.0							
BTG10-2	72.5	26.6	35.4	49.8	33.1	100.0						
TT2-4	73	21.6	27.7	23.6	35.7	23.0	100.0					
BTG4-1	71.6	41.1	48.5	35.9	31.0	39.8	33.0	100.0				
LK6-12	72.6	20.2	21.2	22.2	18.6	23.8	35.4	35.9	100.0			
KM1-7	72.6	19.7	19.6	24.1	23.4	19.4	21.7	20.2	19.7	100.0		
BTG2-3	71.6	21.9	41.8	36.9	40.7	31.5	37.6	39.8	33.4	16.7	100.0	
TT1-11	72.8	25.3	36.1	26.8	38.8	28.4	25.9	44.3	29.1	21.1	38.4	100.0
M. chersina JCM 9459^T	72.9	36.4	42.0	50.6	38.9	48.4	50.0	38.7	42.8	22.5	52.4	48.1
M. coerulea JCM 3175^T	71.7	26.4	28.4	34.7	25.0	34.6	37.6	32.7	33.5	16.9	48.2	37.2
M. purpureochromogenes JCM 3156^T	73.0	28.0	23.1	22.9	18.9	24.6	36.6	28.2	30.4	16.1	38.4	43.2
M. echinospora JCM 3073^T	71.7	21.0	29.5	29.8	31.4	26.9	33.1	24.9	27.3	16.9	32.7	33.1
M. carbonacea JCM 3139^T	73.3	27.2	24.7	27.7	19.1	18.4	30.3	27.3	21.1	16.9	26.6	32.8
M. chalcea JCM 3031^T	71.9	27.0	**91.0**	49.7	52.9	45.3	43.3	33.4	28.6	23.3	43.3	41.5
M. inositola JCM 6239^T	71.4	29.0	26.7	22.2	27.2	24.6	34.4	34.0	26.1	12.9	36.3	38.0
M. olivasterospora JCM 7348^T	71.9	24.0	23.8	13.8	23.2	22.3	30.6	28.2	26.0	19.3	32.1	35.6
M. nigra JCM 8973^T	71.7	26.0	28.9	38.8	23.7	26.9	41.1	26.0	29.8	12.9	34.9	37.3
M. halophytica JCM 3125^T	72.5	36.0	41.4	36.1	27.2	30.7	47.1	45.2	39.1	20.9	40.1	53.1
M. aurantiaca JCM 10878^T	71.6	28.0	46.7	41.9	**79.2**	47.6	39.5	32.7	34.7	20.1	44.0	40.7
M. rosaria JCM 3159^T	72.9	24.0	25.9	22.2	27.0	23.8	34.4	28.0	24.8	14.5	31.7	34.9
M. matsumotoense JCM 9104^T	71.0	26.0	29.1	20.1	14.2	22.3	25.5	23.2	17.4	14.5	25.8	32.1
M. pallida JCM 3133^T	71.1	28.0	24.0	20.1	23.2	16.1	31.2	27.8	20.5	21.7	28.4	35.3

phylogenetic tree was constructed from evolutionary distances by using neighbor-joining method in the MEGA software program version 2.1.

Phylogenetic analysis of the almost complete 16S rDNA sequences of the tested organisms with the corresponding sequences of all of the type strains of validly described *Micromonospora* species, selected sequences of *Actinoplanes* species, and the 16S rDNA sequence of *Streptomyces ambofaciens* (as an outgroup) showed that the representatives of *Micromonospora* groups I, II, III, IV, V, VI, VII, VIII, IX, X, and XI could be distinguished both for one another (Figure 19; Table 20 and 22). It is evident from figure 19 that the groups II, IV, VI, VII, VIII, IX, and X strains form distinct phyletic lines in the 16S *Micromonospora* rDNA tree, and the groups I, III, V, and XI strains form subclade with *M. chalcea* DSM 43026^T, *M. aurantiaca* DSM 43813^T, *M. matsumotoense* IMSNU 22003^T, and *M. coerulea* DSM 43143^T, respectively.

The two strains of *Micromonospora* group I (LK5-4 amd KM3-1) shared 16S rDNA nucleotide similarities within the range 99.9%, values which correspond to 2 nucleotide differences. These organisms were most closely associated with *M. chalcea* DSM 43026^T in the neighbor-joining analysis by a moderately high bootstrap value and shared the highest similarity percentage of 99.7 and 99.6 with *M. chalcea* DSM 43026^T, respectively. Strain LK5-4^T showed almost identical physiological and biochemical properties to *M. chalcea* DSM 43026^T (=JCM 3031^T). In addition, the level of DNA-DNA similarity between strains LK5-4^T and *M. chalcea* DSM 43026^T (=JCM 3031^T) was 91.0%. Hence, this strain should be identified as *M. chalcea*.

The two strains of *Micromonospora* group II (KM4-29 and KM4-24) showed identical 16S rDNA nucleotide sequences and were most closely associated with identical 16S rDNA nucleotide sequences of *Micromonospora* group IV (BTG10-2 and BTG7-3), and was supported by a high bootstrap value of 93%. These two groups shared a 16S rDNA similarity of 99.8%. In contrast, a low level of DNA homology was found between *Micromonospora* group II and group IV. The 16S rDNA similarities between the group II strains and all of validly described *Micromonospora* were within the range 97.7% (*M. olivasterospora*) to 99.0% (*M. chalcea*), which corresponds to the range 34 to 17 nucleotide differences, respectively, whereas the 16S rDNA similarities between the group IV strains and all of validly described *Micromonospora* ranged from 97.7% (*M. olivasterospora*) to

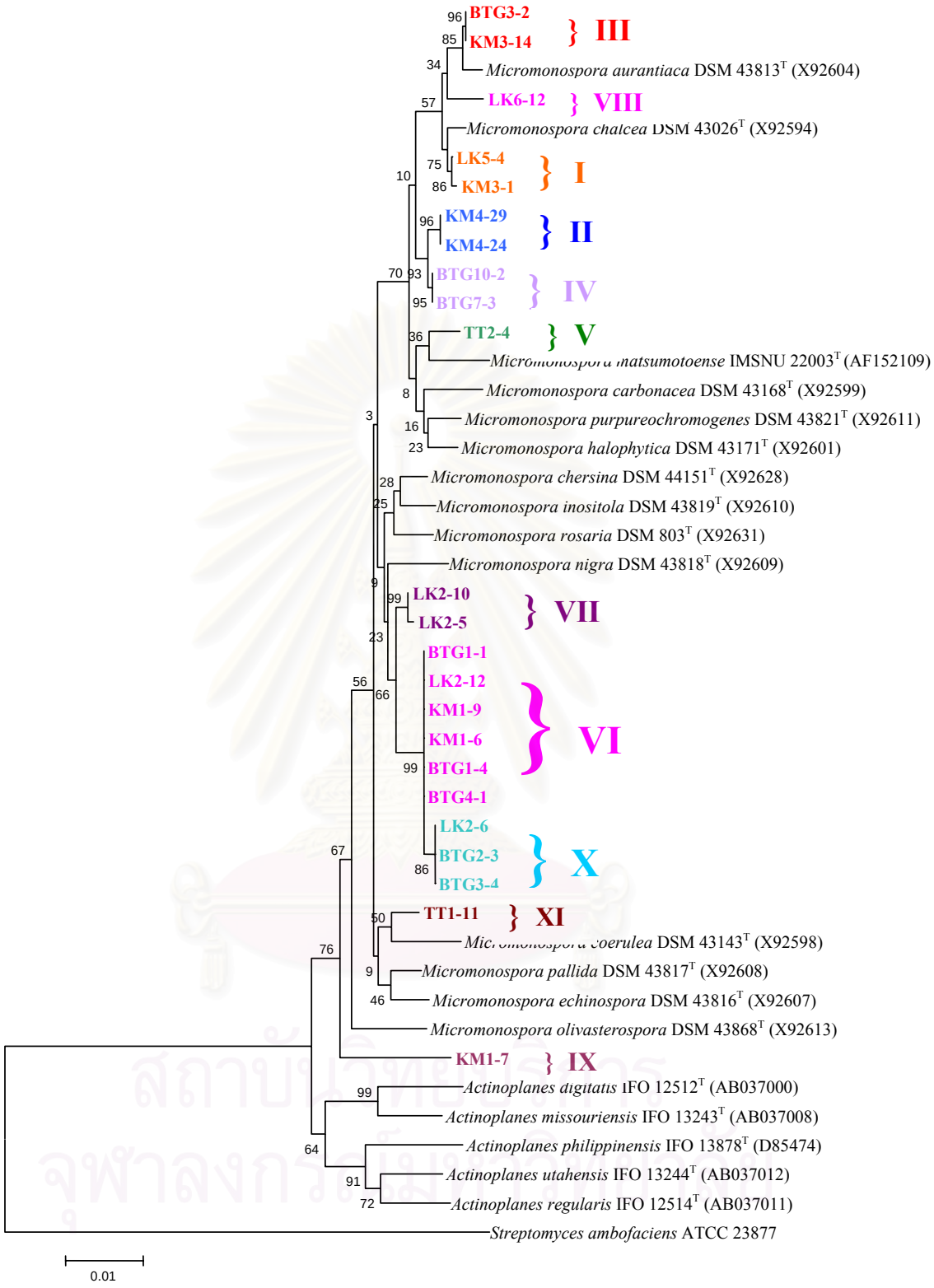


Figure 19. Unroot neighbor-joining tree base on nearly complete 16S rDNA sequences, showing the position of the representative *Micromonospora* strains in the *Micromonospora* tree.

99.2% (*M. chalcea*), which corresponds to the range 36 to 13 nucleotide differences, respectively. Strain KM4-29^T showed a different physiological and biochemical pattern compared to the phylogenetically closest species, *Micromonospora* group IV strain (BTG10-2^T). In particular, the maximum NaCl concentration for growth and utilization of D-mannitol, salicin and D-galactose effectively discriminates strain KM4-29^T from strain BTG10-2^T. The level of DNA-DNA similarity between strains KM4-29^T, BTG10-2^T and the other *Micromonospora* groups as well as all of type strains of validly described *Micromonospora* species, except *M. gallica*, ranged from 13.8-50.6%. These representative strains were left unidentified.

The representative strains of *Micromonospora* group III (BTG3-2 and KM3-14^T) had identical 16S rDNA nucleotide sequences. These organisms were most closely associated with *M. aurantiaca* DSM 43813^T and which supported by a bootstrap value of 85% in the neighbor-joining run. The 16S rDNA similarities between the group II strains and all of validly described *Micromonospora* are within the range 97.7% (*M. olivasterospora* DSM 43868^T) to 99.7% (*M. aurantiaca*), which corresponds to the range 35 to 4 nucleotide differences, respectively. Strain KM3-14^T had almost identical physiological and biochemical properties which were distinguished in the hydrolysis of starch and the maximum NaCl concentration for growth from *M. aurantiaca* DSM 43813^T (=JCM 10878^T). However, the level of DNA-DNA similarity between strains KM3-14^T and *M. aurantiaca* DSM 43813^T (=JCM 10878^T) was 79.2%. Hence, this strain should be identified as *M. aurantiaca*.

The representative strain of *Micromonospora* group V (TT2-4^T) showed the most closely associated with *M. matsumotoense* IMSNU 22003^T in the neighbor-joining analysis but this relationship was not supported by a high bootstrap value in the neighbor-joining run. The 16S rDNA similarities between strain TT2-4^T and all of validly described *Micromonospora* ranged from 97.7% (*M. olivasterospora*) to 98.9% (*M. purpureochromogenes*), which corresponds to the range 36 to 18 nucleotide differences, respectively. Strain TT2-4^T showed difference in physiological and biochemical properties from those of the phylogenetically closest species, *M. matsumotoense* IMSNU 22003^T. In particular, the result of nitrate reduction, the maximum NaCl concentration for growth and utilization of L-rhamnose, D-melibiose, salicin and D-ribose effectively discriminates strain TT2-4^T from *M. matsumotoense* IMSNU 22003^T. In addition, DNA similarity values between strain TT2-4^T and the

14 type strains of the other validly described species, together with the other *Micromonospora* groups were only 50.0% or less. These representative strain were left unidentified.

The six representative *Micromonospora* group VI strains, BTG1-1, KM1-6, BTG1-4, KM1-9, BTG4-1, and LK2-12, had identical 16S rDNA nucleotide sequences. These organisms were most closely associated with the *Micromonospora* group X that contained three identical 16S rDNA nucleotide sequence strains, BTG2-3, BTG3-4, and LK2-6, which was suporrted by a high bootstrap value of 99%. These two groups shared a high 16S rDNA similarity of 99.9% which corresponds to 1 nucleotide differences but showed a low level of DNA homology. The 16S rDNA similarities between *Micromonospora* group VI and all of validly described *Micromonospora* species ranged from 97.9% (*M. carbonacea*) to 99.0% (*M. chersina*), which corresponds to the range 32 to 14 nucleotide differences, respectively, whereas the 16S rDNA similarities between *Micromonospora* group X and all of validly described *Micromonospora* species ranged from 97.8% (*M. aurantiaca*) to 98.8% (*M. rosaria*), which corresponds to the range 30 to 15 nucleotide differences, respectively. Members of *Micromonospora* group VI strain BTG4-1^T showed physiological and biochemical properties which were distinguished in the maximum NaCl concentration for growth (4% for BTG4-1^T and 3% for BTG2-3^T) and utilization of D-mannitol, D-ribose, L-rhamnose, glycerol, lactose, D-galactose, L-arabinose, and cellobiose from the phylogenetically clostest *Micromonospora* group X strains (BTG2-3^T). The levels of DNA-DNA similarity between strains BTG4-1^T, BTG2-3^T, and the other *Micromonospora* groups as well as all of type strains of validly described *Micromonospora* species, except *M. gallica*, ranged from 20.2-48.5%. These representative strains were left unidentified.

The representatives of *Micromonospora* group VII, strains LK2-10 and LK2-5, shared a high 16S rDNA similarity of 99.9% which corresponds to 1 nucleotide differences; this value was almost exclusively in the conserved region of the molecule. These organisms were most closely associated with *Micromonospora* group VI which was supported by a moderately bootstrap value in the neighbor-joining analysis, and were shared a 16S rDNA similarity within the range of 99.4% to 99.5%, respectively. The 16Sr DNA similarities between strain LK2-10 and all of validly type strains of genus *Micromonospora* are within the range 98.1% (*M.*

aurantiaca) to 99.0% (*M. nigra*), respectively, which correspond to the range 27 to 14 nucleotide differences, respectively, where as the 16Sr DNA similarities between strain LK2-5 and all of validly described *Micromonospora* species are within the range 98.0% (*M. aurantiaca*) to 99.0% (*M. nigra*), respectively, which correspond to the range 28 to 16 nucleotide differences, respectively. *Micromonospora* group VII, strains LK2-10^T and LK2-5, showed almost identical physiological and biochemical properties which were distinguished in growth at 45˚C and utilization of D-ribose, L-rhamnose, and L-arabinose from *Micromonospora* group VI, strain BTG4-1^T. Colour of substrate mycelium of strain LK2-10^T was yellowish white, which is distinguishable from the color of all of the type strains of *Micromonospora* that generally indicate orange colour. The levels of DNA-DNA similarity between strains LK2-10^T and LK 2-5 ranged from 89-92%, while the levels of DNA-DNA similarity of them and all of type strains of validly described *Micromonospora* species, except *M. gallica*, together with the other *Micromonospora* groups, ranged from 19.5-41.1%. The representative strains in these group were unidentified.

These three subclades (groups VI, VII, and X) formed a distinct phyletic line with *M. nigra*, but this relationship was not supported by a high bootstrap value in the neighbor-joining run. The *Micromonospora* groups VI, VII, and X could be distinguished both for one another by using the evidences of DNA-DNA hybridization experiment that demonstrated a low level of DNA homology.

The *Micromonospora* group VIII, strain LK6-12, formed a distinct phyletic line with the type strains of genus *Micromonospora*. However, the position of strain LK6-12 in the *Micromonospora* tree varies depending on the treeing algorithm because this strain showed a low bootstrap value in neighbor-joining tree. Strain LK6-12 was most closely related to the type strain of *M. aurantiaca*, and shared a 16S rDNA nucleotide similarity value of 99.1%, which corresponds to 19 nucleotide differences. *Micromonospora* group VIII, strain LK6-12^T, showed the physiological and biochemical properties which were distinguished in hydrolysis of starch, tyrosine decomposition, nitrate reduction, and utilization of D-mannitol, L-rhamnose, D-melibiose, glycerol, salicin, lactose, D-galactose, D-fructose, and L-arabinose from *Micromonospora aurantiaca* JCM 10878^T. The level of DNA-DNA similarity between strains LK6-12^T and all of type strains of validly described *Micromonospora*

species, except *M. gallica*, together with the other *Micromonospora* groups, ranged from 17.4-42.8%. The strain inthis group was unidentified.

Micromonospora group IX, strain KM1-7, formed a distinct phyletic line towards the base of the evolutionary branch that encompasses the type strains of *Micromonospora* species. Strain KM1-7 was most closely related to the type strain of *M. olivasterospora*, and which was supported by a moderately high bootstrap value of 76%; the two organisms shared a 16S rDNA nucleotide similarity value of 97.5%, which corresponds to 38 nucleotide differences. This strain showed the properties on starch hydrolysis, nitrate reduction, growth at 45 ˚C, the maximum NaCl tolerant for growth, and utilization of L-arabinose that were distinguished from *Micromonospora olivasterospora* JCM 7348^{T}. The levels of DNA-DNA similarity between strains KM1-7^{T} and all of type strains of validly described *Micromonospora* species, except *M. gallica*, together with the other *Micromonospora* groups, ranged from 12.9-24.1%. This representative strain was left unidentified.

From Figure 19, it is appearant that the *Micromonospora* group XI, strain TT1-11, was most closely related to *M. coerulea*, an association that is supported by a moderately high bootstrap value of 50% in the neighbor-joining analysis. The type strains of *M. coerulea* and strain TT1-11 shared a 16S rDNA sequence similarity of 98.8%, which corresponds to 23 nucleotide differences. Strain TT1-11^{T} showed a different physiological and biochemical patterns as compared to the phylogenetically closest species, *M. coerulea*. In particular, the utilization of L-arabinose and D-ribose effectively discriminates strain TT1-11^{T} from *M. coerulea.* In addition, DNA similarity values between strain TT1-11^{T} and the 14 type strains of the other validly described species, together with the other *Micromonospora* groups, were only 53.1% or less. The strain in this group was unidentified.

Based on the phenotypic and genotypic characteristics, the *Micromonospora* groups I and III strains should be identified as *M. chalcea* and *M. aurantiaca*, respectively. The other nine groups, should be classified as a new species of the genus *Micromonospora*. The differential characteristics and the summary of the percentage of 16S rDNA sequence similarity between the representative strains and the validly type strains of *Micromonospora* are shown in Tables 21 and 22, respectively.

CLUSTAL X (1.83) multiple sequence alignment.

```
BTG2-3          --------------------AGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGA
BTG3-4          --------------------AGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGA
LK2-6           ------------------TCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGA
BTG1-4          ------------------TCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGA
KM1-9           -CTAGTTTGATCC-TGGCTCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGA
LK2-12          --TAGTTTGATCC-TGGNTCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGA
KM1-6           --TAGTTTGATCC-TGGCTCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGA
BTG4-1          --TAGTTTGATCC-TGGCTCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGA
BTG1-1          --TAGTTTGATCC-TGGCTCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGA
BTG3-2          -TAGTTGATCCCT-TGGNTCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGA
X92604          -----TGATCC---TGGCTCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGA
KM3-14          ------------------TCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGA
KM3-1           TAGTTTGATCCC--TTGGCCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGA
X92594          -----TGATCC---TGGCTCAGGCCGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGA
X92601          -----TGATCC---TGGCTCAGGCCGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGA
X92611          -----TGATCC---TGGCGCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGA
KM4-29          --TAGTTTGATCC-TGGCTCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGA
KM4-24          --TAGTTTGATCC-TGGNTCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGA
LK6-12          ---TAGTTGATCC-CTGGCTAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGA
LK5-4           ------------------------------------------------------CGA
TT2-4           ------------------------------------------------------TCGA
BTG10-2         -TAGTTTGATCCN-TGGCTCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGA
BTG7-3          --TAGTTTGATCC-TGGCTCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGA
AF152109        -AGAGTTTGATCA-TGGCTCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGA
X92599          -------TGATCC-TGGCTCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGA
X92613          -------TGATCC-TGGCTCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGA
TT1-11          -AGAGTTTGATCA-TGGCTCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGA
X92598          -------TGATCC-TGGCTCAGGCCGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGA
X92608          -------TGATCC-TGGCGCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGA
X92607          -------TGATCC-TGGCGCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGA
X92610          -------TGATCC-TGGCGCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGA
X92609          -------TGATCC-TGGCGCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGA
X92631          -------TGATCC-TGGCTCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGA
X92628          -------TGATCC-TGGCTCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGA
LK2-10          ------------------CAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGA
LK2-5           ------------------TCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGA
KM1-7           --TAGTTTGATCCTTGGNTCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGA
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Figure 20. Comparison of 16S rDNA nucleotide sequences between the representative *Micromonospora* strains and the validly described *Micromonospora* species.

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BTG2-3          GCGGAAGGCCCTTCGGGGTACTCGAGCGGCGAACGGGTGAGTCACGTGAGCACC
BTG3-4          GCGGAAGGCCCTTCGGGGTACTCGAGCGGCGAACGGGTGAGTCACGTGAGCACC
LK2-6           GCGGAAGGCCCTTCGGGGTACTCGAGCGGCGAACGGGTGAGTCACGTGAGCACC
BTG1-4          GCGGAAGGCCCTTCGGGGTACTCGAGCGGCGAACGGGTGATACACGTGAGCACC
KM1-9           GCGGAAGGCCCTTCGGGGTACTCGAGCGGCGAACGGGTGATACACGTGAGCACC
LK2-12          GCGGAAGGCCCTTCGGGGTACTCGAGCGGCGAACGGGTGATACACGTGAGCACC
KM1-6           GCGGAAGGCCCTTCGGGGTACTCGAGCGGCGAACGGGTGATACACGTGAGCACC
BTG4-1          GCGGAAGGCCCTTCGGGGTACTCGAGCGGCGAACGGGTGATACACGTGAGCACC
BTG1-1          GCGGAAGGCCCTTCGGGGTACTCGAGCGGCGAACGGGTGATACACGTGAGCACC
BTG3-2          GCGGAAGGCCCTTCGGGGTACTCGAGCGGCGAACGGGTGATACACGTGAGCACC
X92604          GCGGAAGGCCCTTCGGGGTACTCGAGCGGCGAACGGGTGATACACGTGAGCACC
KM3-14          GCGGAAGGCCCTTCGGGGTACTCGAGCGGCGAACGGGTGATACACGTGAGCACC
KM3-1           GCGGAAGGCCCTTCGGGGTACTCGAGCGGCGAACGGGTGATACACGTGAGCACC
X92594          ACGGAAGGCCCTTCGGGGTACTCGAGCGGCGAACGGGTGATACACGTGAGCACC
X92601          ACGGAAGGCCCTTCGGGGTACTCGAGCGGCGAACGGGTGATACACGTGAGCACC
X92611          GCGGAAGGCCCTTCGGGGTACTCGAGCGGCGAACGGGTGATACACGTGAGCACC
KM4-29          GCGGAAGGCCCTTCGGGGTACTCGAGCGGCGAACGGGTGATACACGTGAGCACC
KM4-24          GCGGAAGGCCCTTCGGGGTACTCGAGCGGCGAACGGGTGATACACGTGAGCACC
LK6-12          GCGGAAGGCCCTTCGGGGTACTCGAGCGGCGAACGGGTGATACACGTGAGCACC
LK5-4           GCGGAAGGCCCTTCGGGGTACTCGAGCGGCGAACGGGTGATACACGTGAGCACC
TT2-4           GCGGAAGGCCCTTCGGGGTACTCGAGCGGCGAACGGGTGATACACGTGAGCACC
BTG10-2         GCGGAAGGCCCTTCGGGGTACTCGAGCGGCGAACGGGTGATACACGTGAGCACC
BTG7-3          GCGGAAGGCCCTTCGGGGTACTCGAGCGGCGAACGGGTGATACACGTGAGCACC
AF152109        GCGGAAGGCCCTTCGGGGTACTCGAGCGGCGAACGGGTGATACACGTGAGCACC
X92599          GCGGAAGGCCCTTCGGGGTACTCGAGCGGCGAACGGGTGATACACGTGAGCACC
X92613          GCGGAAGGCCCTTCGGGGTACTCGAGCGGCGAACGGGTGATACACGTGAGCACC
TT1-11          GCGGAAGGCCCTTCGGGGTACTCGAGCGGCGAACGGGTGATACACGTGAGCACC
X92598          GCGGAAGGCCCTTCGGGGTACTCGAGCGGCGAACGGGTGATACACGTGAGCACC
X92608          GCGGAAGGCCCTTCGGGGTACTCGAGCGGCGAACGGGTGATACACGTGAGCACC
X92607          GCGGAAGGCCCTTCGGGGTACTCGAGCGGCGAACGGGTGATACACGTGAGCACC
X92610          GCGGAAGGCCCTTCGGGGTACTCGAGCGGCGAACGGGTGATACACGTGAGCACC
X92609          GCGGAAGGCCCTTCGGGGTACTCGAGCGGCGAACGGGTGATACACGTGAGCACC
X92631          GCGGAAGGCCCTTCGGGGTACTCGAGCGGCGAACGGGTGATACACGTGAGCACC
X92628          GCGGAAGGCCCTTCGGGGTACTCGAGCGGCGAACGGGTGATACACGTGAGCACC
LK2-10          GCGGAAGGCCCTTCGGGGTACTCGAGCGGCGAACGGGTGATACACGTGAGCACC
LK2-5           GCGGAAGGCCCTTCGGGGTACTCGAGCGGCGAACGGGTGATACACGTGAGCACC
KM1-7           GCGGAAGGCCCTTCGGGGTACTCGAGCGGCGAACGGGTGATACACGTGAGTACC
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Figure 20. (Continued)

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BTG2-3          TGCCCTAGGCTTTGGGATAACCCCGGGAAACCGGGGCTAATACCGAATAGGACCTGGC
BTG3-4          TGCCCTAGGCTTTGGGATAACCCCGGGAAACCGGGGCTAATACCGAATAGGACCTGGC
LK2-6           TGCCCTAGGCTTTGGGATAACCCCGGGAAACCGGGGCTAATACCGAATAGGACCTGGC
BTG1-4          TGCCCTAGGCTTTGGGATAACCCCGGGAAACCGGGGCTAATACCGAATAGGACCTGGC
KM1-9           TGCCCTAGGCTTTGGGATAACCCCGGGAAACCGGGGCTAATACCGAATAGGACCTGGC
LK2-12          TGCCCTAGGCTTTGGGATAACCCCGGGAAACCGGGGCTAATACCGAATAGGACCTGGC
KM1-6           TGCCCTAGGCTTTGGGATAACCCCGGGAAACCGGGGCTAATACCGAATAGGACCTGGC
BTG4-1          TGCCCTAGGCTTTGGGATAACCCCGGGAAACCGGGGCTAATACCGAATAGGACCTGGC
BTG1-1          TGCCCTAGGCTTTGGGATAACCCCGGGAAACCGGGGCTAATACCGAATAGGACCTGGC
BTG3-2          TGCCCCAGGCTTTGGGATAACCCCGGGAAACCGGGGCTAATACCGAATATGACCTCTG
X92604          TCCCCCAGGCTTTGGGATAACCCCGGGAAACCGGGGCTAATACCGAATATGACCTCTG
KM3-14          TGCCCCAGGCTTTGGGATAACCCCGGGAAACCGGGGCTAATACCGAATATGACCTCTG
KM3-1           TGCCCCAGGCTTTGGGATAACCCCGGGAAACCGGGGCTAATACCGAATATGACCTCCG
X92594          TGCCCCAGGCTTTGGGATAACCCCGGGAAACCGGGGCTAATACCGAATATGACCTTGC
X92601          TCCCCTAGGCTTTGGGATAACCCCGGGAAACCGGGGCTAATACCGAATATGACCTCCG
X92611          TCCCCTAGGCTTTGGGATAACCCCGGGAAACCGGGGCTAATACCGAATATGACTTCTG
KM4-29          TGCCCTAGGCTTTGGGATAACCCCGGGAAACCGGGGCTAATACCGAATATGACCACAT
KM4-24          TGCCCTAGGCTTTGGGATAACCCCGGGAAACCGGGGCTAATACCGAATATGACCACAT
LK6-12          TGCCCCAGGCTTTGGGATAACCCCGGGAAACCGGGGCTAATACCGAATATGACCTTGC
LK5-4           TGCCCCAGGCTTTGGGATAACCCCGGGAAACCGGGGCTAATACCGAATATGACCTTGC
TT2-4           TGCCCTAGGCTTTGGGATAACCCCGGGAAACCGGGGCTAATACCGAATATGACCTTTC
BTG10-2         TGCCCTAGGCTTTGGGATAACCCCGGGAAACCGGGGCTAATACCGAATATGACCTCGC
BTG7-3          TGCCCTAGGCTTTGGGATAACCCCGGGAAACCGGGGCTAATACCGAATATGACCTCGC
AF152109        TGCCCTAGGCTTTGGGATAACCCTCGGAAACGGGGGCTAATACCGAATATTACTTGAC
X92599          TCCCCTAGGCTTTGGGATAACCCTCGGAAACGGGGGCTAATACCGGATACAACCTTTG
X92613          TCCCCTAGGCTTTGGGATAACCCTCGGAAACGGGGGCTAATACCGAATATTACTGCTG
TT1-11          TGCCCTAGGCTTTGGGATAACCCTCGGAAACGGGGGCTAATACCGAATATGACTACTG
X92598          TCCCCTAGCCTTTGGGATAACCCCGGGAAACCGGGGCTAATACCGAATAGGACTGCTG
X92608          TCCCCTAGGCTTTGGGATAACCCCGGGAAACCGGGGCTAATACCGAATATGACCTGCG
X92607          TGCCCTAGGCTTTGGGATAACCCCGGGAAACCGGGCCTAATACCGAATAGGACCTTCG
X92610          TCCCCTAGGCTTTGGGATAACCCCGGGAAACCGGGGCTAATACCGAATAGAACCTGGC
X92609          TCCCCCAGGCTTTGGGATAACCCCGGGAAACCGGGGCTAATACCGAATATGACCTTCG
X92631          TCCCCTAGGCTTTGGGATAACCCCGGGAAACCGGGGCTAATACCGAATAGGACCTGAC
X92628          TCCCCTAGGCTTTGGGATAACCCCGGGAAACCGGGGCTAATACCGAATAGGACCTGGC
LK2-10          TGCCCTAGGCTTTGGGATAACCCCGGGAAACCGGGGCTAATACCGAATAGGACCTCCT
LK2-5           TGCCCTAGGCTTTGGGATAACCCCGGGAAACCGGGGCTAATACCGAATAGGACCTCCT
KM1-7           TGCCCTGCGCTTTGGGATAACCCTCGGAAACGGGGGCTAATACCGGATATGATCTCCT
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Figure 20. (Continued)

```
BTG2-3          ACCGCATGGTGTTGGGTGGAAAGTTTTTCGGCCTGGGATGGGCTCGCGGCCTATCAGCT
BTG3-4          ACCGCATGGTGTTGGGTGGAAAGTTTTTCGGCCTGGGATGGGCTCGCGGCCTATCAGCT
LK2-6           ACCGCATGGTGTTGGGTGGAAAGTTTTTCGGCCTGGGATGGGCTCGCGGCCTATCAGCT
BTG1-4          ACCGCATGGTGTTGGGTGGAAAGTTTTTCGGCCTGGGATGGGCTCGCGGCCTATCAGCT
KM1-9           ACCGCATGGTGTTGGGTGGAAAGTTTTTCGGCCTGGGATGGGCTCGCGGCCTATCAGCT
LK2-12          ACCGCATGGTGTTGGGTGGAAAGTTTTTCGGCCTGGGATGGGCTCGCGGCCTATCAGCT
KM1-6           ACCGCATGGTGTTGGGTGGAAAGTTTTTCGGCCTGGGATGGGCTCGCGGCCTATCAGCT
BTG4-1          ACCGCATGGTGTTGGGTGGAAAGTTTTTCGGCCTGGGATGGGCTCGCGGCCTATCAGCT
BTG1-1          ACCGCATGGTGTTGGGTGGAAAGTTTTTCGGCCTGGGATGGGCTCGCGGCCTATCAGCT
BTG3-2          ACCGCATGGTTGGTGGTGGAAAGTTTTTCGGCTTGGGATGGGCTCGCGGCCTATCAGCT
X92604          ACCGCATGGTTGGTGGTGGAAAGTTTTTCGGCTTGGGATGGGCTCGCGGCCTATCAGCT
KM3-14          ACCGCATGGTTGGTGGTGGAAAGTTTTTCGGCTTGGGATGGGCTCGCGGCCTATCAGCT
KM3-1           ATCGCATGGTCGGTGGTGGAAAGTTTTTCGGCTTGGGATGGGCTCGCGGCCTATCAGCT
X92594          ACCGCATGGTGTTTGGTGGAAAGTTTTTCGGCTTGGGATGGGCTCGCGCCCTATCAGCT
X92601          ATCGCATGGTTGGTGGTGGAAAGTTTTTCGGCCTGGGATGGGCTCGCGGCCTATCAGCT
X92611          CCCGCATGGGTGGTGGTGGAAAGTTTTTCGGCCTGGGATGGGCTCGCGGCCTATCAGCT
KM4-29          GTCGCATGGTGTGTGGTGGAAAGTTTTTCGGCCTGGGATGGGCTCGCGGCCTATCAGCT
KM4-24          GTCGCATGGTGTGTGGTGGAAAGTTTTTCGGCCTGGGATGGGCTCGCGGCCTATCAGCT
LK6-12          ATCGCATGATGTCTGGTGGAAAGTTTTTCGGCTTGGGATGGGCTCGCGGCCTATCAGCT
LK5-4           ACCGCATGGTGTGTGGTGGAAAGTTTTTCGGCTTGGGATGGGCTCGCGGCCTATCAGCT
TT2-4           CTCGCATGAGGTTTGGTGGAAAGTTTTTCGGCCTGGGATGGGCTCGCGGCCTATCAGCT
BTG10-2         ATCGCATGGTGTGTGGTGGAAAGTTTTTCGGCCTGGGATGGGCTCGCGGCCTATCAGCT
BTG7-3          ATCGCATGGTGTGTGGTGGAAAGTTTTTCGGCCTGGGATGGGCTCGCGGCCTATCAGCT
AF152109        CTCGCATGGGGTTGGGTGGAAAGTTTTTCGGCCTGGGATGGGCTCGCGGCCTATCAGCT
X92599          GTCGCATGACTGGGGGTGGAAAGTTTTTCGGCCTGGGATGGGCTCGCGGCCTATCAGCT
X92613          GCCGCATGGCTGGTGGTGGAAAGTTTTTCGGCCTGGGATGGGCTCGCGGCCTATCAGCT
TT1-11          ATCGCATGGTTGGTGGTGGAAAGTTTTTCGGCCTGGGATGGGCTCGCGGCCTATCAGCT
X92598          GTCGCATGGCTGGTGGTGGAAAGTTTTTCGGCCTGGGATGGGCTCGCGCCCTATCAGCT
X92608          GTCGCATGACTGTTGGTGGAAAGTTTTTCGGCTTGGGATGGGCTCGCGGCCTATCAGCT
X92607          GTCGCATGACTGTTGGTGGAAAGTTTTTCGGCCTGGGATGGGCTCGCGGCCTATCAGCT
X92610          CTCGCATGAGTCTGGGTGGAAAGTTTTTCGGCCTGGGATGGGCTCGCGGCCTATCAGCT
X92609          GCCGCATGGTGGAGGGTGGAAAGTTCTTCGGCTTGGGATGGGCTCGCGGCCTATCAGCT
X92631          TCCGCATGGGGTTGGGTGGAAAGTTTTTCGGCCTGGGATGGGCTCGCGGCCTATCAGCT
X92628          CTCGCATGAGGTTGGGTGGAAAGTTTTTCGGCCTGGGATGGGCTCGCGGCCTATCAGCT
LK2-10          GTCGCATGGTGGGGGGTGGAAAGTTTTTCGGCCTGGGATGGGCTCGCGGCCTATCAGCT
LK2-5           GTCGCATGGTGGGGGGTGGAAAGTTTTTCGGCCTGGGATGGGCTCGCGGCCTATCAGCT
KM1-7           GCCGCATGGTGGGGGGTGGAAAGTTTTTCGGCGTGGGATGGGCTCGCGGCCTATCAGCT
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Figure 20. (Continued)

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BTG2-3          TGTTGGTGGGGTGATGGCCTACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCG
BTG3-4          TGTTGGTGGGGTGATGGCCTACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCG
LK2-6           TGTTGGTGGGGTGATGGCCTACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCG
BTG1-4          TGTTGGTGGGGTGATGGCCTACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCG
KM1-9           TGTTGGTGGGGTGATGGCCTACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCG
LK2-12          TGTTGGTGGGGTGATGGCCTACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCG
KM1-6           TGTTGGTGGGGTGATGGCCTACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCG
BTG4-1          TGTTGGTGGGGTGATGGCCTACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCG
BTG1-1          TGTTGGTGGGGTGATGGCCTACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCG
BTG3-2          TGTTGGTGGGGTGATGGCCTACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCG
X92604          TGTTGGTGGGGTGATGGCCTACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCG
KM3-14          TGTTGGTGGGGTGATGGCCTACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCG
KM3-1           TGTTGGTGGGGTGATGGCCTACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCG
X92594          TGTTGGTGGGGTGATGGCCTACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCG
X92601          TGTTGGTGGGGTGATGGCCTACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCG
X92611          TGTTGGTGGGGTGATGGCCTACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCG
KM4-29          TGTTGGTGGGGTGATGGCCTACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCG
KM4-24          TGTTGGTGGGGTGATGGCCTACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCG
LK6-12          TGTTGGTGGGGTGATGGCCTACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCG
LK5-4           TGTTGGTGGGGTGATGGCCTACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCG
TT2-4           TGTTGGTGGGGTGATGGCCTACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCG
BTG10-2         TGTTGGTGGGGTGATGGCCTACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCG
BTG7-3          TGTTGGTGGGGTGATGGCCTACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCG
AF152109        TGTTGGTGGGGTGATGGCCTACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCG
X92599          TGTTGGTGGGGTGATGGCCTACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCG
X92613          TGTTGGTGGGGTGATGGCCTACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCG
TT1-11          TGTTGGTGGGGTGATGGCCTACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCG
X92598          TGTTGGTGGGGTGATGGCCTACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCG
X92608          TGTTGGTGGGGTGATGGCCTACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCG
X92607          TGTTGGTGGGGTGATGGCCTACCAAGCCGACGACGGGTAGCCGGCCTGAGAGGGCGACCG
X92610          TGTTGGTGGGGTGATGGCCTACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCG
X92609          TGTTGGTGGGGTGATGGCCTACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCG
X92631          TGTTGGTGGGGTGATGGCCTACCAAGCCGACGACGGGTAGCCGGCCTGAGAGGGCGACCG
X92628          TGTTGGTGGGGTGATGGCCTACCAAGCCGACGACGGGTAGCCGGCCTGAGAGGGCGACCG
LK2-10          TGTTGGTGGGGTGATGGCCTACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCG
LK2-5           TGTTGGTGGGGTGATGGCCTACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCG
KM1-7           TGTTGGTGGGGTGATGGCCTACCAAGGCGGTGACGGGTAGCCGGCCTGAGAGGGCGACCG
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Figure 20. (Continued)

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BTG2-3          GCCACACTGGGACTGAGAACGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTG
BTG3-4          GCCACACTGGGACTGAGAACGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTG
LK2-6           GCCACACTGGGACTGAGAACGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTG
BTG1-4          GCCACACTGGGACTGAGAACGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTG
KM1-9           GCCACACTGGGACTGAGAACGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTG
LK2-12          GCCACACTGGGACTGAGAACGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTG
KM1-6           GCCACACTGGGACTGAGAACGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTG
BTG4-1          GCCACACTGGGACTGAGAACGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTG
BTG1-1          GCCACACTGGGACTGAGAACGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTG
BTG3-2          GCCACACTGGGACTGAGAACGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTG
X92604          GCCACACTGGGACTGAGAACGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTG
KM3-14          GCCACACTGGGACTGAGAACGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTG
KM3-1           GCCACACTGGGACTGAGAACGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTG
X92594          GCCACACTGGGACTGAGAACGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTG
X92601          GCCACACTGGGACTGAGAACGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTG
X92611          GCCACACTGGGACTGAGAACGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTG
KM4-29          GCCACACTGGGACTGAGAACGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTG
KM4-24          GCCACACTGGGACTGAGAACGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTG
LK6-12          GCCACACTGGGACTGAGAACGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTG
LK5-4           GCCACACTGGGACTGAGAACGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTG
TT2-4           GCCACACTGGGACTGAGAACGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTG
BTG10-2         GCCACACTGGGACTGAGAACGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTG
BTG7-3          GCCACACTGGGACTGAGAACGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTG
AF152109        GCCACACTGGGACTGAGAACGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTG
X92599          GCCACACTGGGACTGAGAACGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTG
X92613          GCCACACTGGGACTGAGAACGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTG
TT1-11          GCCACACTGGGACTGAGAACGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTG
X92598          GCCACACTGGGACTGAGAACGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTG
X92608          GCCACACTGGGACTGAGAACGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTG
X92607          GCCACACTGGGACTGAGAACGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTG
X92610          GCCACACTGGGACTGAGAACGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTG
X92609          GCCACACTGGGACTGAGAACGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTG
X92631          GCCACACTGGGACTGAGAACGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTG
X92628          GCCACACTGGGACTGAGAACGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTG
LK2-10          GCCACACTGGGACTGAGAACGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTG
LK2-5           GCCACACTGGGACTGAGAACGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTG
KM1-7           GCCACACTGGGACTGAGAACGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTG
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Figure 20. (Continued)

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BTG2-3          CACAATGGGCGGAAGCCTGATGCACGACCCGCGTGAGGGATGACGGCCTTCGGGTTG
BTG3-4          CACAATGGGCGGAAGCCTGATGCACGACCCGCGTGAGGGATGACGGCCTTCGGGTTG
LK2-6           CACAATGGGCGGAAGCCTGATGCACGACCCGCGTGAGGGATGACGGCCTTCGGGTTG
BTG1-4          CACAATGGGCGGAAGCCTGATGCACGACCCGCGTGAGGGATGACGGCCTTCGGGTTG
KM1-9           CACAATGGGCGGAAGCCTGATGCACGACCCGCGTGAGGGATGACGGCCTTCGGGTTG
LK2-12          CACAATGGGCGGAAGCCTGATGCACGACCCGCGTGAGGGATGACGGCCTTCGGGTTG
KM1-6           CACAATGGGCGGAAGCCTGATGCACGACCCGCGTGAGGGATGACGGCCTTCGGGTTG
BTG4-1          CACAATGGGCGGAAGCCTGATGCACGACCCGCGTGAGGGATGACGGCCTTCGGGTTG
BTG1-1          CACAATGGGCGGAAGCCTGATGCACGACCCGCGTGAGGGATGACGGCCTTCGGGTTG
BTG3-2          CACAATGGGCGGAAGCCTGATGCACGACCCGCGTGAGGGATGACGGCCTTCGGGTTG
X92604          CACAATGGGCGGAAGCCTGATGCACGACCCGCGTGAGGGATGACGGCCTTCGGGTTG
KM3-14          CACAATGGGCGGAAGCCTGATGCACGACCCGCGTGAGGGATGACGGCCTTCGGGTTG
KM3-1           CACAATGGGCGGAAGCCTGATGCACGACCCGCGTGAGGGATGACGGCCTTCGGGTTG
X92594          CACAATGGGCGGAAGCCTGATGCACGACCCGCGTGAGGGATGACGGCCTTCGGGTTG
X92601          CACAATGGGCGGAAGCCTGATGCACGACCCGCGTGAGGGATGACGGCCTTCGGGTTG
X92611          CACAATGGGCGGAAGCCTGATGCACGACCCGCGTGAGGGATGACGGCCTTCGGGTTG
KM4-29          CACAATGGGCGGAAGCCTGATGCACGACCCGCGTGAGGGATGACGGCCTTCGGGTTG
KM4-24          CACAATGGGCGGAAGCCTGATGCACGACCCGCGTGAGGGATGACGGCCTTCGGGTTG
LK6-12          CACAATGGGCGGAAGCCTGATGCACGACCCGCGTGAGGGATGACGGCCTTCGGGTTG
LK5-4           CACAATGGGCGGAAGCCTGATGCACGACCCGCGTGAGGGATGACGGCCTTCGGGTTG
TT2-4           CACAATGGGCGGAAGCCTGATGCACGACCCGCGTGAGGGATGACGGCCTTCGGGTTG
BTG10-2         CACAATGGGCGGAAGCCTGATGCACGACCCGCGTGAGGGATGACGGCCTTCGGGTTG
BTG7-3          CACAATGGGCGGAAGCCTGATGCACGACCCGCGTGAGGGATGACGGCCTTCGGGTTG
AF152109        CACAATGGGCGGAAGCCTGATGCACGACCCGCGTGAGGGATGACGGCCTTCGGGTTG
X92599          CACAATGGGCGGAAGCCTGATGCACGACCCGCGTGAGGGATGACGGCCTTCGGGTTG
X92613          CACAATGGGCGGAAGCCTGATGCACGACCCGCGTGAGGGATGACGGCCTTCGGGTTG
TT1-11          CACAATGGGCGGAAGCCTGATGCACGACCCGCGTGAGGGATGACGGCCTTCGGGTTG
X92598          CACAATGGGCGGAAGCCTGATGCACGACCCGCGTGAGGGATGACGGCCTTCGGGTTG
X92608          CACAATGGGCGGAAGCCTGATGCACGACCCGCGTGAGGGATGACGGCCTTCGGGTTG
X92607          CACAATGGGCGGAAGCCTGATGCACGACCCGCGTGAGGGATGACGGCCTTCGGGTTG
X92610          CACAATGGGCGGAAGCCTGATGCACGACCCGCGTGAGGGATGACGGCCTTCGGGTTG
X92609          CACAATGGGCGGAAGCCTGATTCACGACCCGCGTGAGGGATGACGGCCTTCGGGTTG
X92631          CACAATGGGCGGAAGCCTGATGCACGACCCGCGTGAGGGATGACGGCCTTCGGGTTG
X92628          CACAATGGGCGGAAGCCTGATGCACGACCCGCGTGAGGGATGACGGCCTTCGGGTTG
LK2-10          CACAATGGGCGGAAGCCTGATGCACGACCCGCGTGAGGGATGACGGCCTTCGGGTTG
LK2-5           CACAATGGGCGGAAGCCTGATGCACGACCCGCGTGAGGGATGACGGCCTTCGGGTTG
KM1-7           CACAATGGGCGGAAGCCTGATGCACGACCCGCGTGAGGGATGACGGCCTTCGGGTTG
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Figure 20. (Continued)

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BTG2-3          TAACCTCTTTCAGCAGGGACGAAGCGTAAGTGACGGTACCTGCAGAAGAAGCGCCGG
BTG3-4          TAACCTCTTTCAGCAGGGACGAAGCGTAAGTGACGGTACCTGCAGAAGAAGCGCCGG
LK2-6           TAACCTCTTTCAGCAGGGACGAAGCGTAAGTGACGGTACCTGCAGAAGAAGCGCCGG
BTG1-4          TAACCTCTTTCAGCAGGGACGAAGCGTAAGTGACGGTACCTGCAGAAGAAGCGCCGG
KM1-9           TAACCTCTTTCAGCAGGGACGAAGCGTAAGTGACGGTACCTGCAGAAGAAGCGCCGG
LK2-12          TAACCTCTTTCAGCAGGGACGAAGCGTAAGTGACGGTACCTGCAGAAGAAGCGCCGG
KM1-6           TAACCTCTTTCAGCAGGGACGAAGCGTAAGTGACGGTACCTGCAGAAGAAGCGCCGG
BTG4-1          TAACCTCTTTCAGCAGGGACGAAGCGTAAGTGACGGTACCTGCAGAAGAAGCGCCGG
BTG1-1          TAACCTCTTTCAGCAGGGACGAAGCGTAAGTGACGGTACCTGCAGAAGAAGCGCCGG
BTG3-2          TAACCTCTTTCAGCAGGGACGAAGCGTAAGTGACGGTACCTGCAGAAGAAGCGCCGG
X92604          TAACCTCTTTCAGCAGGGACGAAGCGTAAGTGACGGTACCTGCAGAAGAAGCGCCGG
KM3-14          TAACCTCTTTCAGCAGGGACGAAGCGTAAGTGACGGTACCTGCAGAAGAAGCGCCGG
KM3-1           TAACCTCTTTCAGCAGGGACGAAGCGTAAGTGACGGTACCTGCAGAAGAAGCGCCGG
X92594          TAACCTCTTTCAGCAGGGACGAAGCGTAAGTGACGGTACCTGCAGAAGAAGCGCCGG
X92601          TAACCTCTTTCAGCAGGGACGAAGCGTAAGTGACGGTACCTGCAGAAGAAGCGCCGG
X92611          TAACCTCTTTCAGCAGGGACGAAGCGTAAGTGACGGTACCTGCAGAAGAAGCGCCGG
KM4-29          TAACCTCTTTCAGCAGGGACGAAGCGTAAGTGACGGTACCTGCAGAAGAAGCGCCGG
KM4-24          TAACCTCTTTCAGCAGGGACGAAGCGTAAGTGACGGTACCTGCAGAAGAAGCGCCGG
LK6-12          TAACCTCTTTCAGCAGGGACGAAGCGCAAGTGACGGTACCTGCAGAAGAAGCACCGG
LK5-4           TAACCTCTTTCAGCAGGGACGAAGCGTAAGTGACGGTACCTGCAGAAGAAGCGCCGG
TT2-4           TAACCTCTTTCAGCAGGGACGAAGCGTAAGTGACGGTACCTGCAGAAGAAGCACCGG
BTG10-2         TAACCTCTTTCAGCAGGGACGAAGCGTAAGTGACGGTACCTGCAGAAGAAGCGCCGG
BTG7-3          TAACCTCTTTCAGCAGGGACGAAGCGTAAGTGACGGTACCTGCAGAAGAAGCGCCGG
AF152109        TAACCTCTTTCAGCAGGGACGAAGCGTAAGTGACGGTACCTGCAGAAGAAGCACCGG
X92599          TAACCTCTTTCAGCAGGGACGAAGCGCAAGTGACGGTACCTGCAGAAGAAGCGCCGG
X92613          TAACCTCTTTCAGCAGGGACGAAGCGCAAGTGACGGTACCTGCAGAAGAAGCGCCGG
TT1-11          TAACCTCTTTCAGCAGGGACGAAGCGAAAGTGACGGTACCTGCAGAAGAAGCGCCGG
X92598          TAACCTCTTTCAGCAGGGACGAAGCGAAAGTGACGGTACCTGCAGAAGAAGCACCGG
X92608          TAACCTCTTTCAGCAGGGACGAAGCGCAAGTGACGGTACCTGCAGAAGAAGCGCCGG
X92607          TAACCTCTTTCAGCAGGGACGAAGCGTAAGTGACGGTACCTGCAGAAGAAGCGCCAG
X92610          TAACCTCTTTCAGCAGGGACGAAGCGTAAGTGACGGTACCTGCAGAAGAAGCGCCGG
X92609          TAACCTCTTTCAGCAGGGACGAAGCGCAAGTGACGGTACCTGCAGAAGAAGCGCCGG
X92631          TAACCTCTTTCAGCAGGGACGAAGCGCAAGTGACGGTACCTGCAGAAGAAGCGCCGG
X92628          TAACCTCTTTCAGCAGGGACGAAGCGTAAGTGACGGTACCTGCAGAAGAAGCGCCGG
LK2-10          TAACCTCTTTCAGCAGGGACGAAGCGTAAGTGACGGTACCTGCAGAAGAAGCGCCGG
LK2-5           TAACCTCTTTCAGCAGGGACGAAGCGTAAGTGACGGTACCTGCAGAAGAAGCGCCGG
KM1-7           TAACCTCTTTCAGTAGGGACGAAGCGGAAGTGACGGTACCTACAGAAGAAGCGCCGG
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Figure 20. (Continued)

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BTG2-3          CAACTACGTGCCAGCAGCCGCGGTAAGACGTAGGGCGCGAGCGTTGTCCGGATTTATTGG
BTG3-4          CAACTACGTGCCAGCAGCCGCGGTAAGACGTAGGGCGCGAGCGTTGTCCGGATTTATTGG
LK2-6           CAACTACGTGCCAGCAGCCGCGGTAAGACGTAGGGCGCGAGCGTTGTCCGGATTTATTGG
BTG1-4          CAACTACGTGCCAGCAGCCGCGGTAAGACGTAGGGCGCGAGCGTTGTCCGGATTTATTGG
KM1-9           CAACTACGTGCCAGCAGCCGCGGTAAGACGTAGGGCGCGAGCGTTGTCCGGATTTATTGG
LK2-12          CAACTACGTGCCAGCAGCCGCGGTAAGACGTAGGGCGCGAGCGTTGTCCGGATTTATTGG
KM1-6           CAACTACGTGCCAGCAGCCGCGGTAAGACGTAGGGCGCGAGCGTTGTCCGGATTTATTGG
BTG4-1          CAACTACGTGCCAGCAGCCGCGGTAAGACGTAGGGCGCGAGCGTTGTCCGGATTTATTGG
BTG1-1          CAACTACGTGCCAGCAGCCGCGGTAAGACGTAGGGCGCGAGCGTTGTCCGGATTTATTGG
BTG3-2          CAACTACGTGCCAGCAGCCGCGGTAAGACGTAGGGCGCGAGCGTTGTCCGGATTTATTGG
X92604          CAACTACGTGCCAGCAGCCGCGGTAAGACGTAGGGCGCGAGCGTTGTCCGGATTTATTGG
KM3-14          CAACTACGTGCCAGCAGCCGCGGTAAGACGTAGGGCGCGAGCGTTGTCCGGATTTATTGG
KM3-1           CAACTACGTGCCAGCAGCCGCGGTAAGACGTAGGGCGCGAGCGTTGTCCGGATTTATTGG
X92594          CAACTACGTGCCAGCAGCCGCGGTAAGACGTAGGGCGCGAGCGTTGTCCGGATTTATTGG
X92601          CAACTACGTGCCAGCAGCCGCGGTAAGACGTAGGGCGCGAGCGTTGTCCGGATTTATTGG
X92611          CAACTACGTGCCAGCAGCCGCGGTAAGACGTAGGGCGCGAGCGTTGTCCGGATTTATTGG
KM4-29          CAACTACGTGCCAGCAGCCGCGGTAAGACGTAGGGCGCGAGCGTTGTCCGGATTTATTGG
KM4-24          CAACTACGTGCCAGCAGCCGCGGTAAGACGTAGGGCGCGAGCGTTGTCCGGATTTATTGG
LK6-12          CAACTACGTGCCAGCAGCCGCGGTAAGACGTAGGGTGCGAGCGTTGTCCGGATTTATTGG
LK5-4           CAACTACGTGCCAGCAGCCGCGGTAAGACGTAGGGCGCGAGCGTTGTCCGGATTTATTGG
TT2-4           CAACTACGTGCCAGCAGCCGCGGTAAGACGTAGGGTGCGAGCGTTGTCCGGATTTATTGG
BTG10-2         CAACTACGTGCCAGCAGCCGCGGTAAGACGTAGGGCGCGAGCGTTGTCCGGATTTATTGG
BTG7-3          CAACTACGTGCCAGCAGCCGCGGTAAGACGTAGGGCGCGAGCGTTGTCCGGATTTATTGG
AF152109        CAACTACGTGCCAGCAGCCGCGGTAAGACGTAGGGTGCGAGCGTTGTCCGGATTTATTGG
X92599          CAACTACGTGCCAGCAGCCGCGGTAAGACGTAGGGCGCGAGCGTTGTCCGGATTTATTGG
X92613          CAACTACGTGCCAGCAGCCGCGGTAAGACGTAGGGCGCGAGCGTTGTCCGGATTTATTGG
TT1-11          CAACTACGTGCCAGCAGCCGCGGTAAGACGTAGGGCGCGAGCGTTGTCCGGATTTATTGG
X92598          CAACTACGTGCCAGCAGCCGCGGTAAGACGTAGGGTGCGAGCGTTGTCCGGAATTATTGG
X92608          CAACTACGTGCCAGCAGCCGCGGTAAGACGTAGGGCGCGAGCGTTGTCCGGATTTATTGG
X92607          CAACTACGTGCCAGCAGCCGCGGTAAGACGTAGGGCGCGAGCGTTGTCCGGATTTATTGG
X92610          CAACTACGTGCCAGCAGCCGCGGTAAGACGTAGGGCGCGAGCGTTGTCCGGATTTATTGG
X92609          CAACTACGTGCCAGCAGCCGCGGTAAGACGTAGGGCGCGAGCGTTGTCCGGATTTATTGG
X92631          CAACTACGTGCCAGCAGCCGCGGTAAGACGTAGGGCGCGAGCGTTGTCCGGATTTATTGG
X92628          CAACTACGTGCCAGCAGCCGCGGTAAGACGTAGGGCGCGAGCGTTGTCCGGATTTATTGG
LK2-10          CAACTACGTGCCAGCAGCCGCGGTAAGACGTAGGGCGCGAGCGTTGTCCGGATTTATTGG
LK2-5           CAACTACGTGCCAGCAGCCGCGGTAAGACGTAGGGCGCGAGCGTTGTCCGGATTTATTGG
KM1-7           CAACTACGTGCCAGCAGCCGCGGTAAGACGTAGGGCGCGAGCGTTGTCCGGATTTATTGG
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Figure 20. (Continued)

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BTG2-3          GCGTAAAGAGCTCGTAGGCGGCTTGTCGCGTCGACTGTGAAAACCCACGGCTCAACCGTG
BTG3-4          GCGTAAAGAGCTCGTAGGCGGCTTGTCGCGTCGACTGTGAAAACCCACGGCTCAACCGTG
LK2-6           GCGTAAAGAGCTCGTAGGCGGCTTGTCGCGTCGACTGTGAAAACCCACGGCTCAACCGTG
BTG1-4          GCGTAAAGAGCTCGTAGGCGGCTTGTCGCGTCGACTGTGAAAACCCACGGCTCAACCGTG
KM1-9           GCGTAAAGAGCTCGTAGGCGGCTTGTCGCGTCGACTGTGAAAACCCACGGCTCAACCGTG
LK2-12          GCGTAAAGAGCTCGTAGGCGGCTTGTCGCGTCGACTGTGAAAACCCACGGCTCAACCGTG
KM1-6           GCGTAAAGAGCTCGTAGGCGGCTTGTCGCGTCGACTGTGAAAACCCACGGCTCAACCGTG
BTG4-1          GCGTAAAGAGCTCGTAGGCGGCTTGTCGCGTCGACTGTGAAAACCCACGGCTCAACCGTG
BTG1-1          GCGTAAAGAGCTCGTAGGCGGCTTGTCGCGTCGACTGTGAAAACCCACGGCTCAACCGTG
BTG3-2          GCGTAAAGAGCTCGTAGGCGGCTTGTCGCGTCGACCGTGAAAACTTGGGGCTCAACCCCA
X92604          GCGTAAAGAGCTCGTAGGCGGCTTGTCGCGTCGACCGTGAAAACTTGGGGCTCAACCCCA
KM3-14          GCGTAAAGAGCTCGTAGGCGGCTTGTCGCGTCGACCGTGAAAACTTGGGGCTCAACCCCA
KM3-1           GCGTAAAGAGCTCGTAGGCGGCTTGTCGCGTCGACCGTGAAAACCTGGGGCTCAACCCCA
X92594          GCGTAAAGAGCTCGTAGGCGGCTTGTCGCGTCGACCGTGAAAACCTGGGGCTCAACCCCA
X92601          GCGTAAAGAGCTCGTAGGCGGCTTGTCGCGTCGACCGTGAAAACTTGGGGCTCAACCCCA
X92611          GCGTAAAGAGCTCGTAGGCGGCTTGTCGCGTCGACCGTGAAAACTTGGGGCTCAACCCCA
KM4-29          GCGTAAAGAGCTCGTAGGCGGCTTGTCGCGTCGACCGTGAAAACCTGGGGCTCAACCCCA
KM4-24          GCGTAAAGAGCTCGTAGGCGGCTTGTCGCGTCGACCGTGAAAACCTGGGGCTCAACCCCA
LK6-12          GCGTAAAGAGCTCGTAGGCGGCTTGTCGCGTCGACCGTGAAAACTTGGGGCTCAACTCCA
LK5-4           GCGTAAAGAGCTCGTAGGCGGCTTGTCGCGTCGACCGTGAAAACCTGGGGCTCAACCCCA
TT2-4           GCGTAAAGAGCTCGTAGGCGGCTTGTCGCGTCGACCGTGAAAACTTGGGGCTCAACTCCA
BTG10-2         GCGTAAAGAGCTCGTAGGCGGCTTGTCGCGTCGACCGTGAAAACCTGGGGCTCAACCCCA
BTG7-3          GCGTAAAGAGCTCGTAGGCGGCTTGTCGCGTCGACCGTGAAAACCTGGGGCTCAACCCCA
AF152109        GCGTAAAGAGCTCGTAGGCGGCTTGTCGCGTCGACCGTGAAAACTTGGGGCTCAACCCCA
X92599          GCGTAAAGAGCTCGTAGGCGGCTTGTCGCGTCGACCGTGAAAACTTGGGGCTCAACCCCA
X92613          GCGTAAAGAGCTCGTAGGCGGCTTGTCGCGTCGACTGTGAAAACCCGCGGCTCAACTGCG
TT1-11          GCGTAAAGAGCTCGTAGGCGGCTTGTCGCGTCGACTGTGAAAACCCGCAGCTCAACTGCG
X92598          GCGTAAAGAGCTCGTAGGCGGCTTGTCGCGTCGACTGTGAAAACCCGCAGCTCAACTGCG
X92608          GCGTAAAGAGCTCGTAGGCGGCTTGTCGCGTCGACTGTGAAAACCCGCGGCTCAACTGCG
X92607          GCGTAAAGAGCTCGTAGGCGGCTTGTCGCGTCGACTGTGAAAACCCGCAGCTCAACTGCG
X92610          GCGTAAAGAGCTCGTAGGCGGCTTGTCGCGTCGACTGTGAAAACCCGCAGCTCAACTGCG
X92609          GCGTAAAGAGCTCGTAGGCGGCTTGTCGCGTCGACTGTGAAAACCCGCAGCTCAACTGCG
X92631          GCGTAAAGAGCTCGTAGGCGGCTTGTCGCGTCGACTGTGAAAACCCGCGGCTCAACTGCG
X92628          GCGTAAAGAGCTCGTAGGCGGCTTGTCGCGTCGACTGTGAAAACCCGCAGCTCAACTGCG
LK2-10          GCGTAAAGAGCTCGTAGGCGGCTTGTCGCGTCGACTGTGAAAACCCGCGGCTCAACCGCG
LK2-5           GCGTAAAGAGCTCGTAGGCGGCTTGTCGCGTCGACTGTGAAAACCCGCGGCTCAACCGCG
KM1-7           GCGTAAAGAGCTCGTAGGCGGCTTGTCGCGTCGACTGTGAAAACCCGCGGCTCAACTGCG
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Figure 20. (Continued)

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BTG2-3          GGCCTGCAGTCATACGGGCAGGCTAGAGTTCGGTAGGGGAGACTGAATTCCTGGTGTA
BTG3-4          GGCCTGCAGTCATACGGGCAGGCTAGAGTTCGGTAGGGGAGACTGAATTCCTGGTGTA
LK2-6           GGCCTGCAGTCATACGGGCAGGCTAGAGTTCGGTAGGGGAGACTGAATTCCTGGTGTA
BTG1-4          GGCCTGCAGTCATACGGGCAGGCTAGAGTTCGGTAGGGGAGACTGAATTCCTGGTGTA
KM1-9           GGCCTGCAGTCATACGGGCAGGCTAGAGTTCGGTAGGGGAGACTGAATTCCTGGTGTA
LK2-12          GGCCTGCAGTCATACGGGCAGGCTAGAGTTCGGTAGGGGAGACTGAATTCCTGGTGTA
KM1-6           GGCCTGCAGTCATACGGGCAGGCTAGAGTTCGGTAGGGGAGACTGAATTCCTGGTGTA
BTG4-1          GGCCTGCAGTCATACGGGCAGGCTAGAGTTCGGTAGGGGAGACTGAATTCCTGGTGTA
BTG1-1          GGCCTGCAGTCATACGGGCAGGCTAGAGTTCGGTAGGGGAGACTGAATTCCTGGTGTA
BTG3-2          AGCCTGCGGTCATACGGGCAGGCTAGAGTTCGGTAGGGGAGACTGAATTCCTGGTGTA
X92604          AGCCTGCGGTCATACGGGCAGGCTAGAGTTCGGTAGGGGAGACTGAATTCCTGGTGTA
KM3-14          AGCCTGCGGTCATACGGGCAGGCTAGAGTTCGGTAGGGGAGACTGAATTCCTGGTGTA
KM3-1           GGCCTGCGGTCATACGGGCAGGCTAGAGTTCGGTAGGGGAGACTGAATTCCTGGTGTA
X92594          GGCCTGCGGTCATACGGGCAGGCTAGAGTTCGGTAGGGGAGACTGAATTCCTGGTGTA
X92601          AGCCTGCGGTCATACGGGCAGGCTAGAGTTCGGTAGGGGAGACTGAATTCCTGGTGTA
X92611          AGCCTGCGGTCATACGGGCAGGCTAGAGTTCGGTAGGGGAGACTGAATTCCTGGTGTA
KM4-29          GGCCTGCGGTCATACGGGCAGGCTAGAGTTCGGTAGGGGAGACTGAATTCCTGGTGTA
KM4-24          GGCCTGCGGTCATACGGGCAGGCTAGAGTTCGGTAGGGGAGACTGAATTCCTGGTGTA
LK6-12          AGCCTGCGGTCATACGGGCAGGCTAGAGTTCGGTAGGGGAGACTGAATTCCTGGTGTA
LK5-4           GGCCTGCGGTCATACGGGCAGGCTAGAGTTCGGTAGGGGAGACTGAATTCCTGGTGTA
TT2-4           AGCCTGCGGTCATACGGGCAGGCTAGAGTTCGGTAGGGGAGACTGAATTCCTGGTGTA
BTG10-2         GGCCTGCGGTCATACGGGCAGGCTAGAGTTCGGTAGGGGAGACTGAATTCCTGGTGTA
BTG7-3          GGCCTGCGGTCATACGGGCAGGCTAGAGTTCGGTAGGGGAGACTGAATTCCTGGTGTA
AF152109        AGCCTGCGGTCATACGGGCAGGCTAGAGTTCAGTAGGGGAGACTGAATTCCTGGTGTA
X92599          AGCCTGCGGTCATACGGGCAGGCTAGAGTTCGGTAGGGGAGACTGAATTCCTGGTGTA
X92613          GGCCTGCAGTCATACGGGCAGGCTAGAGTTCGGTAGGGGAGACTGAATTCCTGGTGTA
TT1-11          GGCCTGCAGTCATACGGGCAGGCTAGAGTTCGGTAGGGGAGACTGAATTCCTGGTGTA
X92598          GGCCTGCAGTCATACGGGCAGGCTAGAGTTCGGTAGGGGAGACTGAATTCCTGGTGTA
X92608          GGCCTGCAGTCATACGGGCAGGCTAGAGTTCGGTAGGGGAGACTGAATTCCTGGTGTA
X92607          GGCCTGCAGTCATACGGGCAGGCTAGAGTTCGGTAGGGGAGACTGAATTCCTGGTGTA
X92610          GGCCTGCAGTCATACGGGCAGGCTAGAGTTCGGTAGGGGAGACTGAATTCCTGGTGTA
X92609          GGCCTGCAGTCATACGGGCAGGCTAGAGTTCGGTAGGGGAGACTGAATTCCTGGTGTA
X92631          GGCCTGCAGTCATACGGGCAGGCTAGAGTTCGGTAGGGGAGACTGAATTCCTGGTGTA
X92628          GGCCTGCAGTCATACGGGCAGGCTAGAGTTCGGTAGGGGAGACTGAATTCCTGGTGTA
LK2-10          GGCCTGCAGTCATACGGGCAGGCTAGAGTTCGGTAGGGGAGACTGAATTCCTGGTGTA
LK2-5           GGCCTGCAGTCATACGGGCAGGCTAGAGTTCGGTAGGGGAGACTGAATTCCTGGTGTA
KM1-7           GGCTTGCAGTCATACGGGCAGGCTAGAGTTCGGTAGGGGAGACTGAATTCCTGGTGTA
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Figure 20. (Continued)

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BTG2-3          GCGGTGAAATGCGCAGATATAGGAGGAACACCGGTGGCGAAGCGGGTCTTGGGCCGA
BTG3-4          GCGGTGAAATGCGCAGATATAGGAGGAACACCGGTGGCGAAGCGGGTCTTGGGCCGA
LK2-6           GCGGTGAAATGCGCAGATATAGGAGGAACACCGGTGGCGAAGCGGGTCTTGGGCCGA
BTG1-4          GCGGTGAAATGCGCAGATATAGGAGGAACACCGGTGGCGAAGCGGGTCTTGGGCCGA
KM1-9           GCGGTGAAATGCGCAGATATAGGAGGAACACCGGTGGCGAAGCGGGTCTTGGGCCGA
LK2-12          GCGGTGAAATGCGCAGATATAGGAGGAACACCGGTGGCGAAGCGGGTCTTGGGCCGA
KM1-6           GCGGTGAAATGCGCAGATATAGGAGGAACACCGGTGGCGAAGCGGGTCTTGGGCCGA
BTG4-1          GCGGTGAAATGCGCAGATATAGGAGGAACACCGGTGGCGAAGCGGGTCTTGGGCCGA
BTG1-1          GCGGTGAAATGCGCAGATATAGGAGGAACACCGGTGGCGAAGCGGGTCTTGGGCCGA
BTG3-2          GCGGTGAAATGCGCAGATATAGGAGGAACACCGGTGGCGAAGCGGGTCTTGGGCCGA
X92604          GCGGTGAAATGCGCAGATATAGGAGGAACACCGGTGGCGAAGCGGGTCTTGGGCCGA
KM3-14          GCGGTGAAATGCGCAGATATAGGAGGAACACCGGTGGCGAAGCGGGTCTTGGGCCGA
KM3-1           GCGGTGAAATGCGCAGATATAGGAGGAACACCGGTGGCGAAGCGGGTCTTGGGCCGA
X92594          GCGGTGAAATGCGCAGATATAGGAGGAACACCGGTGGCGAAGCGGGTCTTGGGCCGA
X92601          GCGGTGAAATGCGCAGATATAGGAGGAACACCGGTGGCGAAGCGGGTCTTGGGCCGA
X92611          GCGGTGAAATGCGCAGATATAGGAGGAACACCGGTGGCGAAGCGGGTCTTGGGCCGA
KM4-29          GCGGTGAAATGCGCAGATATAGGAGGAACACCGGTGGCGAAGCGGGTCTTGGGCCGA
KM4-24          GCGGTGAAATGCGCAGATATAGGAGGAACACCGGTGGCGAAGCGGGTCTTGGGCCGA
LK6-12          GCGGTGAAATGCGCAGATATANGAGGAACACCGGTGGCGAAGCGGGTCTTGGGCCGA
LK5-4           GCGGTGAAATGCGCAGATATAGGAGGAACACCGGTGGCGAAGCGGGTCTTGGGCCGA
TT2-4           GCGGTGAAATGCGCAGATATAGGAGGAACACCGGTGGCGAAGCGGGTCTTGGGCCGA
BTG10-2         GCGGTGAAATGCGCAGATATAGGAGGAACACCGGTGGCGAAGCGGGTCTTGGGCCGA
BTG7-3          GCGGTGAAATGCGCAGATATAGGAGGAACACCGGTGGCGAAGCGGGTCTTGGGCCGA
AF152109        GCGGTGAAATGCGCAGATATAGGAGGAACACCGGTGGCGAAGCGGGTCTTGGGCCGA
X92599          GCGGTGAAATGCGCAGATATAGGAGGAACACCGGTGGCGAAGCGGGTCTTGGGCCGA
X92613          GCGGTGAAATGCGCAGATATAGGAGGAACACCGGTGGCGAAGCGGGTCTTGGGCCGA
TT1-11          GCGGTGAAATGCGCAGATATAGGAGGAACACCGGTGGCGAAGCGGGTCTTGGGCCGA
X92598          GCGGTGAAATGCGCAGATATAGGAGGAACACCGATGGCGAAGCAGGTCTTGGGCCGA
X92608          GCGGTGAAATGCGCAGATATAGGAGGAACACCGGTGGCGAAGCGGGTCTTGGGCCGA
X92607          GCGGTGAAATGCGCAGATATAGGAGGAACACCGGTGGCGAAGCGGGTCTTGGGCCGA
X92610          GCGGTGAAATGCGCAGATATAGGAGGAACAACGGTGGCGAAGCGGGTCTTGGGCCGA
X92609          GCGGTGAAATGCGCAGATATAGGAGGAACACCGGTGGCGAAGCGGGTCTTGGGCCGA
X92631          GCGGTGAAATGCGCAGATATAGGAGGAACACCGGTGGCGAAGCGGGTCTTGGGCCGA
X92628          GCGGTGAAATGCGCAGATATAGGAGGAACACCGGTGGCGAAGCGGGTCTTGGGCCGA
LK2-10          GCGGTGAAATGCGCAGATATAGGAGGAACACCGGTGGCGAAGCGGGTCTTGGGCCGA
LK2-5           GCGGTGAAATGCGCAGATATAGGAGGAACACCGGTGGCGAAGCGGGTCTTGGGCCGA
KM1-7           GCGGTGAAATGCGCAGATATAGGAGGAACACCGGTGGCGAAGCGGGTCTTGGGCCGA
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Figure 20. (Continued)

```
BTG2-3          TACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCC
BTG3-4          TACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCC
LK2-6           TACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCC
BTG1-4          TACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCC
KM1-9           TACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCC
LK2-12          TACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCC
KM1-6           TACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCC
BTG4-1          TACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCC
BTG1-1          TACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCC
BTG3-2          TACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCC
X92604          TACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCC
KM3-14          TACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCC
KM3-1           TACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCC
X92594          TACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCC
X92601          TACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCC
X92611          TACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCC
KM4-29          TACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCC
KM4-24          TACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCC
LK6-12          TACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCC
LK5-4           TACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCC
TT2-4           TACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCC
BTG10-2         TACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCC
BTG7-3          TACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCC
AF152109        TACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCC
X92599          TACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCC
X92613          TACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCC
TT1-11          TACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCC
X92598          TACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCC
X92608          TACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCC
X92607          TACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCC
X92610          TACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCC
X92609          TACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCC
X92631          TACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCC
X92628          TACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCC
LK2-10          TACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCC
LK2-5           TACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCC
KM1-7           TACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCC
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Figure 20. (Continued)

```
BTG2-3          ACGCTGTAACGTTGGGCGCTAGGTGTGGGGGGCCTCTCCGGTTCCTGTGCCGCACT
BTG3-4          ACGCTGTAACGTTGGGCGCTAGGTGTGGGGGGCCTCTCCGGTTCCTGTGCCGCACT
LK2-6           ACGCTGTAACGTTGGGCGCTAGGTGTGGGGGGCCTCTCCGGTTCCTGTGCCGCACT
BTG1-4          ACGCTGTAACGTTGGGCGCTAGGTGTGGGGGGCCTCTCCGGTTCCTGTGCCGCACT
KM1-9           ACGCTGTAACGTTGGGCGCTAGGTGTGGGGGGCCTCTCCGGTTCCTGTGCCGCACT
LK2-12          ACGCTGTAACGTTGGGCGCTAGGTGTGGGGGGCCTCTCCGGTTCCTGTGCCGCACT
KM1-6           ACGCTGTAACGTTGGGCGCTAGGTGTGGGGGGCCTCTCCGGTTCCTGTGCCGCACT
BTG4-1          ACGCTGTAACGTTGGGCGCTAGGTGTGGGGGGCCTCTCCGGTTCCTGTGCCGCACT
BTG1-1          ACGCTGTAACGTTGGGCGCTAGGTGTGGGGGGCCTCTCCGGTTCCTGTGCCGCACT
BTG3-2          ACGCTGTAACGTTGGGCGCTAGGTGTGGGGGGCCTCTCCGGTTCCTGTGCCGCACT
X92604          ACGCTGTAACGTTGGGCGCTAGGTGTGGGGGGCCTCTCCGGTTCCTGTGCCGCACT
KM3-14          ACGCTGTAACGTTGGGCGCTAGGTGTGGGGGGCCTCTCCGGTTCCTGTGCCGCACT
KM3-1           ACGCTGTAACGTTGGGCGCTAGGTGTGGGGGGCCTCTCCGGTTCCTGTGCCGCACT
X92594          ACGCTGTAACGTTGGGCGCTAGGTGTGGGGGGCCTCTCCGGTTCCTGTGCCGCACT
X92601          ACGCTGTAACGTTGGGCGCTAGGTGTGGGGGGCCTCTCCGGTTCTTGTGCCGCACT
X92611          ACGCTGTAACGTTGGGCGCTAGGTGTGGGGGGCCTCTCCGGTTCCTGTGCCGCACT
KM4-29          ACGCTGTAACGTTGGGCGCTAGGTGTGGGGGGCCTCTCCGGTTCCTGTGCCGCACT
KM4-24          ACGCTGTAACGTTGGGCGCTAGGTGTGGGGGGCCTCTCCGGTTCCTGTGCCGCACT
LK6-12          ACGCTGTAACGTTGGGCGCTAGGTGTGGGGGGCCTCTCCGGTTCCTGTGCCGCACT
LK5-4           ACGCTGTAACGTTGGGCGCTAGGTGTGGGGGGCCTCTCCGGTTCCTGTGCCGCACT
TT2-4           ACGCTGTAACGTTGGGCGCTAGGTGTGGGGGGCCTCTCCGGTTCCTGTGCCGCACT
BTG10-2         ACGCTGTAACGTTGGGCGCTAGGTGTGGGGGGCCTCTCCGGTTCCTGTGCCGCACT
BTG7-3          ACGCTGTAACGTTGGGCGCTAGGTGTGGGGGGCCTCTCCGGTTCCTGTGCCGCACT
AF152109        ACGCTGTAACGTTGGGCGCTAGGTGTGGGGGGCCTCTCCGGTTTCTGTGCCGCACT
X92599          ACGCTGTAACGTTGGGCGCTAGGTGTGGGGGGCCTCTCCGGTTCTTGTGCCGCACT
X92613          ACGCTGTAACGTTGGGCGCTAGGTGTGGGGGGCCTCTCCGGTTCCTGTGCCGCACT
TT1-11          ACGCTGTAACGTTGGGCGCTAGGTGTGGGGGGCCTCTCCGGTTCCTGTGCCGCACT
X92598          ACGCTGTAACGTTGGGCGCTAGGTGTGGGGGACCTCTCCGGTTCCTGTGCCGCACT
X92608          ACGCTGTAACGTTGGGCGCTAGGTGTGGGGGGCCTCTCCGGTTCTTGTGCCGCACT
X92607          ACGCTGTAACGTTGGGCGCTAGGTGTGGGGGGCCTCTCCGGTTCTTGTGCCGCACT
X92610          ACGCTGTAACGTTGGGCGCTAGGTGTGGGGGGCCTCTCCGGTTCCTGTGCCGCACT
X92609          ACGCTGTAACGTTGCGCGCTAGGTGTGGGGGGCCTCTCCGGTTCCTGTGCCGCACT
X92631          ACGCTGTAACGTTGGGCGCTAGGTGTGGGGGGCCTCTCCGGTTCTTGTGCCGCACT
X92628          ACGCTGTAACGTTGGGCGCTAGGTGTGGGGGGCCTCTCCGGTTCCTGTGCCGCACT
LK2-10          ACGCTGTAACGTTGGGCGCTAGGTGTGGGGGGCCTCTCCGGTTCCTGTGCCGCACT
LK2-5           ACGCTGTAACGTTGGGCGCTAGGTGTGGGGGGCCTCTCCGGTTCCTGTGCCGCACT
KM1-7           ACGCTGTAACGTTGGGCGCTAGGTGTGGGGGGCCTCTCCGGTTCTTGTGCCGCACT
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Figure 20. (Continued)

```
BTG2-3          AACGCATTAGCGCCCCGCCTGGGGAGTACGGCCGCAGGCTAAACTCAAAGGAATTG
BTG3-4          AACGCATTAGCGCCCCGCCTGGGGAGTACGGCCGCAGGCTAAACTCAAAGGAATTG
LK2-6           AACGCATTAGCGCCCCGCCTGGGGAGTACGGCCGCAGGCTAAACTCAAAGGAATTG
BTG1-4          AACGCATTAGCGCCCCGCCTGGGGAGTACGGCCGCAGGCTAAACTCAAAGGAATTG
KM1-9           AACGCATTAGCGCCCCGCCTGGGGAGTACGGCCGCAGGCTAAACTCAAAGGAATTG
LK2-12          AACGCATTAGCGCCCCGCCTGGGGAGTACGGCCGCAGGCTAAACTCAAAGGAATTG
KM1-6           AACGCATTAGCGCCCCGCCTGGGGAGTACGGCCGCAGGCTAAACTCAAAGGAATTG
BTG4-1          AACGCATTAGCGCCCCGCCTGGGGAGTACGGCCGCAGGCTAAACTCAAAGGAATTG
BTG1-1          AACGCATTAGCGCCCCGCCTGGGGAGTACGGCCGCAGGCTAAACTCAAAGGAATTG
BTG3-2          AACGCATTAGCGCCCCGCCTGGGGAGTACGGCCGCAGGCTAAACTCAAAGGAATTG
X92604          AACGCATTAGCGCCCCGCCTGGGGAGTACGGCCGCAGGCTAAACTCAAAGGAATTG
KM3-14          AACGCATTAGCGCCCCGCCTGGGGAGTACGGCCGCAGGCTAAACTCAAAGGAATTG
KM3-1           AACGCATTAGCGCCCCGCCTGGGGAGTACGGCCGCAGGCTAAACTCAAAGGAATTG
X92594          AACGCATTAGCGCCCCGCCTGGGGAGTACGGCCGCAGGCTAAACTCAAAGGAATTG
X92601          AACGCATTAGCGCCCCGCCTGGGGAGTACGGCCGCAGGCTAAACTCAAAGGAATTG
X92611          AACGCATTAGCGCCCCGCCTGGGGAGTACGGCCGCAGGCTAAACTCAAAGGAATTG
KM4-29          AACGCATTAGCGCCCCGCCTGGGGAGTACGGCCGCAGGCTAAACTCAAAGGAATTG
KM4-24          AACGCATTAGCGCCCCGCCTGGGGAGTACGGCCGCAGGCTAAACTCAAAGGAATTG
LK6-12          AACGCATTAGCGCCCCGCCTGGGGAGTACGGCCGCAGGCTAAACTCAAAGGAATTG
LK5-4           AACGCATTAGCGCCCCGCCTGGGGAGTACGGCCGCAGGCTAAACTCAAAGGAATTG
TT2-4           AACGCATTAGCGCCCCGCCTGGGGAGTACGGCCGCAGGCTAAACTCAAAGGAATTG
BTG10-2         AACGCATTAGCGCCCCGCCTGGGGAGTACGGCCGCAGGCTAAACTCAAAGGAATTG
BTG7-3          AACGCATTAGCGCCCCGCCTGGGGAGTACGGCCGCAGGCTAAACTCAAAGGAATTG
AF152109        AACGCATTAGCGCCCCGCCTGGGGAGTACGGCCGCAGGCTAAACTCAAAGGAATTG
X92599          AACGCATTAGCGCCCCGCCTGGGGAGTACGGCCGCAGGCTAAACTCAAAGGAATTG
X92613          AACGCATTAGCGCCCCGCCTGGGGAGTACGGCCGCAGGCTAAACTCAAAGGAATTG
TT1-11          AACGCATTAGCGCCCCGCCTGGGGAGTACGGCCGCAGGCTAAACTCAAAGGAATTG
X92598          AACGCATTAGCGCCCCGCCTGGGGAGTACGGCCGCAGGCTAAACTCAAAGGAATTG
X92608          AACGCATTAGCGCCCCGCCTGGGGAGTACGGCCGCAGGCTAAACTCAAAGGAATTG
X92607          AACGCATTAGCGCCCCGCCTGGGGAGTACGGCCGCAGGCTAAACTCAAAGGAATTG
X92610          AACGCATTAGCGCCCCGCCTGGGGAGTACGGCCGCAGGCTAAACTCAAAGGAATTG
X92609          AACGCATTAGCGCCCCGCCTGGGGAGTACGGCCGCAGGCTAAACTCAAAGGAATTG
X92631          AACGCATTAGCGCCCCGCCTGGGGAGTACGGCCGCAGGCTAAACTCAAAGGAATTG
X92628          AACGCATTAGCGCCCCGCCTGGGGAGTACGGCCGCAGGCTAAACTCAAAGGAATTG
LK2-10          AACGCATTAGCGCCCCGCCTGGGGAGTACGGCCGCAGGCTAAACTCAAAGGAATTG
LK2-5           AACGCATTAGCGCCCCGCCTGGGGAGTACGGCCGCAGGCTAAACTCAAAGGAATTG
KM1-7           AACGCATTAGCGCCCCGCCTGGGGAGTACGGCCGCAGGCTAAACTCAAAGGAATTG
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Figure 20. (Continued)

```
BTG2-3          ACGGGGGCCCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTT
BTG3-4          ACGGGGGCCCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTT
LK2-6           ACGGGGGCCCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTT
BTG1-4          ACGGGGGCCCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTT
KM1-9           ACGGGGGCCCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTT
LK2-12          ACGGGGGCCCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTT
KM1-6           ACGGGGGCCCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTT
BTG4-1          ACGGGGGCCCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTT
BTG1-1          ACGGGGGCCCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTT
BTG3-2          ACGGGGGCCCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTT
X92604          ACGGGGGCCCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTT
KM3-14          ACGGGGGCCCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTT
KM3-1           ACGGGGGCCCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTT
X92594          ACGGGGGCCCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTT
X92601          ACGGGGGCCCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTT
X92611          ACGGGGGCCCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTT
KM4-29          ACGGGGGCCCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTT
KM4-24          ACGGGGGCCCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTT
LK6-12          ACGGGGGCCCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTT
LK5-4           ACGGGGGCCCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTT
TT2-4           ACGGGGGCCCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTT
BTG10-2         ACGGGGGCCCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTT
BTG7-3          ACGGGGGCCCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTT
AF152109        ACGGGGGCCCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTT
X92599          ACGGGGGCCCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTT
X92613          ACGGGGGCCCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTT
TT1-11          ACGGGGGCCCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTT
X92598          ACGGGGGCCCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTT
X92608          ACGGGGGCCCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTT
X92607          ACGGGGGCCCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTT
X92610          ACGGGGGCCCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTT
X92609          ACGGGGGCCCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTT
X92631          ACGGGGGCCCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTT
X92628          ACGGGGGCCCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTT
LK2-10          ACGGGGGCCCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTT
LK2-5           ACGGGGGCCCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGACCCTT
KM1-7           ACGGGGGCCCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTT
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Figure 20. (Continued)

```
BTG2-3          ACCTGGGTTTGACATGGCCGCAAAACCTGCAGAGATGTGGGGTCCTTCGGGGGCGGTCAC
BTG3-4          ACCTGGGTTTGACATGGCCGCAAAACCTGCAGAGATGTGGGGTCCTTCGGGGGCGGTCAC
LK2-6           ACCTGGGTTTGACATGGCCGCAAAACCTGCAGAGATGTGGGGTCCTTCGGGGGCGGTCAC
BTG1-4          ACCTGGGTTTGACATGGCCGCAAAACCTGCAGAGATGTGGGGTCCTTCGGGGGCGGTCAC
KM1-9           ACCTGGGTTTGACATGGCCGCAAAACCTGCAGAGATGTGGGGTCCTTCGGGGGCGGTCAC
LK2-12          ACCTGGGTTTGACATGGCCGCAAAACCTGCAGAGATGTGGGGTCCTTCGGGGGCGGTCAC
KM1-6           ACCTGGGTTTGACATGGCCGCAAAACCTGCAGAGATGTGGGGTCCTTCGGGGGCGGTCAC
BTG4-1          ACCTGGGTTTGACATGGCCGCAAAACCTGCAGAGATGTGGGGTCCTTCGGGGGCGGTCAC
BTG1-1          ACCTGGGTTTGACATGGCCGCAAAACCTGCAGAGATGTGGGGTCCTTCGGGGGCGGTCAC
BTG3-2          ACCTGGGTTTGACATGGCCGCAAAACTGTCAGAGATGGCAGGTCCTTCGGGGGCGGTCAC
X92604          ACCTGGGTTTGACATGGCCGCAAAACTGTCAGAGATGGCAGGTCCTTCGGGGGCGGTCAC
KM3-14          ACCTGGGTTTGACATGGCCGCAAAACTGTCAGAGATGGCAGGTCCTTCGGGGGCGGTCAC
KM3-1           ACCTGGGTTTGACATGGCCGCAAAACTGTCAGAGATGGCAGGTCCTTCGGGGGCGGTCAC
X92594          ACCTGGGTTTGACATGGCCGCAAAACTGTCAGAGATGGCAGGTCCTTCGGGGGCGGTCAC
X92601          ACCTGGGTTTGACATGGCCGCAAAACCGGCAGAGATGTCGGGTCCTTCGGGGGCGGTCAC
X92611          ACCTGGGTTTGACATGGCCGCAAAACTCACAGAGATGTGAGGTCCTTCGGGGGCGGTCAC
KM4-29          ACCTGGGTTTGACATGGCCGCAAAACTTGCAGAGATGTAAGGTCCTTCGGGGGCGGTCAC
KM4-24          ACCTGGGTTTGACATGGCCGCAAAACTTGCAGAGATGTAAGGTCCTTCGGGGGCGGTCAC
LK6-12          ACCTGGGTTTGACATGGCCGCAAAACTGTCAGAGATGGCAGGTCCTTCGGGGGCGGTCAC
LK5-4           ACCTGGGTTTGACATGGCCGCAAAACTGTCAGAGATGGCAGGTCCTTCGGGGGCGGTCAC
TT2-4           ACCTGGGTTTGACATGGCCGCAAAACCTCCAGAGATGGGGGGTCCTTCGGGGGCGGTCAC
BTG10-2         ACCTGGGTTTGACATGGCCGCAAAACTTGCAGAGATGTAAGGTCCTTCGGGGGCGGTCAC
BTG7-3          ACCTGGGTTTGACATGGCCGCAAAACTTGCAGAGATGTAAGGTCCTTCGGGGGCGGTCAC
AF152109        ACCTGGGTTTGACATGGCCGCAAAACTTGCAGAGATGTAAGGTCCTTCGGGGGCGGTCAC
X92599          ACCTGGGTTTGACATGGCCGCAAAACCTCCAGAGATGGGGGGTCCTTCGGGGGCGGTCAC
X92613          ACCTGGGTTTGACATCGCCGGAAATCCTCCAGAGATGGGGGGTCCTTCGGGGCCGGTGAC
TT1-11          ACCTGGGTTTGACATGGCCGCAAAACTCGCAGAGATGTGAGGTCCTTCGGGGGCGGTCAC
X92598          ACCTGGGTTTGACATGGCCGCAAAACTCACAGAGATGTGAGGTCCTTCGGGGGCGGTCAC
X92608          ACCTGGGTTTGACATGGCCGCAAAACCTGCAGAGATGTAGGGTCCTTCGGGGGCGGTCAC
X92607          ACCTGGGTTTGACATGGCCGCAAAACCGGCAGAGATGTCGGGTCCTTCGGGGGCGGTCAC
X92610          ACCTGGGTTTGACATGGCCGCAAAACCGGCAGAGATGTCGGGTCCTTCGGGGGCGGTCAC
X92609          ACCTGGGTTTGACATGGCCGCAAAACCTGCAGAGATGTGGGGTCCTTCGGGGGCGGTCAC
X92631          ACCTGGGTTTGACATGGCCGCAAAACTGTCAGAGATGGCAGGTCCTTCGGGGGCGGTCAC
X92628          ACCTGGGTTTGACATGGCCGCAAAACTCGCAGAGATGTGAGGTCCTTCGGGGGCGGTCAC
LK2-10          ACCTGGGTTTGACATGGCCGCAAAACCTGCAGAGATGTGGGGTCCTTCGGGGGCGGTCAC
LK2-5           ACCTGGGTTTGACATGGCCGCAAAACCTGCAGAGATGTGGGGTCCTTCGGGGGCGGTCAC
KM1-7           ACCTGGGTTTGACATGGCCGCAAAACCTCCAGAGATGGGGGGTCCTTCGGGGGCGGTCAC
                *************** **** *** *   ********   ************ **** **
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Figure 20. (Continued)

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BTG2-3          AGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGA
BTG3-4          AGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGA
LK2-6           AGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGA
BTG1-4          AGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGA
KM1-9           AGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGA
LK2-12          AGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGA
KM1-6           AGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGA
BTG4-1          AGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGA
BTG1-1          AGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGA
BTG3-2          AGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGA
X92604          AGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGA
KM3-14          AGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGA
KM3-1           AGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGA
X92594          AGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGA
X92601          AGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGA
X92611          AGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGA
KM4-29          AGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGA
KM4-24          AGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGA
LK6-12          AGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGA
LK5-4           AGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGA
TT2-4           AGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGA
BTG10-2         AGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGA
BTG7-3          AGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGA
AF152109        AGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGA
X92599          AGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGA
X92613          AGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGA
TT1-11          AGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGA
X92598          AGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGA
X92608          AGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGA
X92607          AGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGA
X92610          AGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGA
X92609          AGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGA
X92631          AGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGA
X92628          AGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGA
LK2-10          AGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGA
LK2-5           AGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGA
KM1-7           AGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGA
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Figure 20. (Continued)

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BTG2-3          GCGCAACCCTCGTTCGATGTTGCCAGCGCGTTATGGCGGGGACTCATCGAAGACTGCCGG
BTG3-4          GCGCAACCCTCGTTCGATGTTGCCAGCGCGTTATGGCGGGGACTCATCGAAGACTGCCGG
LK2-6           GCGCAACCCTCGTTCGATGTTGCCAGCGCGTTATGGCGGGGACTCATCGAAGACTGCCGG
BTG1-4          GCGCAACCCTCGTTCGATGTTGCCAGCGCGTTATGGCGGGGACTCATCGAAGACTGCCGG
KM1-9           GCGCAACCCTCGTTCGATGTTGCCAGCGCGTTATGGCGGGGACTCATCGAAGACTGCCGG
LK2-12          GCGCAACCCTCGTTCGATGTTGCCAGCGCGTTATGGCGGGGACTCATCGAAGACTGCCGG
KM1-6           GCGCAACCCTCGTTCGATGTTGCCAGCGCGTTATGGCGGGGACTCATCGAAGACTGCCGG
BTG4-1          GCGCAACCCTCGTTCGATGTTGCCAGCGCGTTATGGCGGGGACTCATCGAAGACTGCCGG
BTG1-1          GCGCAACCCTCGTTCGATGTTGCCAGCGCGTTATGGCGGGGACTCATCGAAGACTGCCGG
BTG3-2          GCGCAACCCTCGTTCGATGTTGCCAGCGCGTTATGGCGGGGACTCATCGAAGACTGCCGG
X92604          GCGCAACCCTCGTTCGATGTTGCCAGCGGGTTATGGCGGGGACTCATCGAAGACTGCCGG
KM3-14          GCGCAACCCTCGTTCGATGTTGCCAGCGCGTTATGGCGGGGACTCATCGAAGACTGCCGG
KM3-1           GCGCAACCCTCGTTCGATGTTGCCAGCGCGTTATGGCGGGGACTCATCGAAGACTGCCGG
X92594          GCGCAACCCTCGTTCGATGTTGCCAGCGCGTTATGGCGGGGACTCATCGAAGACTGCCGG
X92601          GCGCAACCCTCGTTCGATGTTGCCAGCGCGTTATGGCGGGGACTCATCGAAGACTGCCGG
X92611          GCGCAACCCTCGTTCGATGTTGCCAGCGCGTTATGGCGGGGACTCATCGAAGACTGCCGG
KM4-29          GCGCAACCCTCGTTCGATGTTGCCAGCGCGTTATGGCGGGGACTCATCGAAGACTGCCGG
KM4-24          GCGCAACCCTCGTTCGATGTTGCCAGCGCGTTATGGCGGGGACTCATCGAAGACTGCCGG
LK6-12          GCGCAACCCTCGTTCGATGTTGCCAGCGCGTTATGGCGGGGACTCATCGAAGACTGCCGG
LK5-4           GCGCAACCCTCGTTCGATGTTGCCAGCGCGTTATGGCGGGGACTCATCGAAGACTGCCGG
TT2-4           GCGCAACCCTCGTTCGATGTTGCCAGCGCGTTATGGCGGGGACTCATCGAAGACTGCCGG
BTG10-2         GCGCAACCCTCGTTCGATGTTGCCAGCGCGTTATGGCGGGGACTCATCGAAGACTGCCGG
BTG7-3          GCGCAACCCTCGTTCGATGTTGCCAGCGCGTTATGGCGGGGACTCATCGAAGACTGCCGG
AF152109        GCGCAACCCTCGTTCGATGTTGCCAGCGCGTTATGGCGGGGACTCATCGAAGACTGCCGG
X92599          GCGCAACCCTCGTTCGATGTTGCCAGCGCGTTATGGCGGGGACTCATCGAAGACTGCCGG
X92613          GCGCAACCCTCGTTCGATGTTGCCAGCGCGTTATGGCGGGGACTCATCGAAGACTGCCGG
TT1-11          GCGCAACCCTCGTTCGATGTTGCCAGCGCGTTATGGCGGGGACTCATCGAAGACTGCCGG
X92598          GCGCAACCCTCGTTCGATGTTGCCAGCGCGTTATGGCGGGGACTCATCGAAGACTGCCGG
X92608          GCGCAACCCTCGTTCGATGTTGCCAGCGCGTTATGGCGGGGACTCATCGAAGACTGCCGG
X92607          GCGCAACCCTCGTTCGATGTTGCCAGCGCGTTATGGCGGGGACTCATCGAAGACTGCCGG
X92610          GCGCAACCCTCGTTCGATGTTGCCAGCGCGTTATGGCGGGGACTCATCGAAGACTGCCGG
X92609          GCGCAACCCTCGTTCGATGTTGCCAGCGCGTTATGGCGGGGACTCATCGAAGACTGCCGG
X92631          GCGCAACCCTCGTTCGATGTTGCCAGCGCGTTATGGCGGGGACTCATCGAAGACTGCCGG
X92628          GCGCAACCCTCGTTCGATGTTGCCAGCGCGTTATGGCGGGGACTCATCGAAGACTGCCGG
LK2-10          GCGCAACCCTCGTTCGATGTTGCCAGCGCGTTATGGCGGGGACTCATCGAAGACTGCCGG
LK2-5           GCGCAACCCTCGTTCGATGTTGCCAGCGCGTTATGGCGGGGACTCATCGAAGACTGCCGG
KM1-7           GCGCAACCCTCGTTCGATGTTGCCAGCGCGTTATGGCGGGGACTCATCGAAGACTGCCGG
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Figure 20. (Continued)

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BTG2-3          GGTCAACTCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGCCCCTTATGTCCAGGGCT
BTG3-4          GGTCAACTCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGCCCCTTATGTCCAGGGCT
LK2-6           GGTCAACTCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGCCCCTTATGTCCAGGGCT
BTG1-4          GGTCAACTCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGCCCCTTATGTCCAGGGCT
KM1-9           GGTCAACTCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGCCCCTTATGTCCAGGGCT
LK2-12          GGTCAACTCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGCCCCTTATGTCCAGGGCT
KM1-6           GGTCAACTCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGCCCCTTATGTCCAGGGCT
BTG4-1          GGTCAACTCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGCCCCTTATGTCCAGGGCT
BTG1-1          GGTCAACTCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGCCCCTTATGTCCAGGGCT
BTG3-2          GGTCAACTCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGCCCCTTATGTCCAGGGCT
X92604          GGTCAACTCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGCCCCTTATGTCCAGGGCT
KM3-14          GGTCAACTCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGCCCCTTATGTCCAGGGCT
KM3-1           GGTCAACTCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGCCCCTTATGTCCAGGGCT
X92594          GGTCAACTCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGCCCCTTATGTCCAGGGCT
X92601          GGTCAACTCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGCCCCTTATGTCCAGGGCT
X92611          GGTCAACTCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGCCCCTTATGTCCAGGGCT
KM4-29          GGTCAACTCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGCCCCTTATGTCCAGGGCT
KM4-24          GGTCAACTCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGCCCCTTATGTCCAGGGCT
LK6-12          GGTCAACTCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGCCCCTTATGTCCAGGGCT
LK5-4           GGTCAACTCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGCCCCTTATGTCCAGGGCT
TT2-4           GGTCAACTCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGCCCCTTATGTCCAGGGCT
BTG10-2         GGTCAACTCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGCCCCTTATGTCCAGGGCT
BTG7-3          GGTCAACTCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGCCCCTTATGTCCAGGGCT
AF152109        GGTCAACTCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGCCCCTTATGTCCAGGGCT
X92599          GGTCAACTCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGCCCCTTATGTCCAGGGCT
X92613          GGTCAACTCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGCCCCTTATGTCCAGGGCT
TT1-11          GGTCAACTCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGCCCCTTATGTCCAGGGCT
X92598          GGTCAACTCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGCCCCTTATGTCCAGGGCT
X92608          GGTCAACTCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGCCCCTTATGTCCAGGGCT
X92607          GGTCAACTCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGCCCCTTATGTCCAGGGCT
X92610          GGTCAACTCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGCCCCTTATGTCCAGGGCT
X92609          GGTCAACTCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGCCCCTTATGTCCAGGGCT
X92631          GGTCAACTCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGCCCCTTATGTCCAGGGCT
X92628          GGTCAACTCGGAGGAAGGTGGGGATGAGGTCAAGTCATCATGCCCCTTATGTCCAGGGCT
LK2-10          GGTCAACTCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGCCCCTTATGTCCAGGGCT
LK2-5           GGTCAACTCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGCCCCTTATGTCCAGGGCT
KM1-7           GGTCAACTCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGCCCCTTATGTCCAGGGCT
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Figure 20. (Continued)

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BTG2-3          TCACGCATGCTACAATGGCCGGTACAATGGGCTGCGATACCGTGAGGTGGAGCGAATCCC
BTG3-4          TCACGCATGCTACAATGGCCGGTACAATGGGCTGCGATACCGTGAGGTGGAGCGAATCCC
LK2-6           TCACGCATGCTACAATGGCCGGTACAATGGGCTGCGATACCGTGAGGTGGAGCGAATCCC
BTG1-4          TCACGCATGCTACAATGGCCGGTACAATGGGCTGCGATACCGTGAGGTGGAGCGAATCCC
KM1-9           TCACGCATGCTACAATGGCCGGTACAATGGGCTGCGATACCGTGAGGTGGAGCGAATCCC
LK2-12          TCACGCATGCTACAATGGCCGGTACAATGGGCTGCGATACCGTGAGGTGGAGCGAATCCC
KM1-6           TCACGCATGCTACAATGGCCGGTACAATGGGCTGCGATACCGTGAGGTGGAGCGAATCCC
BTG4-1          TCACGCATGCTACAATGGCCGGTACAATGGGCTGCGATACCGTGAGGTGGAGCGAATCCC
BTG1-1          TCACGCATGCTACAATGGCCGGTACAATGGGCTGCGATACCGTGAGGTGGAGCGAATCCC
BTG3-2          TCACGCATGCTACAATGGCCGGTACAATGGGCTGCGATACCGTGAGGTGGAGCGAATCCC
X92604          TCACGCATGCTACAATGGTCGGTACAATGGGCTGCGATACCGTGAGGTGGAGCGAATCCC
KM3-14          TCACGCATGCTACAATGGCCGGTACAATGGGCTGCGATACCGTGAGGTGGAGCGAATCCC
KM3-1           TCACGCATGCTACAATGGCCGGTACAATGGGCTGCGATACCGTGAGGTGGAGCGAATCCC
X92594          TCACGCATGCTACAATGGCCGGTACAATGGGCTGCGATACCGTGAGGTGGAGCGAATCCC
X92601          TCACGCATGCTACAATGGCCGGTACAATGGGCTGCGATACCGTGAGGTGGAGCGAATCCC
X92611          TCACGCATGCTACAATGGCCGGTACAATGGGCTGCGATACCGTGAGGTGGAGCGAATCCC
KM4-29          TCACGCATGCTACAATGGCCGGTACAATGGGCTGCGATACCGTGAGGTGGAGCGAATCCC
KM4-24          TCACGCATGCTACAATGGCCGGTACAATGGGCTGCGATACCGTGAGGTGGAGCGAATCCC
LK6-12          TCACGCATGCTACAATGGTCGGTACAATGGGCTGCGATACCGTGAGGTGGAGCGAATCCC
LK5-4           TCACGCATGCTACAATGGCCGGTACAATGGGCTGCGATACCGTGAGGTGGAGCGAATCCC
TT2-4           TCACGCATGCTACAATGGCCGGTACAATGGGCTGCGATACCGTGAGGTGGAGCGAATCCC
BTG10-2         TCACGCATGCTACAATGGCCGGTACAATGGGCTGCGATACCGTGAGGTGGAGCGAATCCC
BTG7-3          TCACGCATGCTACAATGGCCGGTACAATGGGCTGCGATACCGTGAGGTGGAGCGAATCCC
AF152109        TCACGCATGCTACAATGGCCGGTACAATGGGTTGCGATACCGTGAGGTGGAGCGAATCCC
X92599          TCACGCATGCTACAATGGCCGGTACAATGGGCTGCGATACCGTGAGGTGGAGCGAATCCC
X92613          TCACGCATGCTACAATGGCCGGTACAATGGGCTGCGATACCGTGAGGTGGAGCGAATCCC
TT1-11          TCACGCATGCTACAATGGCCGGTACAATGGGCTGCGATACCGTGAGGTGGAGCGAATCCC
X92598          TCACGCATGCTACAATGGCCGGTACAATGGGCTGCGATACCGTGAGGTGGAGCGAATCCC
X92608          TCACGCATGCTACAATGGCCGGTACAATGGGCTGCGATACCGTAAGGTGGAGCGAATCCC
X92607          TCACGCATGCTACAATGGCCGGTACAATGGGCTGCGATACCGTGAGGTGGAGCGAATCCC
X92610          TCACGCATGCTACAATGGCCGGTACAATGGGCTGCGATACCGTGAGGTGGAGCGAATCCC
X92609          TCACGCATGCTACAATGGCCGGTACAATGGGCTGCGATACCGTGAGGTGGAGCGAATCCC
X92631          TCACGCATGCTACAATGGCCGGTACAATGGGCTGCGATACCGTGAGGTGGAGCGAATCCC
X92628          TCACGCATGCTACAATGGCCGGTACAATGGGCTGCGATACCGTGAGGTGGAGCGAATCCC
LK2-10          TCACGCATGCTACAATGGCCGGTACAATGGGCTGCGATACCGTGAGGTGGAGCGAATCCC
LK2-5           TCACGCATGCTACAATGGCCGGTACAATGGGCTGCGATACCGTGAGGTGGAGCGAATCCC
KM1-7           TCACGCATGCTACAATGGCCGGTACAATGGGCTGCGATACCGTGAGGTGGAGCGAATCCC
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Figure 20. (Continued)

```
BTG2-3          AAAAAGCCGGTCTCAGTTCGGATCGGGGTCTGCAACTCGACCCCGTGAAGTCGGAGTCGC
BTG3-4          AAAAAGCCGGTCTCAGTTCGGATCGGGGTCTGCAACTCGACCCCGTGAAGTCGGAGTCGC
LK2-6           AAAAAGCCGGTCTCAGTTCGGATCGGGGTCTGCAACTCGACCCCGTGAAGTCGGAGTCGC
BTG1-4          AAAAAGCCGGTCTCAGTTCGGATCGGGGTCTGCAACTCGACCCCGTGAAGTCGGAGTCGC
KM1-9           AAAAAGCCGGTCTCAGTTCGGATCGGGGTCTGCAACTCGACCCCGTGAAGTCGGAGTCGC
LK2-12          AAAAAGCCGGTCTCAGTTCGGATCGGGGTCTGCAACTCGACCCCGTGAAGTCGGAGTCGC
KM1-6           AAAAAGCCGGTCTCAGTTCGGATCGGGGTCTGCAACTCGACCCCGTGAAGTCGGAGTCGC
BTG4-1          AAAAAGCCGGTCTCAGTTCGGATCGGGGTCTGCAACTCGACCCCGTGAAGTCGGAGTCGC
BTG1-1          AAAAAGCCGGTCTCAGTTCGGATCGGGGTCTGCAACTCGACCCCGTGAAGTCGGAGTCGC
BTG3-2          AAAAAGCCGGTCTCAGTTCGGATCGGGGTCTGCAACTCGACCCCGTGAAGTCGGAGTCGC
X92604          AAAAAGCCGGTCTCAGTTCGGATCGGGGTCTGCAACTCGACCCCGTGAAGTCGGAGTCGC
KM3-14          AAAAAGCCGGTCTCAGTTCGGATCGGGGTCTGCAACTCGACCCCGTGAAGTCGGAGTCGC
KM3-1           AAAAAGCCGGTCTCAGTTCGGATCGGGGTCTGCAACTCGACCCCGTGAAGTCGGAGTCGC
X92594          AAAAAGCCGGTCTCAGTTCGGATCGGGGTCTGCAACTCGACCCCGTGAAGTCGGAGTCGC
X92601          AAAAAGCCGGTCTCAGTTCGGATCGGGGTCTGCAACTCGACCCCGTGAAGTCGGAGTCGC
X92611          AAAAAGCCGGTCTCAGTTCGGATCGGGGTCTGCAACTCGACCCCGTGAAGTCGGAGTCGC
KM4-29          AAAAAGCCGGTCTCAGTTCGGATCGGGGTCTGCAACTCGACCCCGTGAAGTCGGAGTCGC
KM4-24          AAAAAGCCGGTCTCAGTTCGGATCGGGGTCTGCAACTCGACCCCGTGAAGTCGGAGTCGC
LK6-12          AAAAAGCCGGTCTCAGTTCGGATCGGGGTCTGCAACTCGACCCCGTGAAGTCGGAGTCGC
LK5-4           AAAAAGCCGGTCTCAGTTCGGATCGGGGTCTGCAACTCGACCCCGTGAAGTCGGAGTCGC
TT2-4           AAAAAGCCGGTCTCAGTTCGGATCGGGGTCTGCAACTCGACCCCGTGAAGTCGGAGTCGC
BTG10-2         AAAAAGCCGGTCTCAGTTCGGATCGGGGTCTGCAACTCGACCCCGTGAAGTCGGAGTCGC
BTG7-3          AAAAAGCCGGTCTCAGTTCGGATCGGGGTCTGCAACTCGACCCCGTGAAGTCGGAGTCGC
AF152109        AAAAAGCCGGTCTCAGTTCGGATCGGGGTCTGCAACTCGACCCCGTGAAGTCGGAGTCGC
X92599          AAAAAGCCGGTCTCAGTTCGGATCGGGGTCTGCAACTCGACCCCGTGAAGTCGGAGTCGC
X92613          AAAAAGCCGGTCTCAGTTCGGATCGGGGTCTGCAACTCGACCCCGTGAAGTCGGAGTCGC
TT1-11          AAAAAGCCGGTCTCAGTTCGGATCGGGGTCTGCAACTCGACCCCGTGAAGTCGGAGTCGC
X92598          AAAAAGCCGGTCTCAGTTCGGATCGGGGTCTGCAACTCGACCCCGTGAAGTCGGAGTCGC
X92608          AAAAAGCCGGTCTCAGTTCGGATCGGGGTCTGCAACTCGACCCCGTGAAGTCGGAGTCGC
X92607          AAAAAGCCGGTCTCAGTTCGGATCGGGGTCTGCAACTCGACCCCGTGAAGTCGGAGTCGC
X92610          AAAAAGCCGGTCTCAGTTCGGATCGGGGTCTGCAACTCGACCCCGTGAAGTCGGAGTCGC
X92609          AAAAAGCCGGTCTCAGTTCGGATCGGGGTCTGCAACTCGACCCCGTGAAGTCGGAGTCGC
X92631          AAAAAGCCGGTCTCAGTTCGGATCGGGGTCTGCAACTCGACCCCGTGAAGTCGGAGTCGC
X92628          AAAAAGCCGGTCTCAGTTCGGATCGGGGTCTGCAACTCGACCCCGTGAAGTCGGAGTCGC
LK2-10          AAAAAGCCGGTCTCAGTTCGGATCGGGGTCTGCAACTCGACCCCGTGAAGTCGGAGTCGC
LK2-5           AAAAAGCCGGTCTCAGTTCGGATCGGGGTCTGCAACTCGACCCCGTGAAGTCGGAGTCGC
KM1-7           AAAAAGCCGGTCTCAGTTCGGATCGGGGTCTGCAACTCGACCCCGTGAAGTCGGAGTCGC
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Figure 20. (Continued)

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BTG2-3          TAGTAATCGCAGATCAGCAACGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCC
BTG3-4          TAGTAATCGCAGATCAGCAACGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCC
LK2-6           TAGTAATCGCAGATCAGCAACGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCC
BTG1-4          TAGTAATCGCAGATCAGCAACGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCC
KM1-9           TAGTAATCGCAGATCAGCAACGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCC
LK2-12          TAGTAATCGCAGATCAGCAACGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCC
KM1-6           TAGTAATCGCAGATCAGCAACGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCC
BTG4-1          TAGTAATCGCAGATCAGCAACGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCC
BTG1-1          TAGTAATCGCAGATCAGCAACGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCC
BTG3-2          TAGTAATCGCAGATCAGCAACGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCC
X92604          TAGTAATCGCAGATCAGCAACGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCC
KM3-14          TAGTAATCGCAGATCAGCAACGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCC
KM3-1           TAGTAATCGCAGATCAGCAACGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCC
X92594          TAGTAATCGCAGATCAGCAACGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCC
X92601          TAGTAATCGCAGATCAGCAACGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCC
X92611          TAGTAATCGCAGATCAGCAACGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCC
KM4-29          TAGTAATCGCAGATCAGCAACGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCC
KM4-24          TAGTAATCGCAGATCAGCAACGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCC
LK6-12          TAGTAATCGCAGATCAGCAACGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCC
LK5-4           TAGTAATCGCAGATCAGCAACGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCC
TT2-4           TAGTAATCGCAGATCAGCAACGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCC
BTG10-2         TAGTAATCGCAGATCAGCAACGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCC
BTG7-3          TAGTAATCGCAGATCAGCAACGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCC
AF152109        TAGTAATCGCAGATCAGCAACGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCC
X92599          TAGTAATCGCAGATCAGCAACGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCC
X92613          TAGTAATCGCAGATCAGCAACGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCC
TT1-11          TAGTAATCGCAGATCAGCAACGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCC
X92598          TAGTAATCGCAGATCAGCAACGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCC
X92608          TAGTAATCGCAGATCAGCAACGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCC
X92607          TAGTAATCGCAGATCAGCAACGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCC
X92610          TAGTAATCGCAGATCAGCAACGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCC
X92609          TAGTAATCGCAGATCAGCAACGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCC
X92631          TAGTAATCGCAGATCAGCAACGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCC
X92628          TAGTAATCGCAGATCAGCAACGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCC
LK2-10          TAGTAATCGCAGATCAGCAACGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCC
LK2-5           TAGTAATCGCAGATCAGCAACGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCC
KM1-7           TAGTAATCGCAGATCAGCAACGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCC
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Figure 20. (Continued)

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BTG2-3          CGTCACGTCACGAAAGTCGGCAACACCCGAAGCCGGTGGCCCAACCCTTGTGGAGGG
BTG3-4          CGTCACGTCACGAAAGTCGGCAACACCCGAAGCCGGTGGCCCAACCCTTGTGGAGGG
LK2-6           CGTCACGTCACGAAAGTCGGCAACACCCGAAGCCGGTGGCCCAACCCTTGTGGAGGG
BTG1-4          CGTCACGTCACGAAAGTCGGCAACACCCGAAGCCGGTGGCCCAACCCTTGTGGAGGG
KM1-9           CGTCACGTCACGAAAGTCGGCAACACCCGAAGCCGGTGGCCCAACCCTTGTGGAGGG
LK2-12          CGTCACGTCACGAAAGTCGGCAACACCCGAAGCCGGTGGCCCAACCCTTGTGGAGGG
KM1-6           CGTCACGTCACGAAAGTCGGCAACACCCGAAGCCGGTGGCCCAACCCTTGTGGAGGG
BTG4-1          CGTCACGTCACGAAAGTCGGCAACACCCGAAGCCGGTGGCCCAACCCTTGTGGAGGG
BTG1-1          CGTCACGTCACGAAAGTCGGCAACACCCGAAGCCGGTGGCCCAACCCTTGTGGAGGG
BTG3-2          CGTCACGTCACGAAAGTCGGCAACACCCGAAGCCGGTGGCCCAACCCTTGTGGAGGG
X92604          CGTCAGGTCACGAAAGTCGGCAACACCCGAAGCCGGTGGCCCAACCCTTGTGGAGGG
KM3-14          CGTCACGTCACGAAAGTCGGCAACACCCGAAGCCGGTGGCCCAACCCTTGTGGAGGG
KM3-1           CGTCACGTCACGAAAGTCGGCAACACCCGAAGCCGGTGGCCCAACCCTTGTGGAGGG
X92594          CGTCAGGTCACGAAAGTCGGCAACACCCGAAGCCGGTGGCCCAACCCTTGTGGAGGG
X92601          CGTCAGGTCACGAAAGTCGGCAACACCCGAAGCCGGTGGCCCAACCCTTGTGGAGGG
X92611          CGTCAGGTCACGAAAGTCGGCAACACCCGAAGCCGGTGGCCCAACCCTTGTGGAGGG
KM4-29          CGTCACGTCACGAAAGTCGGCAACACCCGAAGCCGGTGGCCCAACCCTTGTGGAGGG
KM4-24          CGTCACGTCACGAAAGTCGGCAACACCCGAAGCCGGTGGCCCAACCCTTGTGGAGGG
LK6-12          CGTCACGTCACGAAAGTCGGCAACACCCGAAGCCGGTGGCCCAACCCTTGTGGAGGG
LK5-4           CGTCACGTCACGAAAGTCGGCAACACCCGAAGCCGGTGGCCCAACCCTTGTGGAGGG
TT2-4           CGTCACGTCACGAAAGTCGGCAACACCCGAAGCCGGTGGCCCAACCCTTGTGGAGGG
BTG10-2         CGTCACGTCACGAAAGTCGGCAACACCCGAAGCCGGTGGCCCAACCCTTGTGGAGGG
BTG7-3          CGTCACGTCACGAAAGTCGGCAACACCCGAAGCCGGTGGCCCAACCCTTGTGGAGGG
AF152109        CGTCACGTCACGAAAGTCGGCAACACCCGAAGCCGGTGGCCCAACCCTTGTGGAGGG
X92599          CGTCAGGTCACGAAAGTCGGCAACACCCGAAGCCGGTGGCCCAACCCTTGTGGAGGG
X92613          CGTCAGGTCACGAAAGTCGGCAACACCCGAAGCCGGTGGCCCAACCCGTAAGGAGGG
TT1-11          CGTCACGTCACGAAAGTCGGCAACACCCGAAGCCGGTGGCCCAACCCTTGTGGAGGG
X92598          CGTCAGGTCACGAAAGTCGGCAACACCCGAAGCCGGTGGCCCAACCCTTGTGGAGGG
X92608          CGTCAGGTCACGAAAGTCGGCAACACCCGAAGCCGGTGGCCCAACCCTTGTGGAGGG
X92607          CGTCACGTCACGAAAGTCGGCAACACCCGAAGCCGGTGGCCCAACCCTTGTGGAGGG
X92610          CGTCAGGTCACGAAAGTCGGCAACACCCGAAGCCGGTGGCCCAACCCTTGTGGAGGG
X92609          CGTCAGGTCACGAAAGTCGGCAACACCCGAAGCCGGTGGCCCAACCCTTGTGGAGGG
X92631          CGTCACGTCACGAAAGTCGGCAACACCCGAAGCCGGTGGCCCAACCCTTGTGGAGGG
X92628          CGTCACGTCACGAAAGTCGGCAACACCCGAAGCCGGTGGCCCAACCCTTGTGGAGGG
LK2-10          CGTCACGTCACGAAAGTCGGCAACACCCGAAGCCGGTGGCCCAACCCTTGTGGAGGG
LK2-5           CGTCACGTCACGAAAGTCGGCAACACCCGAAGCCGGTGGCCCAACCCTTGTGGAGGG
KM1-7           CGTCACGTCACGAAAGTCGGCAACACCCGAAGCCCATGGCCTAACCTTTTCCGGGGG
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Figure 20. (Continued)

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BTG2-3          AGCCGTCAAGGTGGGGCTGGCGATTGGGACGAATCTAACAAGG
BTG3-4          AGCCGTCAAGGTGGGGCTGGCGATTGGGACGAATCTAACAAGG
LK2-6           AGCCGTCAAGGTGGGGCTGGCGATTGGGACGAATCTAACAAGG
BTG1-4          AGCCGTCAAGGTGGGGCTGGCGATTGGGACGAATCTAACAAGG
KM1-9           AGCCGTCAAGGTGGGGCTGGCGATTGGGACGAATCTAACAAGG
LK2-12          AGCCGTCAAGGTGGGGCTGGCGATTGGGACGAATCTAACAAGG
KM1-6           AGCCGTCAAGGTGGGGCTGGCGATTGGGACGAATCTAACAAGG
BTG4-1          AGCCGTCAAGGTGGGGCTGGCGATTGGGACGAATCTAACAAGG
BTG1-1          AGCCGTCAAGGTGGGGCTGGCGATTGGGACGAATCTAACAAGG
BTG3-2          AGCCGTCAAGGTGGGGCTGGCGATTGGGACGAATCTAACAAGG
X92604          AGCCGTCAAGGTGGGGCTGGCGATTGGGACGAATCTAACAAGG
KM3-14          AGCCGTCAAGGTGGGGCTGGCGATTGGGACGAATCTAACAAGG
KM3-1           AGCCGTCAAGGTGGGGCTGGCGATTGGGACGAATCTAACAAGG
X92594          AGCCGTCAAGGTGGGGCTGGCGATTGGGACGAATCTAACAAGG
X92601          AGCCGTCAAGGTGGGGCTGGCGATTGGAACGAATCTAACAAGG
X92611          AGCCGTCAAGGTGGGGCTGGCGATTGGGACGAATCTAACAAGG
KM4-29          AGCCGTCAAGGTGGGGCTGGCGATTGGGACGAATCTAACAAGG
KM4-24          AGCCGTCAAGGTGGGGCTGGCGATTGGGACGAATCTAACAAGG
LK6-12          AGCCGTCAAGGTGGGGCTGGCGATTGGGACGAATCTAACAAGG
LK5-4           AGCCGTCAAGGTGGGGCTGGCGATTGGGACGAATCTAACAAGG
TT2-4           AGCCGTCAAGGTGGGGCTGGCGATTGGGACGAATCTAACAAGG
BTG10-2         AGCCGTCAAGGTGGGGCTGGCGATTGGGACGAATCTAACAAGG
BTG7-3          AGCCGTCAAGGTGGGGCTGGCGATTGGGACGAATCTAACAAGG
AF152109        AGCCGTCAAGGTGGGGCTGGCGATTGGGACGAATCTAACAAGG
X92599          AGCCGTCAAGGTGGGGCTGGCGATTGGGACGAATCTAACAAGG
X92613          AGCCGTCAAGGTGGGGCTGGCGATTGGGACGAATCTAACAAGG
TT1-11          AGCCGTCAAGGTGGGGCTGGCGATTGGGACGAATCTAACAAGG
X92598          AGCCGTCAAGGTGGGGCTGGCGATTGGNACGAATCTAACAAGG
X92608          AGCCGTCAAGGTGGGGCTGGCGATTGGGACGAATCTAACAAGG
X92607          AGCCGTCAAGGTGGTGCTGGCGATTGGGACGAATCTAACAAGG
X92610          AGCCGTCAAGGTGGGGCTGGCGATTGGGACGAATCTAACAAGG
X92609          AGCTGTCAAGGTGGGGCTGGCGATTGGGACGAATCTAACAAGG
X92631          AGCCGTCAAGGTGGGGCTGGCGATTGGGACGAATCTAACAAGG
X92628          AGCCGTCAAGGTGGGGCTGGCGATTGGGACGAATCTAACAAGG
LK2-10          AGCCGTCAAGGTGGGGCTGGCGATTGGGACGAATCTAACAAGG
LK2-5           AGCCGTCAAGGTGGGGCTGGCGATTGGGACGAATCTAACAAGG
KM1-7           AGTGGTCAAGGTGGGGCTGGCGATTGGGACGAATCTAACAAGG
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Figure 20. (Continued)

X92604 = *M. aurantiaca* DSM 43813^{T}

X92608= *M. pallida* DSM 43817^{T}

X92594= *M. chalcea* DSM 43026^{T}

X92607= *M. echinospora* DSM 43816^{T}

X92601= *M. halophytica* DSM 43171^{T}

X92610= *M. inositola* DSM 43819^{T}

X92611= *M. purpureochromogenes* DSM 43821^{T}

X92609= *M. nigra* DSM 43818^{T}

AF152109= *M. matsumotoense* IMSNU 22003^{T}

X92631= *M. rosaria* DSM 803^{T}

X92599= *M. carbonacea* DSM 43168 ^{T}

X92628= *M. chersina* DSM 44151^{T}

X92613= *M. olivasterospora* DSM 43868^{T}

X92598= *M. coerulea* DSM 43143^{T}

Table 21. Differential characteristics among the representative strains and validly described *Micromonospora* species.

Characteristic	LK5-4	KM4-29	KM3-14	BTG10-2	TT2-4	BTG4-1	LK2-10	LK6-12	KM1-7	BTG2-3	TT1-11	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Nitrate reduction	+	-	+	-	-	w	+	-	-	-	-	-	-	-	-	+	-	+	-	+	+	+	+	+	+
Peptonization of milk	+	+	+	+	+	+	+	w	w	w	w	+	+	-	w	+	+	+	+	+	+	w	+	+	+
Starch hydrolysis	+	+	w	+	+	+	+	+	-	+	+	+	+	w	-	+	+	+	+	-	+	+	+	+	+
Gelatin liquefaction	+	w	+	+	+	+	+	w	w	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Decomposition of:																									
Tyrosine	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
Growth at :																									
40 ˚C	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
45 ˚C	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-
Max. NaCl tolerance(%)	6	6	5	5	5	4	4	4	4	3	2	1.5	5	1.5	1.5	3	3	3	2	4	4	4	4	3	3
Utilization of :																									
D-Mannitol	w	-	-	+	-	+	w	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
D-Ribose	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-	w	w	w	-	-	-	-	-	+
L-Rhamnose	w	+	-	+	w	-	+	w	w	+	-	-	-	-	-	+	-	-	+	-	-	-	-	-	+
D-Melibiose	+	+	+	+	+	+	+	-	w	w	+	+	+	+	+	w	-	-	+	+	+	+	+	+	-
D-Raffinose	+	+	w	+	+	+	+	+	-	+	+	+	+	w	w	-	-	+	-	w	+	+	-	+	-
Glycerol	w	+	-	+	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	w	-	+	-
Salicin	-	+	-	-	+	+	+	+	-	w	+	w	-	-	-	-	-	-	-	-	+	-	-	+	-
Lactose	+	+	-	+	+	+	+	w	+	-	+	+	+	+	w	w	-	+	+	-	+	+	w	+	-
D-Galactose	+	-	+	+	+	+	w	-	+	-	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+
L-Arabinose	-	-	w	-	+	w	-	-	-	-	+	-	w	-	-	+	+	+	w	w	w	+	-	-	+
Cellobiose	+	+	+	w	+	+	+	w	+	-	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+
D-Fructose	w	-	+	-	w	-	-	-	w	-	+	+	w	w	+	+	-	+	-	+	w	+	w	+	+

1, *M. coerulea* JCM 3175^T; 2, *M. chalcea* JCM 3031^T; 3, *M. inositola* JCM 6239^T; 4, *M. purpureochromogenes* JCM 3156^T; 5, *M. olivasterospora* JCM 7348^T; 6, *M. echinospora* JCM 3073^T; 7, *M. matsumotoense* JCM 9401^T; 8, *M. rosaria* JCM 3159^T; 9, *M. aurantiaca* JCM 10878^T; 10. *M. nigra* JCM 8973^T; 11. *M. halophytica* JCM 3125^T; 12. *M. chersina* JCM 9459^T; 13. *M. carbonacea* JCM 3139^T; 14. *M. pallida* JCM 3133^T

+, positive; -, negative, w, weakly negative.

Table 22. Similarity percentage of the representative *Micromonospora* strains.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43
1. AB037012	100	98.3	97.7	97.4	97.1	96.6	96.3	96.6	96.4	96.9	96.4	96.8	96.7	96.7	96.7	96.9	96.9	96.4	96.4	96.6	96.3	96.8	96.8	96.8	96.9	96.9	96.9	96.9	96.9	96.9	97	96.9	97.2	96.3	96.8	96.8	96.9	96.6	96.6	96.6	97.1	96.3	89
2. AB037011	98.3	100	97.7	97.1	97.3	96.7	96.4	96.7	96.6	96.7	96.7	96.6	96.4	96.7	96.7	96.7	96.7	96.6	96.6	96.8	96.6	96.4	96.4	96.4	96.6	96.6	96.6	96.6	96.6	96.6	96.9	96.8	97.1	96.3	96.7	96.6	96.6	96.3	96.3	96.5	97	96.3	89
3. D85474	97.7	97.7	100	96.2	96.4	96.6	96.3	96.6	96.3	96.5	96.6	96.6	96.3	96.8	96.8	96.6	96.6	96.6	96.7	96.8	96.9	96.4	96.4	96.4	96.6	96.6	96.6	96.6	96.6	96.6	96.7	96.6	96.6	95.9	96.3	96.2	96.4	96.2	96.1	96.1	96.3	96.1	88.9
4. AB037000	97.4	97.1	96.2	100	98.1	96	95.7	96	95.9	96.2	96.5	96.1	96.1	96.4	96.4	96.5	96.5	96.3	96.1	96.4	96.3	96.6	96.6	96.6	96.7	96.7	96.7	96.7	96.7	96.7	96.8	96.7	97	96.4	96.6	96.9	96.8	96.5	96.5	96.1	96.9	96.8	88.7
5. AB037008	97.1	97.3	96.4	98.1	100	96.3	96.1	96.3	96.1	96.6	96.3	96.5	96.3	96.6	96.6	96.7	96.7	96.3	96.3	96.3	96.4	97	97	97	97.1	97.1	97.1	97.1	97.1	97.1	97.1	97.1	97.4	96.4	97	96.9	96.6	96.5	96.5	96.6	97.3	97.1	89.2
6. BTG3-2	96.6	96.7	96.6	96	96.3	100	99.7	100	99.3	99.6	98.8	99.6	99.3	99	99	99.1	99.1	99.3	99.1	98.3	98.5	98.1	98.1	98.1	98.2	98.2	98.2	98.2	98.2	98.2	98.4	98.3	98.5	97.7	98.4	97.9	98.1	98	97.9	98	97.7	96.9	89
7. X92604	96.3	96.4	96.3	95.7	96.1	99.7	100	99.7	99.1	99.3	98.5	99.3	99.2	98.7	98.7	98.8	98.8	99.3	99.1	98	98.5	97.8	97.8	97.8	98	98	98	98	98	98	98.1	98	98.2	97.7	98.2	97.7	98.1	97.7	97.9	98	97.7	96.6	88.7
8. KM3-14	96.6	96.7	96.6	96	96.3	100	99.7	100	99.3	99.6	98.8	99.6	99.3	99	99	99.1	99.1	99.3	99.1	98.3	98.5	98.1	98.1	98.1	98.2	98.2	98.2	98.2	98.2	98.2	98.4	98.3	98.5	97.7	98.4	97.9	98.1	98	97.9	98	97.7	96.9	89
9. LK6-12	96.4	96.6	96.3	95.9	96.1	99.3	99.1	99.3	100	99.3	99.1	99.3	99.1	98.8	98.8	99	99	98.5	98.6	98.5	98	97.9	97.9	97.9	98	98	98	98	98	98	98	98	98.1	97.7	98.2	98.1	98	97.8	97.9	97.7	97.1	96.6	88.8
10. LK5-4	96.9	96.7	96.5	96.2	96.6	99.6	99.3	99.6	99.3	100	98.7	99.9	99.7	99.3	99.3	99.5	99.5	98.9	98.8	98.3	98.1	98.5	98.5	98.5	98.6	98.6	98.6	98.6	98.6	98.6	98.5	98.5	98.5	97.7	98.7	98.2	98.1	98	98	98.2	97.6	97.1	89.2
11. TT2-4	96.4	96.7	96.6	96.5	96.3	98.8	98.5	98.8	99.1	98.7	100	98.6	98.5	98.8	98.8	98.9	98.9	98.9	98.7	98.8	98.7	98.2	98.2	98.2	98.4	98.4	98.4	98.4	98.4	98.4	98.5	98.4	98.1	97.8	98.2	98.4	98.3	98.1	98.2	97.9	97.7	97	88.9
12. KM3-1	96.8	96.6	96.6	96.1	96.5	99.6	99.3	99.6	99.3	99.9	98.6	100	99.6	99.2	99.2	99.4	99.4	98.9	98.8	98.2	98.1	98.4	98.4	98.4	98.5	98.5	98.5	98.5	98.5	98.5	98.5	98.4	98.5	97.7	98.6	98.2	98.1	98	98	98.1	97.6	97	89.1
13. X92594	96.7	96.4	96.3	96.1	96.3	99.3	99.2	99.3	99.1	99.7	98.5	99.6	100	99	99	99.2	99.2	98.8	98.8	98.2	98	98.3	98.3	98.3	98.5	98.5	98.5	98.5	98.5	98.5	98.2	98.2	98.2	97.7	98.5	98.1	98.1	97.8	98	98.1	97.4	96.8	89.1
14. KM4-29	96.7	96.7	96.8	96.4	96.6	99	98.7	99	98.8	99.3	98.8	99.2	99	100	100	99.8	99.8	99	98.9	98.8	98.3	98.5	98.5	98.5	98.7	98.7	98.7	98.7	98.7	98.7	99	98.9	98.7	98	98.5	98.5	98.5	98.2	98.1	98.2	97.7	97.1	89
15. KM4-24	96.7	96.7	96.8	96.4	96.6	99	98.7	99	98.8	99.3	98.8	99.2	99	100	100	99.8	99.8	99	98.9	98.8	98.3	98.5	98.5	98.5	98.7	98.7	98.7	98.7	98.7	98.7	99	98.9	98.7	98	98.5	98.5	98.5	98.2	98.1	98.2	97.7	97.1	89
16. BTG10-2	96.9	96.7	96.6	96.5	96.7	99.1	98.8	99.1	99	99.5	98.9	99.4	99.2	99.8	99.8	100	100	99	99	98.8	98.3	98.8	98.8	98.8	98.9	98.9	98.9	98.9	98.9	98.9	99	98.9	98.8	98	98.5	98.6	98.5	98.2	98.2	98.2	97.7	97.1	89.1
17. BTG7-3	96.9	96.7	96.6	96.5	96.7	99.1	98.8	99.1	99	99.5	98.9	99.4	99.2	99.8	99.8	100	100	99	99	98.8	98.3	98.8	98.8	98.8	98.9	98.9	98.9	98.9	98.9	98.9	99	98.9	98.8	98	98.5	98.6	98.5	98.2	98.2	98.2	97.7	97.1	89.1
18. X92611	96.4	96.6	96.6	96.3	96.3	99.3	99.3	99.3	98.5	98.9	98.9	98.9	98.8	99	99	99	99	100	99.2	98.5	98.8	98.1	98.1	98.1	98.2	98.2	98.2	98.2	98.2	98.2	98.5	98.4	98.5	98.3	98.3	98.4	98.3	97.9	98.2	98.1	98	96.8	88.7
19. X92601	96.4	96.6	96.7	96.1	96.3	99.1	99.1	99.1	98.6	98.8	98.7	98.8	98.8	98.9	98.9	99	99	99.2	100	98.2	98.8	98.2	98.2	98.2	98.3	98.3	98.3	98.3	98.3	98.3	98.7	98.6	98.5	98	98.2	98	98.7	98.4	98.4	98.2	97.7	96.9	89
20. AF152109	96.6	96.8	96.8	96.4	96.3	98.3	98	98.3	98.5	98.3	98.8	98.2	98.2	98.8	98.8	98.8	98.8	98.5	98.2	100	98.3	97.9	97.9	97.9	98	98	98	98	98	98	98.1	98	98.2	97.4	98	98	97.7	97.4	97.7	97.4	97.3	96.6	88.7
21. X92599	96.3	96.6	96.9	96.3	96.4	98.5	98.5	98.5	98	98.1	98.7	98.1	98	98.3	98.3	98.3	98.3	98.8	98.8	98.3	100	97.7	97.7	97.7	97.9	97.9	97.9	97.9	97.9	97.9	98.3	98.2	98.1	97.6	97.8	97.7	98.3	97.9	98.1	97.7	98.2	97.4	89.3
22. BTG2-3	96.8	96.4	96.4	96.6	97	98.1	97.8	98.1	97.9	98.5	98.2	98.4	98.3	98.5	98.5	98.8	98.8	98.1	98.2	97.9	97.7	100	100	100	99.9	99.9	99.9	99.9	99.9	99.9	99.3	99.3	98.6	98	98.8	98.8	98.5	98.5	98.7	98.6	97.9	97.4	89.2
23. BTG3-4	96.8	96.4	96.4	96.6	97	98.1	97.8	98.1	97.9	98.5	98.2	98.4	98.3	98.5	98.5	98.8	98.8	98.1	98.2	97.9	97.7	100	100	100	99.9	99.9	99.9	99.9	99.9	99.9	99.3	99.3	98.6	98	98.8	98.8	98.5	98.5	98.7	98.6	97.9	97.4	89.2
24. LK2-6	96.8	96.4	96.4	96.6	97	98.1	97.8	98.1	97.9	98.5	98.2	98.4	98.3	98.5	98.5	98.8	98.8	98.1	98.2	97.9	97.7	100	100	100	99.9	99.9	99.9	99.9	99.9	99.9	99.3	99.3	98.6	98	98.8	98.8	98.5	98.5	98.7	98.6	97.9	97.4	89.2
25. BTG1-4	96.9	96.6	96.6	96.7	97.1	98.2	98	98.2	98	98.6	98.4	98.5	98.5	98.7	98.7	98.9	98.9	98.2	98.3	98	97.9	99.9	99.9	99.9	100	100	100	100	100	100	99.5	99.4	98.8	98.1	98.9	99	98.7	98.6	98.8	98.8	98	97.6	89.3
26. KM1-9	96.9	96.6	96.6	96.7	97.1	98.2	98	98.2	98	98.6	98.4	98.5	98.5	98.7	98.7	98.9	98.9	98.2	98.3	98	97.9	99.9	99.9	99.9	100	100	100	100	100	100	99.5	99.4	98.8	98.1	98.9	99	98.7	98.6	98.8	98.8	98	97.6	89.3
27. LK2-12	96.9	96.6	96.6	96.7	97.1	98.2	98	98.2	98	98.6	98.4	98.5	98.5	98.7	98.7	98.9	98.9	98.2	98.3	98	97.9	99.9	99.9	99.9	100	100	100	100	100	100	99.5	99.4	98.8	98.1	98.9	99	98.7	98.6	98.8	98.8	98	97.6	89.3
28. KM1-6	96.9	96.6	96.6	96.7	97.1	98.2	98	98.2	98	98.6	98.4	98.5	98.5	98.7	98.7	98.9	98.9	98.2	98.3	98	97.9	99.9	99.9	99.9	100	100	100	100	100	100	99.5	99.4	98.8	98.1	98.9	99	98.7	98.6	98.8	98.8	98	97.6	89.3
29. BTG4-1	96.9	96.6	96.6	96.7	97.1	98.2	98	98.2	98	98.6	98.4	98.5	98.5	98.7	98.7	98.9	98.9	98.2	98.3	98	97.9	99.9	99.9	99.9	100	100	100	100	100	100	99.5	99.4	98.8	98.1	98.9	99	98.7	98.6	98.8	98.8	98	97.6	89.3
30. BTG1-1	96.9	96.6	96.6	96.7	97.1	98.2	98	98.2	98	98.6	98.4	98.5	98.5	98.7	98.7	98.9	98.9	98.2	98.3	98	97.9	99.9	99.9	99.9	100	100	100	100	100	100	99.5	99.4	98.8	98.1	98.9	99	98.7	98.6	98.8	98.8	98	97.6	89.3
31. LK2-10	97	96.9	96.7	96.8	97.1	98.4	98.1	98.4	98	98.5	98.5	98.5	98.2	99	99	99	99	98.5	98.7	98.1	98.3	99.3	99.3	99.3	99.5	99.5	99.5	99.5	99.5	99.5	100	99.9	99.1	98.6	98.8	99	99	99	98.9	99	98.3	98	89.5
32. LK2-5	96.9	96.8	96.6	96.7	97.1	98.3	98	98.3	98	98.5	98.4	98.4	98.2	98.9	98.9	98.9	98.9	98.4	98.6	98	98.2	99.3	99.3	99.3	99.4	99.4	99.4	99.4	99.4	99.4	99.9	100	99	98.5	98.8	98.9	99	98.9	98.8	99	98.2	97.9	89.4
33. TT1-11	97.2	97.1	96.6	97	97.4	98.5	98.2	98.5	98.1	98.5	98.1	98.5	98.2	98.7	98.7	98.8	98.8	98.5	98.5	98.2	98.1	98.6	98.6	98.6	98.8	98.8	98.8	98.8	98.8	98.8	99.1	99	100	98.8	98.5	98.9	98.8	98.8	98.6	98.6	98.5	97.8	89
34. X92598	96.3	96.3	95.9	96.4	96.4	97.7	97.7	97.7	97.7	97.7	97.8	97.7	97.7	98	98	98	98	98.3	98	97.4	97.6	98	98	98	98.1	98.1	98.1	98.1	98.1	98.1	98.6	98.5	98.8	100	98.2	98.5	98.5	98.4	98.4	98.2	97.9	96.8	88.7
35. X92631	96.8	96.7	96.3	96.6	97	98.4	98.2	98.4	98.2	98.7	98.2	98.6	98.5	98.5	98.5	98.5	98.5	98.3	98.2	98	97.8	98.8	98.8	98.8	98.9	98.9	98.9	98.9	98.9	98.8	98.8	98.5	98.2	100	99.2	98.6	98.8	98.8	98.4	98	97.4	89.3
36. X92628	96.8	96.6	96.2	96.9	96.9	97.9	97.7	97.9	98.1	98.2	98.4	98.2	98.1	98.5	98.5	98.6	98.6	98.4	98	98	97.7	98.8	98.8	98.8	98.8	99	99	99	99	99	99	98.9	98.8	98.5	99.2	100	98.7	98.8	99.2	98.5	97.7	97.1	89
37. X92608	96.9	96.6	96.4	96.8	96.6	98.1	98.1	98.1	98	98.1	98.3	98.1	98.1	98.5	98.5	98.5	98.5	98.3	98.7	97.7	98.3	98.5	98.5	98.5	98.7	98.7	98.7	98.7	98.7	98.7	99	99	98.8	98.5	98.6	98.7	100	99.1	99	98.9	98.4	97.5	89.1
38. X92607	96.6	96.3	96.2	96.5	96.5	98	97.7	98	97.8	98	98.1	98	97.8	98.2	98.2	98.2	98.2	97.9	98.4	97.4	97.9	98.5	98.5	98.5	98.6	98.6	98.6	98.6	98.6	98.6	99	98.9	98.8	98.4	98.8	98.8	99.1	100	99	98.4	97.9	97.2	89
39. X92610	96.6	96.3	96.1	96.5	96.5	97.9	97.9	97.9	97.9	98	98.2	98	98	98.1	98.1	98.2	98.2	98.2	98.4	97.7	98.1	98.7	98.7	98.7	98.8	98.8	98.8	98.8	98.8	98.8	98.9	98.8	98.6	98.4	98.8	99.2	99	99	100	98.6	98	97	89
40. X92609	96.6	96.5	96.1	96.1	96.6	98	98	98	97.7	98.2	97.9	98.1	98.1	98.2	98.2	98.2	98.2	98.1	98.2	97.4	97.7	98.6	98.6	98.6	98.8	98.8	98.8	98.8	98.8	98.8	99	99	98.6	98.2	98.4	98.5	98.9	98.4	98.6	100	98.1	97.4	89.1
41. X92613	97.1	97	96.3	96.9	97.3	97.7	97.7	97.7	97.1	97.6	97.7	97.6	97.4	97.7	97.7	97.7	97.7	98	97.7	97.3	98.2	97.9	97.9	97.9	97.9	98	98	98	98	98	98.3	98.2	98.5	97.9	98	98.1	98.4	97.9	98	98.1	100	97.5	89.1
42. KM1-7	96.3	96.3	96.1	96.8	97.1	96.9	96.6	96.9	96.6	97.1	97	97	96.8	97.1	97.1	97.1	97.1	96.8	96.9	96.6	97.4	97.4	97.4	97.4	97.6	97.6	97.6	97.6	97.6	97.6	98	97.9	97.8	96.8	97.4	97.1	97.5	97.2	97	97.6	97.5	100	88.9
43. M27245	89	89	88.9	88.7	89.2	89	88.7	89	88.8	89.2	88.9	89.1	89.1	89	89	89.1	89.1	88.7	89	88.7	89.3	89.2	89.2	89.2	89.3	89.3	89.3	89.3	89.3	89.3	89.5	89.4	89	88.7	89.3	89	89.1	89	89	89.1	89.1	88.9	100

7. Characteristics of two known *Micromonospora* species and nine novel *Micromonospora* species (Tables 11-21)

7.1 Characteristics of *Micromonospora* Group I strains

Aerobic, Gram-positive, mesophilic, non-motile actinomycete that forms well-developed and branched substrate hyphae. Colonies are deep orange in ISP medium no. 2, turning to black after sporulation. Single spores are formed on the substrate hyphae. Aerial mycelium is absent. Utilizes D-glucose, D-melibiose, D-mannitol, D-raffinose, L-rhamnose, glycerol, lactose, D-galactose, cellobiose, D-fructose, and D-xylose, but not salicin, L-arabinose, and D-ribose. Hydrolysis of starch, gelatin liquefaction, milk peptonization and nitrate reduction are positive. Well growth is observed between 25 and 30°C. No growth occurs above 40°C. The range of NaCl concentration for growth is 5-6%. Cell wall contains glutamic acid, glycine, alanine, *meso*-diaminopimelic acid. The acyl type of the cell wall is glycolyl. The characteristic whole-cell sugars are xylose and arabinose. The phospholipid profile contains diphosphatidylglycerol, phosphatidylinositolmannosides, phosphatidylinositol and phosphatidylethanolamine, but not phosphatidylcholine. The major cellular fatty acids are iso-$C_{16:0}$, anteiso-$C_{17:0}$, and iso-$C_{15:0}$, and a small amount of iso-$C_{17:0}$, and anteiso-$C_{15:0}$ are also present. Mycolic acids are absent. The predominant menaquinones are MK-10(H_4), and MK-10(H_6). The G+C content of the DNA is 72.3 mol%. Habitat is soil.

7.2 Characteristics of *Micromonospora* Group III strains

Aerobic, Gram-positive, mesophilic, non-motile actinomycete that forms well-developed and branched substrate hyphae. Colonies are light yellowish brown in ISP medium no. 2, turning to dark yellowish brown after sporulation. Single spores are formed on the substrate hyphae. Aerial mycelium is absent. Utilizes D-glucose, D-melibiose, D-raffinose, D-galactose, cellobiose, D-fructose, and D-xylose, but not D-mannitol, L-rhamnose, glycerol, lactose, salicin, L-arabinose, and D-ribose. Hydrolysis of starch, gelatin liquefaction, milk peptonization and nitrate reduction are positive. Well growth is observed between 25 and 30°C. No growth occurs above 40°C. The range of NaCl concentration for growth is 4-5%. Cell wall contains glutamic acid, glycine, alanine, *meso*-diaminopimelic acid. The acyl type of the cell wall is glycolyl. The characteristic whole-cell sugars are xylose and arabinose. The

phospholipid profile contains diphosphatidylglycerol, phosphatidylinositolmannosides, phosphatidylinositol and phosphatidylethanolamine, but not phosphatidylcholine. The major cellular fatty acids are iso-$C_{16:0}$, anteiso-$C_{17:0}$, and iso-$C_{15:0}$, and a small amount of iso-$C_{17:0}$, and anteiso-$C_{15:0}$ are also present. Mycolic acids are absent. The predominant menaquinones are MK-10(H_4), and MK-9(H_4). The G+C content of the DNA is 72.4 mol%. Habitat is soil.

7.3 Characteristics of *Micromonospora* Group II strains

Aerobic, Gram-positive, mesophilic, non-motile actinomycete that forms well-developed and branched substrate hyphae. Colonies are vivid orange in ISP medium no. 2. Single spores are formed on the substrate hyphae. Aerial mycelium is absent. The spores are non-motile and their surface appears smooth. Pale yellow soluble pigment is produced in ISP medium no. 2. Utilizes D-glucose, D-melibiose, D-raffinose, salicin, lactose, glycerol, L-rhamnose, cellobiose, and D-xylose but not D-galactose, D-mannitol, D-ribose, L-arabinose, D-fructose. Hydrolysis of starch, milk peptonization and gelatin liquefaction are positive. Nitrate reduction is negative. Well growth is observed between 25 and 30°C. No growth occurs above 40°C. The range of NaCl concentration for growth is 5-6%. Cell wall contains glutamic acid, glycine, alanine and *meso*-diaminopimelic acid. The acyl type of the cell wall is glycolyl. The characteristic whole-cell sugars are xylose and arabinose. The phospholipid profile contains diphosphatidylglycerol, phosphatidylinositolmannosides, phosphatidylinositol and phosphatidylethanolamine, but not phosphatidylcholine. The major cellular fatty acids are iso-$C_{16:0}$, $C_{17:1}$ ($\omega 8c$), iso-$C_{15:0}$, $C_{17:0}$ and a small amount of iso-$C_{17:0}$, anteiso-$C_{17:0}$ and anteiso-$C_{15:0}$ are also present. Mycolic acids are absent. The predominant menaquinones are MK-10(H_4) and MK-10(H_6). The G+C content of the DNA is 72.4 mol%. Habitat is soil.

7.4 Characteristics of *Micromonospora* Group IV strains

Aerobic, Gram-positive, mesophilic, non-motile actinomycete that forms well-developed and branched substrate hyphae. Colonies are vivid orange in ISP medium no. 2. Single spores are formed on the substrate hyphae. Aerial mycelium is absent. The spores are non-motile and their surface appears smooth. Pale yellow soluble pigment is produced in ISP medium no. 2 and nutrient agar. Utilizes D-glucose, D-melibiose, D-raffinose, lactose, glycerol, L-rhamnose, cellobiose, D-galactose, D-

mannitol and D-xylose but not salicin, D-ribose, L-arabinose, D-fructose. Hydrolysis of starch, milk peptonization and gelatin liquefaction are positive. Nitrate reduction is negative. Well growth is observed between 25 and 30 °C. No growth occurs above 40-45 °C. The range of NaCl concentration for growth is 4-5%. Cell wall contains glutamic acid, glycine, alanine and *meso*-diaminopimelic acid. The acyl type of the cell wall is glycolyl. The characteristic whole-cell sugars are xylose and arabinose. The phospholipid profile contains diphosphatidylglycerol, phosphatidylinositolmannosides, phosphatidylinositol and phosphatidylethanolamine, but not phosphatidylcholine. The major cellular fatty acids are iso-$C_{15:0}$, iso-$C_{16:0}$, anteiso-$C_{15:0}$, and a small amount of iso-$C_{17:0}$, anteiso-$C_{17:0}$, $C_{17:1}$ ($\omega 8c$), $C_{17:0}$, and anteiso-$C_{15:0}$ are also present. Mycolic acids are absent. The predominant menaquinones are MK-10(H_4) and MK-10(H_6). The G+C content of the DNA is 72.3 mol%. Habitat is soil.

7.5 Characteristics of *Micromonospora* Group V strains

Aerobic, Gram-positive, mesophilic, non-motile actinomycete that forms well-developed and branched substrate hyphae. Colonies are vivid orange in ISP medium no. 2. Single spores are formed on the substrate hyphae. Aerial mycelium is absent. The spores are non-motile and their surface appears smooth. Yellow soluble pigment is produced in ISP medium no. 2. Utilizes D-glucose, D-melibiose, D-raffinose, salicin, lactose, L-rhamnose, L-arabinose, D-galactose, D-fructose, cellobiose and D-xylose but not D-mannitol, D-ribose and glycerol. Hydrolysis of starch, milk peptonization and gelatin liquefaction are positive. Nitrate reduction is negative. Well growth is observed between 25 and 30°C. No growth occurs above 40°C. The maximum NaCl concentration for growth is 5%. Cell wall contains glutamic acid, glycine, alanine and *meso*-diaminopimelic acid. The acyl type of the cell wall is glycolyl. The characteristic whole-cell sugars are xylose and arabinose. The phospholipid profile contains diphosphatidylglycerol, phosphatidylinositolmannosides, phosphatidylinositol and phosphatidylethanolamine, but not phosphatidylcholine. The major cellular fatty acids are iso-$C_{16:0}$, iso-$C_{15:0}$, anteiso-$C_{17:0}$, $C_{17:0}$, and a small amount of iso-$C_{17:0}$ and anteiso-$C_{15:0}$ are also present. Mycolic acids are absent. The predominant menaquinones are MK-10(H_4). The G+C content of the DNA is 72.9 mol%. Habitat is soil.

7.6 Characteristics of Group VI strains

Aerobic, Gram-positive, mesophilic, non-motile actinomycete that forms well-developed and branched substrate hyphae. Colonies are grayish yellow in ISP medium no. 2, turning to black after sporulation. Single spores are formed on the substrate hyphae. Aerial mycelium is absent. The spores are non-motile and their surface appears rough and nodular. Pale yellow soluble pigment is produced in ISP medium no. 2 and nutrient agar. Utilizes D-glucose, D-melibiose, D-raffinose, salicin, lactose, glycerol, D-galactose, D-mannitol, D-ribose, L-arabinose, cellobiose and D-xylose but not L-rhamnose and D-fructose. Hydrolysis of starch, milk peptonization, nitrate reduction and gelatin liquefaction are positive. Well growth is observed between 25 and 30°C. No growth occurs above 40°C. The maximum NaCl concentration for growth is 4%. Cell wall contains glutamic acid, glycine, alanine, and *meso*-diaminopimlic acid. The acyl type of the cell wall is glycolyl. The characteristic whole-cell sugars are xylose and arabinose. The phospholipid profile contains diphosphatidylglycerol, phosphatidylinositolmannosides, phosphatidylinositol and phosphatidylethanolamine, but not phosphatidylcholine. The major cellular fatty acids are iso-$C_{15:0}$, iso-$C_{17:0}$ iso-$C_{16:0}$, $C_{17:0}$ and a small amount of anteiso-$C_{17:0}$, and anteiso-$C_{15:0}$ are also present. Mycolic acids are absent. The predominant menaquinones are MK-10(H_8), MK-10(H_6), MK-10(H_4) and MK-9(H_8) . The G+C content of the DNA is 71.5 mol%. Habitat is soil.

7.7 Characteristics of Group VII strains

Aerobic, Gram-positive, mesophilic, non-motile actinomycete that forms well-developed and branched substrate hyphae. Colonies are yellowish white and turn to greyish black after sporulation in ISP medium no. 2. Single spores are formed on substrate hyphae. Aerial mycelium is absent. The spore surface appears rough and nodular. Spores are non-motile. Pale yellow soluble pigment is produced in ISP medium no. 2, no. 3 and nutrient agar. Nitrate is reduced to nitrite. Utilizes D-glucose, L-rhamnose, D-melibiose, D-raffinose, glycerol, salicin, lactose, cellobiose and D-xylose; weakly utilizes D-mannitol, D-galactose but not L-arabinose, D-fructose and D-ribose. Peptonization of milk, hydrolysis of starch and gelatin liquefaction are positive. Optimal temperature for growth is between 25-30^{o}C. No growth occurs above 45^{o}C. The maximum NaCl concentration for growth is 4%. Cell wall contains glutamic acid, glycine, alanine and *meso*-diaminopimelic acid in

the molar ratio of 1:0.9:0.5:1.1. The acyl type of the cell wall is the glycolyl type. Mycolic acids are absent. The predominant menaquinone is MK-9(H_4). The characteristic whole-cell sugars are xylose and arabinose. The phospholipids profile contains diphosphatidylglycerol, phosphatidylinositolmannosides, phosphatidylinositol and phosphatidylethanolamine, but not phosphatidylcholine. The major fatty acid pattern of the type strain consists of iso-$C_{15:0}$, iso-$C_{16:0}$, iso-$C_{17:0}$, anteiso-$C_{15:0}$, $C_{17:0}$, anteiso-$C_{17:0}$. The G+C content of the DNA is 71.5 mol%. Habitat is soil.

7.8 Characteristics of Group VIII strains

Aerobic, Gram-positive, mesophilic, non-motile actinomycete that forms well-developed and branched substrate hyphae. Colonies are deep orange in ISP medium no. 2, turning to dark grayish brown after sporulation. Single spores are formed on the substrate hyphae. Aerial mycelium is absent. The spores are non-motile and their surface appears rough. Pale yellow soluble pigment is produced in ISP medium no. 2. Utilizes D-glucose, D-raffinose, salicin, lactose, glycerol, D-mannitol, L-rhamnose, cellobiose and D-xylose but not D-melibiose, D-galactose, L-arabinose, D-ribose and D-fructose. Hydrolysis of starch, milk peptonization, and gelatin liquefaction are positive. Nitrate reduction is negative. Well growth is observed between 25 and 30°C. No growth occurs above 40°C. The maximum NaCl concentration for growth is 4%. Cell wall contains glutamic acid, glycine, alanine and *meso*-diaminopimelic acid. The acyl type of the cell wall is glycolyl. The characteristic whole-cell sugars are xylose and arabinose. The phospholipid profile contains diphosphatidylglycerol, phosphatidylinositolmannosides, phosphatidylinositol and phosphatidylethanolamine, but not phosphatidylcholine. The major cellular fatty acids are iso-$C_{15:0}$, iso-$C_{16:0}$, iso-$C_{17:1}$ ($\omega 9c$), $C_{17:1}$ ($\omega 8c$), anteiso-$C_{17:0}$, and a small amount of iso-$C_{17:0}$, $C_{17:0}$ and anteiso-$C_{15:0}$ are also present. Mycolic acids are absent. The predominant menaquinones are MK-10(H_4), MK-10(H_6) and MK-9(H_4) . The G+C content of the DNA is 72.5 mol%. Habitat is soil.

7.9 Characteristics of Group IX strains

Aerobic, Gram-positive, mesophilic, non-motile actinomycete that forms well-developed and branched substrate hyphae. Colonies are strong reddish orange in ISP medium no. 2. Single spores are formed on the substrate hyphae. Aerial mycelium is

absent. Yellowish orange soluble pigment is produced in ISP medium no. 2. Utilizes D-glucose, lactose, L-rhamnose, D-melibiose, D-galactose, D-fructose, cellobiose and D-xylose but not D-mannitol, D-raffinose, glycerol, salicin, L-arabinose and D-ribose. Milk peptonization, and gelatin liquefaction are weakly positive. Hydrolysis of starch, nitrate reduction are negative. Well growth is observed between 25 and 30°C. No growth occurs above 40°C. The maximum NaCl concentration for growth is 4%. Cell wall contains glutamic acid, glycine, alanine, and *meso*-diaminopimelic acid. The acyl type of the cell wall is glycolyl. The characteristic whole-cell sugars are xylose and galactose. The phospholipid profile contains diphosphatidylglycerol, phosphatidylinositolmannosides, phosphatidylinositol and phosphatidylethanolamine, but not phosphatidylcholine. The major cellular fatty acids are iso-$C_{15:0}$, iso-$C_{16:0}$, anteiso-$C_{17:0}$, anteiso-$C_{15:0}$, and a small amount of iso-$C_{17:0}$ and $C_{17:0}$ are also present. Mycolic acids are absent. The predominant menaquinones are MK-9(H_6) and MK-9(H_8). The G+C content of the DNA is 72.5 mol%. Habitat is soil.

7.10 Characteristics of Group X strains

Aerobic, Gram-positive, mesophilic, non-motile actinomycete that forms well-developed and branched substrate hyphae. Colonies are grayish yellow in ISP medium no. 2, turning to black after sporulation. Single spores are formed on the substrate hyphae. Aerial mycelium is absent. The spores are non-motile and their surface appears rough and nodular. Pale yellow soluble pigment is produced in ISP medium no. 2. Utilizes D-glucose, D-melibiose, D-raffinose, salicin, L-rhamnose, and D-xylose but not lactose, glycerol, D-galactose, D-mannitol, D-ribose, L-arabinose, cellobiose and D-fructose. Hydrolysis of starch, milk peptonization and gelatin liquefaction are positive. Nitrate reduction is negative. Well growth is observed between 25 and 30°C. No growth occurs above 40°C. The range of NaCl concentration for growth is 3-4%. Cell wall contains glutamic acid, glycine, alanine and *meso*-diaminopimelic acid. The acyl type of the cell wall is glycolyl. The characteristic whole-cell sugars are xylose and arabinose. The phospholipid profile contains diphosphatidylglycerol, phosphatidylinositolmannosides, phosphatidylinositol and phosphatidylethanolamine, but not phosphatidylcholine. The major cellular fatty acids are $C_{17:0}$, anteiso-$C_{15:0}$, iso-$C_{16:0}$, anteiso-$C_{17:0}$, and a small amount of iso-$C_{15:0}$, iso-$C_{17:0}$ and anteiso-$C_{15:0}$ are also present. Mycolic acids

are absent. The predominant menaquinones are MK-10(H_6) and MK-10(H_4). The G+C content of the DNA is 71.5 mol%. Habitat is soil.

7.11 Characteristics of Group XI strains

Aerobic, Gram-positive, mesophilic, non-motile actinomycete that forms well-developed and branched substrate hyphae. Colonies are vivid orange in ISP medium no. 2, turning to brownish black or black after sporulation. Single spores are formed on the substrate hyphae. Aerial mycelium is absent. The spores are non-motile and their surface appears rough. Brown soluble pigment is produced in ISP medium no. 2. Utilizes D-glucose, D-melibiose, D-raffinose, salicin, lactose, D-galactose, cellobiose, D-ribose, L-arabinose, D-fructose, and D-xylose, but not L-rhamnose, glycerol, and D-mannitol. Hydrolysis of starch and gelatin liquefaction are positive, milk peptonization is weakly positive, and nitrate reduction is negative. Well growth is observed between 25 and 30°C. No growth occurs above 40°C. The maximum NaCl concentration for growth is 2%. Cell wall contains glutamic acid, glycine, alanine, *meso*- and 3-OH-diaminopimelic acid. The acyl type of the cell wall is glycolyl. The characteristic whole-cell sugars are xylose and arabinose. The phospholipid profile contains diphosphatidylglycerol, phosphatidylinositolmannosides, phosphatidylinositol and phosphatidylethanolamine, but not phosphatidylcholine. The major cellular fatty acids are iso-$C_{16:0}$ and iso-$C_{15:0}$, and a small amount of iso-$C_{17:0}$, anteiso-$C_{17:0}$ and anteiso-$C_{15:0}$ are also present. Mycolic acids are absent. The predominant menaquinones are MK-10(H_4), MK-9(H_4), and MK-10(H_6). The G+C content of the DNA is 72.8 mol%. Habitat is soil. The type strain is strain TT1-11^T.

8. Structure elucidation of micromonosporin A and compound 2

Micromonosporin A was isolated from the ethyl acetate extract of YM fermentation broth of *Micromonospora* sp. TT1-11 after several chromatographic techniques. The ethyl acetate extract of the strain TT1-11 gave the major compound, Micromonosporin A (9,11,13-trihydroxy-14,19,24-trimethyl-1-azacyclotetracosa-3,5,7,15,17,19,21-heptaen-2-one, 259 mg, 6.71% (w/w) of the ethyl acetate extract or 0.43% (w/v) of the YM fermentation broth (20L)). The chemical structures of this isolated compound were elucidated by analyses of their spectroscopic data including UV, IR, MS, and NMR spectral data.

8.1 Structure elucidation of micromonosporin A

Micromonosporin A (Figure 21) was obtained as an off-white powder showing an optical rotation $[\alpha]^{25}_{D}$ -248.4 (c, 0.1, MeOH). The IR absorption spectrum displayed characteristic bands at 3,430 cm^{-1} (O-H stretching) and 1,631 cm^{-1} (C=O stretching, amide band) (Figure 46). The UV spectrum in MeOH of micromonosporin A exhibited λ_{max} (ε) at 281(4.70), 291(4.77), 306(4.51), and 322(4.33) nm (Figure 45). The ESI-TOF-MS of this compound exhibited the pseudomolecular ion peak $[M+Na]^+$ at *m/z* 450.2611 (calculated for $C_{26}H_{37}O_4N$+Na at 450.2620) (Figure 47).

The 400 MHz ^{1}H NMR spectrum of micromonosporin A (Table 23) in DMSO-d_6 (Figure 48) displayed characteristic signals for N-H of an amide at δ_H 7.57 (1H, d, *J* = 8.6), three oxygenated methine protons at δ_H 4.73 (1H, d, *J* = 4.5), 4.80 (1H, d, *J* = 2.7) and 5.08 (1H, d, *J* = 3.2), two methyl doublets at δ_H 1.03 (3H, d, *J* = 6.7) and 1.14 (3H, d, *J* = 6.6), one methyl singlet at δ_H 1.74, three nonequivalent methylenes at δ_H 1.09 (m) and 1.61 (m), δ_H 2.21 (m) and 2.32 (m) and δ_H 1.27 (m) and 1.66 (m), two methines at δ_H 2.18 (m) and 3.72 (m), and thirteen olefinic protons at δ_H 5.37 (1H, t, *J* = 9.8), δ_H 5.59 (1H, ddd, *J* = 15.0, 9.5, 5.7), δ_H 5.80(1H, d, *J* = 15.4), δ_H 5.84 (1H, dd, *J* = 15.2, 7.9), δ_H 5.94 (1H, t, *J* = 10.9), δ_H 5.99 (1H, d, *J* = 11.2), δ_H 5.62 (1H, d, *J* = 15.3), δ_H 6.14 (1H, dd, *J* = 15.2, 10.6), δ_H 6.21 (1H, dd, *J* = 15.1, 10.9), δ_H 6.34 (m), δ_H 6.35 (m), δ_H 6.39 (m) and δ_H 6.83 (1H, dd, *J* = 15.4, 10.9). The ^{13}C and DEPT NMR spectral data (Figures 51 and 53) indicated that micromonosporin A contains twenty-six carbons, including an amide carbonyl at δ_H 165.6 (s), thirteen olifenic methine carbons at δ_H 122.7 (d), δ_H 125.9 (d), δ_H 127.3 (d), δ_H 129.1 (d), δ_H 129.5 (d), δ_H 130.6 (d), δ_H 131.1 (d), δ_H 131.2 (d),δ_H 135.3 (d), δ_H 137.3 (d), δ_H 137.6 (d), δ_H 137.6 (d) and δ_H 139.9 (d) and one sp^2 quaternary carbon at δ_H 133.1 (s) assignable to seven double bonds, two methine carbons at δ_H 44.5 (t) and δ_H 44.9 (d), three methyl carbons at δ_H 12.4 (q), δ_H 16.8 (q) and δ_H 21.0 (q), three methylenes at δ_H 39.3 (t), δ_H 40.1 (t) and δ_H 46.3 (t), three oxygenated methines at δ_H 63.6 (d), δ_H 68.6 (d) and δ_H 75.0 (d). These accounted for eight of the nine degrees of unsaturation required by the molecular formular, therefore establishing micromonosporin A to be a monocyclic skeleton. The ^{1}H,^{1}H-COSY spectrum of micromonosporin A (Figure 59) revealed partial structures from H-C(3) to H-C(18) (including 14-CH_3), and from H-C(20) to H-C(24) (including 24-CH_3), and also showed the correlation between NH (at δ_H

7.57) (d, J = 8.6) and H-C(24), readily placing an amide bond next to H-C(24). The HMBC spectrum of micromonosporin A (Figure 56) allowed the assignment of 19-CH_3, demonstrating correlations from 19-CH_3 to C-atoms C(18), C(19) and C(20). Allylic couping from 19-CH_3 to H-C(20) was also observed from the ^{1}H,^{1}H-COSY spectrum of micromonosporin A. The HMBC spectrum well assembled a cyclic lactam structure of micromonosporin A, displaying the correlations from both NH proton and H-C(3) to C(2). The IR spectrum of the compound showed an absorption peak at 1631 cm^{-1}, confirming the presence of a conjugated amide C=O group. Based on these spectral data, the structure of micromonosporin A was established as 9,11,13-trihydroxy-14,19,24-trimethyl-1-azacyclotetracosa-3,5,7,15,17,19,21-heptaen-2-one. Complete assignments of H- and C-atom of micromonosporin A were achieved by analyses of ^{1}H,^{1}H-COSY, HMQC and HMBC spectra (Figures 59, 54 and 56).

The geometries of double bonds at C-3=C-4, C-5=C-6, and C-7=C-8 were determined by analyses of coupling constant values. The values of J(3,4), J(5,6) and J(7,8) were 15.4, 15.1, and 10.9 Hz, respectively, indicating (*E*)-, (*E*)-, and (*Z*)-configurations of these double bonds. Furthermore, the values of J(15,16), J(17,18) and J(21,22) were ca. 15 Hz, suggesting (*E*)-configuration of the corresponding C=C bonds. The NOESY spectral data (Figure 62) of micromonosporin A revealed the proximity of 19-CH_3 and H-C(21), implying that a C-19=C-20 bond was also (*E*)-configured. Unfortunately, the configurations at stereogenic C-atoms in micromonosporin A, i.e., C-9, C-11, C-13, C-14 and C-24 could not be established by analyses of coupling constants and NOESY-spectral data.

8.2 Structure elucidation of compound 2

In an attempt to derivatize micromonosporin A, the more stable form was succeeded by the transformation of micromonosporin A to compound **2** using Pd/C hydrogenation.

The hydrogenated product **2** (9,11,13-trihydroxy-14,19,24-trimethyl-1-azacyclotetracosan-2-one, Figure 21) was obtained as colorless viscous liquid, showing optical rotation $[\alpha]^{25}_D$ -7.73 (c, 0.1, MeOH). The IR absorption spectrum (Figure 68) displayed characteristic bands at 3,298 cm^{-1} (O-H stretching) and 1,642 cm^{-1} (C=O stretching, an amide band). The UV spectrum in MeOH of compound **2**

(Figure 67) exhibited λ_{max} (ε) at 203 (3.73) nm. The ESI-TOF-MS of this compound (Figure 69) exhibited the pseudomolecular ion peak $[M+Na]^+$ at *m/z* 464.3712 (calculated for $C_{26}H_{51}O_4N$+Na at 464.3716).

The molecular formula of compound **2** was deduced from the ESITOF mass spectrum as $C_{26}H_{51}O_4N$, showing an accurate mass at *m/z* 464.3712 $(M+Na)^+$. The ^1H NMR spectrum (Figure 70) of compound **2** in $CDCl_3$ displayed characteristic signals for an amide proton at δ_H 5.37 (1H, d, *J* = 8.4), three oxygenated methine protons at δ_H 3.69 (1H, m), 3.88 (1H, m), 4.08 (1H, m), several methylene protons between δ_H 1.14 and δ_H 1.71 (35H, m), three methyl doublets at δ_H 0.84 (3H, d, *J* = 6.5), 0.92 (3H, d, *J* = 6.7) and 1.12 (3H, d, *J* = 6.5), non-equivalent methylene protons at δ_H 2.10 (1H, m) and δ_H 2.20 (1H, m), and a methine proton at δ_H 3.99 (1H, m).

Compound **2** (viscous oil) was esterified with 3,5-dinitrobenzoyl chloride to give the corresponding ester derivative (solid). Unfortunately, this attempt failed to crystallize the 3,5-dinitrobenzoyl derivative of compound **2** for use in the X-Ray analysis; this might be because the sample was not homogeneous and probably a mixture of two diastereoisomeric products having (*R*)- and (*S*)-configurations at the newly created stereogenic center C-19 after hydrogenation of micromonosporin A.

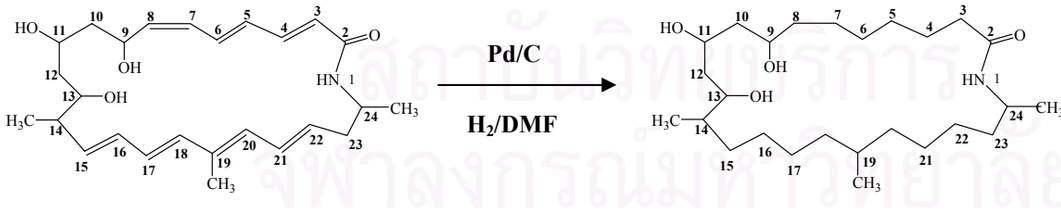

Figure 21. Hydrogenation of micromonosporin A to form compound **2**.

Table 23. 400 MHz ^{1}H- and 100 MHz ^{13}C-NMR spectral data for micromonosporin A in DMSO-d_6.

Atom	**δ_C** [a]	**δ_H**
C(2)	165.6 (s)	-
H-C(3)	125.9 (d)	5.80 (d, *J* = 15.4)
H-C(4)	137.6 (d)	6.83 (dd, *J* = 15.4, 10.9)
H-C(5)	137.3 (d)	6.21 (dd, *J* = 15.1, 10.9)
H-C(6)	122.7 (d)	6.35 (m)
H-C(7)	127.3 (d)	5.94 (t, *J* = 10.9)
H-C(8)	135.3 (d)	5.37 (t, *J* = 9.8)
H-C(9)	63.6 (d)	4.34 (m)
H-C(10)	46.3 (t)	1.27 (m); 1.66 (m)
H-C(11)	68.3 (d)	3.88 (m)
H-C(12)	40.1 (t)	1.09 (m); 1.61 (m)
H-C(13)	75.0 (d)	3.48 (m)
H-C(14)	44.5 (t)	2.18 (m)
H-C(15)	139.9 (d)	5.84 (dd, *J* = 15.2, 7.9)
H-C(16)	129.5 (d)	6.14 (dd, *J* = 15.2, 10.6)
H-C(17)	137.6 (d)	6.34 (m)
H-C(18)	129.1 (d)	6.20 (d, *J* = 15.3)
C(19)	133.1 (s)	-
H-C(20)	131.2 (d)	5.99 (d, *J* = 11.2)
H-C(21)	130.6 (d)	6.39 (m)
H-C(22)	131.1 (d)	5.59 (ddd, *J* = 15.0, 9.5, 5.7)
H-C(23)	39.3 (t)	2.21 (m); 2.32 (m)
H-C(24)	44.9 (d)	3.72 (m)
14-CH_3	16.8 (q)	1.03 (d, *J* = 6.7)
19-CH_3	12.4 (q)	1.74 (s)
24-CH_3	21.0 (q)	1.14 (d, *J* = 6.6)
NH	-	7.57 (d, *J* = 8.6)
9-OH	-	4.73 (d, *J* = 4.5)
11-OH	-	4.80 (d, *J* = 2.7)
13-OH	-	5.08 (d, *J* = 3.2)

[a]) Multiplicity was determined by analyses of DEPT spectrum; *J* in Hz

9. Biological activities

The crude ethyl acetate extract of the YM fermentation broth of *Micromonospora* sp. TT1-11 showed antimicrobial activity against *Bacillus subtilis* ATCC 6633 and *Staphylococcus aureus* ATCC 25923 with inhibition zones of 12.5 and 14.7 mm at the concentration of 1 mg/disc, respectively. The subsequent isolation for the bioactive compounds produced by this strain was performed. The new compound, micromonosporin A, was isolated from the YM fermentation broth as a major product.

As mention earlier, micromonosporin A (9,11,13-trihydroxy-14,19,24-trimethyl-1-azacyclotetracosa-3,5,7,15,17,19,21-heptaen-2-one) was very unstable and spontaneously degraded into many unidentified products, and therefore, its biological activities could not be evaluated. However, the derivative **2** (9,11,13-trihydroxy-14,19,24-trimethyl-1-azacyclotetracosan-2-one) displayed weak antimicrobial activity and also exhibited antimalarial activity at IC_{50} of 3.1 µg/mL and antimycobacterial activity with the MIC value of 50 µg/mL.

CHAPTER V

CONCLUSION

Peat swamp forest soils in the southern area of Thailand are the interested source for screening of antibiotic-producing bacteria. They are acid-sulfate soils which occur in fresh-water marshy land with the pH ranging from 3.3 to 6.4. As part of the research on screening of actinomycetes strains from soils (17 samples) in peat swamp forests collected in Trang, Pattaloong, Yala, and Narathiwat provinces, fifty-two isolates of *Micromonospora* were isolated. On the basis of morphological, cultural, physiological, and biochemical characteristics including chemotaxonomic properties, the isolates were identified as *Micromonospora*. They produced well-developed and branched substrate hyphae but no aerial hyphae. Spores were borne singly on the substrate hyphae. Their spores were smooth, rough and nodular on the surface and non-motile. The colours of the substrate mycelium were yellowish white to strong reddish orange. All strains showed the same pattern of chemotaxonomic characteristics which were similar to those of members of the genus *Micromonospora*. In general, the cell walls of *Micromonospora* are peptidoglycan type A1γ′. The acyl type of cell wall muramic acid was glycolyl. Cell wall hydrolysates contained glutamic acid, glycine, alanine, and diaminopimelic acid, and the isomer of diaminopimelic acid was *meso*, indicating that these strains have wall chemotype II. They contained glucose, xylose, arabinose, galactose, mannose, and ribose as whole-cell sugars (pattern D) but rhamnose was absent. Characteristic phospholipids were diphosphatidylglycerol, phosphatidylinositol, phosphatidylinositolmannosides, and phosphatidylethanolamine, but not phosphatidylcholine. This pattern corresponds to phospholipid type II. Their major cellular fatty acids were iso-$C_{15:0}$, iso-$C_{16:0}$, iso-$C_{17:0}$, anteiso-$C_{15:0}$, $C_{17:0}$, and anteiso-$C_{17:0}$ (type 3b). Mycolic acids were absent. The predominant menaquinones were MK-9(H_4), MK-10(H_4), or MK-9(H_6). The range of G+C content of the DNA was 71.0 to 73.0 mol%. Phylogenetic analysis of the almost complete 16S rDNA sequences revealed that the strains were placed within the clade of the genus *Micromonospora*. Based on the DNA-DNA similarity, 16S rDNA and some physiological and biochemical properties, all strains could be separated into

eleven groups. Group I strains were identified as *M. chalcea* while Group III strains were *M. aurantiaca*. The remaining nine groups (Groups II, IV, V, VI, VII, VIII, IX, X, and XI) were recognized as new species of *Micromonospora*. In this study, the names *Micromonospora eburnea* sp. nov., and *Micromonospora aurantionigra* sp. nov. are proposed for Group VII (2 strains) and Group XI (1 strain), respectively.

M. chalcea strains in Group I, *M. aurantiaca* strains in Group III, strains in Group IV, and Group VI were distributed in the soils collected from Yala, Pattaloong and Narathiwat provinces whereas the *Micromonospora* strains in Groups II, V, and XI were distributed in the soils collected from Pattaloong province. The *Micromonospora* groups VII and VIII were found in Yala. Furthermore, the strains in Group IX were distributed in the soils collected from Pattaloong and Trang and the strains in Group X were isolated from the soils collected in Narathiwat and Yala.

The primary screening revealed that seven strains in Groups VI, VII, and XI showed antimicrobial activity against *S. aureus* ATCC 25923, *B. subtilis* ATCC 6633, and *M. luteus* ATCC 9341. TT1-11 strain (Group XI) was selected for secondary metabolite production due to its ethylacetate crude extract exhibited significant antimicrobial activity. The ethyl acetate extract yielded a new polyene macrolide lactam (Micromonosporin A; 9,11,13-trihydroxy-14,19,24-trimethyl-1-azacyclotetracosa-3,5,7,15,17,19,21-heptaen-2-one) as a major compound. Unfortunately, Micromonosporin A was very unstable and has no antimicrobial activity. However, the hydrogenation product of Micromonosporin A (9,11,13-trihydroxy-14,19,24-trimethyl-1-azacyclotetracosan-2-one) displayed weak antibacterial activity and also exhibited antimalarial activity at IC_{50} of 3.1 µg/mL and antimycobacterial activity with the *MIC* of 50 µg/mL.

In this study, two known and nine novel species of *Micromonospora* strains were isolated from Thai peat swamp forest soils whereas fourteen validly described species were found in soils collected in India, Japan, USA, and the Union of Soviet Socialist Republics (USSR) (Sveshnikova *et al*., 1969). *Micromonospora* strains generally produced lactone macrolides, anthraquinones, and alkaloids, etc., while our selected strain produced a new polyene macrolide lactam. This evidence showed that the new soil sources are useful for the investigation of new microorganisms and their secondary metabolites.

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APPENDIX I

Culture media

All media were dispensed and sterilized in autoclave for 15 min at 15 pounds pressure (121˚C) for media except carbon utilization test which was sterilized at 110 pounds for 110 ˚C for 10 min. All media were prepared in 100 mL of distilled water.

1. Sodium-caseinate agar (SCN)

Sodium caseinate	0.2 g
Glucose	0.1 g
K_2HPO_4	0.02 g
$MgSO_4$	0.02 g
$FeSO_4$	trace amount
Agar	1.5 g

2. Yeast extract-malt extract agar (YMA), ISP medium no.2

Yeast extract	0.4 g
Malt extract	1.0 g
Glucose	0.4 g
Agar	1.5 g

pH 7.3

3. Oatmeal agar, ISP medium no. 3

Oatmeal	20.0 g
Agar	18.0 g

Cook or steam 20 g of oatmeal in 1,000 mL distilled water for 20 minutes. Filter through cheese cloth and add distilled water to restore volume of filtrate to

1,000 mL. Add 1 mL of trace salts solution (A) and adjust to pH 7.2 with NaOH and finally, add 18 g of agar; liquefy by steaming at 100 ˚C for 15-20 minutes.

4. <u>Inorganic salts-starch agar, ISP medium no. 4</u>

Soluble starch	1.0 g
K_2HPO_4	0.1 g
$MgSO_4.7H_2O$	0.1 g
NaCl	0.1 g
$(NH_4)_2SO_4$	0.2 g
$CaCO_3$	0.2 g
Trace salts solution (A)	0.1 mL
Agar	2.0 g

pH 7.0-7.4

5. <u>Glycerol-asparagine agar, ISP medium no.5</u>

Glycerol	1.0 g
L-Asparagine	0.1 g
K_2HPO_4	0.1 g
Trace salts solution (A)	0.1 mL
Agar	2.0 g

6. <u>Tyrosine agar, ISP medium no. 7</u>

Glycerol	1.5 g
L-Tyrosine (Difco)	0.05 g
L-Asparagine (Difco)	0.1 g
K_2HPO_4	0.05 g

$MgSO_4.7H_2O$	0.05 g
NaCl	0.05 g
$FeSO_4.7H_2O$	0.01 g
Trace salts solution (A)	0.1 mL
Agar	2.0 g

pH 7.2-7.4

Trace salt solution (A)

$FeSO_4.7H_2O$	0.1 g
$MnCl_2.4H_2O$	0.1 g
$ZnSO_4.7H_2O$	0.1 g
Distilled water	100 mL

7. Peptone KNO_3 broth

Peptone	1.0 g
KNO_3	0.1 g
NaCl	0.5 g

pH 7.0

8. Carbon utilization medium, ISP medium no. 9

Carbohydrate	1.0 g
$(NH_4)_2.SO_4$	0.264 g
$K_2HPO_4.3H_2O$	0.565 g
KH_2PO_4 anhydrous	0.238 g
$MgSO_4.7H_2O$	0.1 g
Pridham and Gottlieb trace salts (B)	0.1 mL
Agar	1.5 g

pH 6.8-7.0

Trace salts solution (B)

$CuSO_4.5H_2O$	0.64 g
$FeSO_4.7H_2O$	0.11 g
$MnCl_2.4H_2O$	0.79 g
$ZnSO_4.7H_2O$	0.15 g

9. Boullion gelatin broth

Peptone	1.0 g
Meat extract	0.5 g
NaCl	0.5 g
Gelatin	15.0 g

pH 7.0-7.2

10. Peptonization and Coagulation test medium

Skim milk (Difco)	10.0 g

11. Mueller-Hinton agar (Difco)

Beef infusion from	30 g
Casamino acid, Technical	1.75 g
Starch	0.15 g
Agar	1.7 g

pH 7.3

12. Sabouraud's dextrose agar (Difco)

Neopeptone	1.0 g

Dextrose	4.0 g
Agar	1.5 g

pH 5.6-5.8

13. <u>Seed medium</u>

Yeast extract	0.4 g
Glucose	0.4 g
Malt extract	1.0 g

pH 7.3

14. <u>Production medium</u>

Yeast extract	0.4 g
Glucose	0.4 g
Malt extract	1.0 g
$CaCO_3$	0.1 g

pH 7.3

15. <u>Peptone-yeast extract iron agar</u>

Bacto-Peptone Iron Agar, dehydrated (Difco)	3.6 g
Bacto-Yeast Extract (Difco)	0.1 g

pH 7.0-7.2

16. <u>Glucose asparagines agar</u>

Glucose	1 g
Asparagine	0.05 g
K_2HPO_4	0.05 g

Bacto-agar	1.5 g

pH 6.8-7.0

17. Nutrient agar

Meat extract	1 g
Peptone	1 g
NaCl	0.1-0.2 g
Agar	1.5 g

18. Czapek's sucrose agar

Sucrose	3 g
K_2HPO_4	0.1 g
$MgSO_4$	0.05 g
KCl	0.05 g
$FeSO_4$	0.001g
Agar	1.5-1.7 g

pH 7.0-7.2

Appendix II

Reagents and Buffers

1. DON Reagent

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

Add conc. H_2SO_4 in 2,7-dihydroxynapthalene (DON) wait util the yellow solution become colorless (24 h). Keep this solution in refrigerator.

2. 6 N HCl

Conc. HCl	60	mL
Distiller water	60	mL

Add conc. HCl into the distilled water.

3. 2 N H_2SO_4

Conc. H_2SO_4	2	mL
Distilled water	34	mL

Add conc. HCl into the distilled water.

4. Ninhydrin solution

Ninhydrin	0.3	g
1-Butanol	100	mL
Glacial acetic acid	3	mL

5. <u>5% trichloro-acetic acid</u>

Trichloro acetic acid	5	g
Distilled water	100	mL

Add conc. HCl into the distilled water.

6. <u>Reagent 1 for fatty acid analysis</u> (Saponification reagent)

Sodium hydroxide	15	g
MeOH (HPLC grade)	50	mL
Mili-Q water	50	mL

Dissolve NaOH pellets in Mili-Q water and add MeOH.

7. <u>Reagent 2 for fatty acid analysis</u> (Methylation reagent)

6 N HCl	65	mL
MeOH (HPLC grade)	55	mL

pH must be below 1.5.

8. <u>Reagent 3 for fatty acid analysis</u> (Extraction solvent)

n-Hexane (HPLC grade or n-Hexane 1000)	50	mL
Methyl-tert-Butyl Ether (HPLC grade)	50	mL

9. <u>Reagent 4 for fatty acid analysis</u> (base wash reagent)

Sodium hydroxide	1.2	g
Mili-Q water	100	mL

10. <u>Reagent 5 for fatty acid analysis</u> (Saturated sodium chloride)

11. Dittmer&Lester reagent

Solution A

MoO_3	4.011	g
25 N H_2SO_4	100	mL

Dissolve 4.011 g of MoO_3 in 100 mL of 25N H_2SO_4 by heating.

Solution B

Molybdenum powder	0.178	g
Solution A	50	mL

Add 0.178 g of molybdenum powder to 50 mL of solution A, and boil it for 15 minutes. After cooling, remove the precipitate by decantation. Before spraying, mix solution A (50 mL) plus solution B (50 mL) plus water (100 mL).

12. Anisaldehyde reagent

Ethanol	90.0	mL
H_2SO_4	5.0	mL
p-Anisaldehyde	5.0	mL
Acetic acid	1.0	mL

13. Dragendorff'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, mix solution A (10 mL) plus solution B (10 mL) plus acetic acid (10 mL).

14. Nitrate reduction test reagent

Sulphanilic acid solution

Sulphanilic acid	0.8 g
5 N Acetic acid	100 mL

Dissolve by gentle heating in a fume hood.

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

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

Dissolve by gentle heating in a fume hood.

Two drops of sulphanilic acid solution and three drops of *N,N*-dimethyl-1-naphthylamine into peptone nitrate broth inoculing with the test microorganisms.

15. Phenol:Chloroform (1:1 v/v)

Crystalline phenol was liquidified in water bath at 65 ˚C and mixed with chloroform in the ratio of 1:1 (v/v). The solution was stored in a light tight bottle.

16. 100xDenhardt solution

Bovine serum albumin	2%
Polyvinylpyrrolidone	2%
Ficoll 400	2%

17. 0.5M EDTA (pH 8.0)

800 mL of distilled water, 186.1 g of disodium ethylenediaminetetraacetate.$2H_2O$ was added and stirred vigorously on a magnetic stirrer. The pH was adjusted to 8.0 with NaOH (20 g of NaOH pellets). The volume was adjusted to 1 litre. The solution was dispensed into aliquots and sterilized by autoclaving for 15 minutes at 15 lb/in^2.

18. 5M NaCl

To 800 mL of Distilled water, 292.2 g of sodium chloride was added and adjusted the volume to 1 litre with distilled water. The solution was sterilized by autoclaving for 15 minutes at 15 lb/in^2.

19. 2xPBS

8 mM Na_2HPO_4

1.5 mM KH_2PO_4

137 mM NaCl

2.7 mM KCl

The 2xPBS was adjusted the pH to 7.0 with 1N NaOH or 1N HCL. The solution was sterilized by autoclaving for 15 minutes at 15 lb/in^2.

20. 10 mg/mL Salmon sperm DNA

A 10 mg of Salmon sperm DNA was dissolved in 1 mL of 10 mM TE buffer pH 7.6. Boiling for 10 minutes, immediately cooling in ice and sonication for 3 minutes.

21. 3 M Sodium acetate pH 5.2

To 800 mL of distilled water, 408.1 g of sodium acetate was added and adjusted the pH to 5.2 with glacial acetic acid. The volume was adjusted to 1 litre. The solution was sterilized by autoclaving for 15 minutes at 15 lb/in^2.

22. 10% Sodium dodecyl sulphate (SDS)

The stock solution of 10% SDS was prepared by dissolved 10 g of sodium dodecyl sulphate in 100 mL sterilized distilled water. Streilization is not required for the preparation of this stock solution.

23. 20xSSC

3 M NaCl

0.1 M Tri-sodiumcitrate

The 20xSSC was adjusted the pH to 7.0 with 1N NaOH. The solution was sterilized by autoclaving for 15 minutes at 15 lb/in^2.

24. 1 M Tris-HCl pH 8.0

The 1M Tris was prepared by dissolving 121.1 g of Tris base in 800 mL of distilled water. The pH was adjusted to the desired value by adding conc. HCL (pH 8.0, 42 mL of HCl). The solution was cooled to room temperature before making final adjustment to the desired pH. The volume of the solution was adjusted to 1 litter with with distilled water and sterilized by autoclaving.

25. RNase A solution

RNase A	20	mg
0.15 M NaCl	10	mL

Dissolve 20 mg of RNase A in 10 mL 0.15 M NaCl and heat at 95 ˚C for 5-10 minutes. Keep RNase A solution in -20 ˚C.

26. RNase T_1 solution

RNase T_1	80	µL
0.1 M Tris-HCl (pH 7.5)	10	mL

Mix 80 µl of RNase T_1 in 10 mL of 0.1 M Tris-HCl (pH 7.5) and heat at 95 ˚C for 5 minutes. Keep RNase T_1 solution in -20 ˚C.

27. Proteinase K

Proteinase K (Sigma)	4	mg
50 mM Tris-HCl (pH 7.5)	1	mL

Use freshly prepared solution.

28. Nuclease P_1 solution

Nuclease P1	0.1	mg
40 mM CH_3COONa+12 mM $ZnSO_4$ (pH5.3)	1	mL

Store at 4 ˚C.

29. Alkaline phosphatase solution

Alkaline phosphatase	2.4	units
0.1 M Tris-HCl (pH 8.1)	1	mL

30. 0.1 M Tris-HCl buffer, pH 9

Tris	1.21 mg
Distilled water	100 mL

Adjust the pH to 9 with HCl.

31. TE buffer

10 mM Tris HCl (pH 8.0)

1 m M Na_2-EDTA (pH 8.0)

32. TE buffer + RNase A

TE buffer	960 mL
RNase A (2 mg/mL)	100 µL

33. Saline-Na_2 EDTA

0.1 M NaCl

50 mM EDTA.2Na (pH 8.0)

34. Reagent and buffer for DNA-DNA hybridization

34.1 Prehybridization solution

100xDenhardt solution	5 mL
10 mg/ml Salmon sperm DNA	1 mL
20xSSC	10 mL
Formamide	50 mL
Distilled water	34 mL

34.2 Hybridization solution

Prehybridization solution	100 mL
Dextran sulfate	5 g

34.3 Solution I

Bovine serum albumin (Fraction V)	0.25 g
Titron X-100	50 µL

PBS	50	mL

34.4 Solution II

Streptavidin-POD	1	µL
Solution I	4	mL

34.5 Solution III

3,3',5,5'-Tetramethylbenzidine (TMB)	100	µL
(10 mg/mL in DMFO)		
0.3% H_2O_2	100	µL
0.4 M Citric acid + o.2 M Na_2HPO_4 buffer	100	µL
pH 6.2 in 10% DMFO		

34.6 2 M H_2SO_4

H_2SO_4	22	mL
Distilled water	178	mL

The solution was sterilized by autoclaving.

35. Ethidium bromide solution (10 mg/mL)

The ethidium bromide solution was prepared by dissolving 1 g of ethidium bromide in 100 mL of distilled water. The solution was stored in light-tight container at room temperature.

36. Gel loading buffer

0.025 g of bromophenol blue was dissolved in 20 mL of 15% glycerol.

37. Tris-acetate EDTA (TAE) buffer

1xTBE buffer was used as an electrophoresis buffer throughout the study. The working solution of 1xTBE buffer was prepared from stock solution of 5xTAE buffer, as followed.

Tris-base	5.4 g
Boric acid	2.75 g
Na_2-EDTA	0.47 g

Distilled water	100 mL

38. <u>Agarose gel</u>

Agarose	1.6 g
1xTBE buffer	200 mL

Appendix III

Primers and Nucleotide sequences of the PCR amplified 16S rDNA

1. <u>List of primers for 16S rDNA PCR amplification and Sequencing</u>

20F	5'-AGTTTGATCCTGGCTC-3'
1541R	5'-AAGGAGGTGATCCAGCC-3'
27F	5'-GTTTGATCCTGGCTCAG-3'
350F	5'-TACGGGAGGCAGCAG-3'
350R	5'-CTGCTGCCTCCCGTAG-3'
780F	5'-GATTAGATACCCTGGTAG-3'
780R	5'-CTACCAGGGTATCTAATCC-3'
1100F	5'-GCAACGAGCGCAACCC-3'
1100R	5'-AGGGTTGCGCTCGTTG-3'
1492R	5'-GGTTACCTTGTTACGACTT-3'

2. Nucleotide sequences of the PCR amplified 16S rDNA

TCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAGCGGAAAGGCCCTTCGGGGTACTCGAGCGGCGAA
CGGGTGAGTACACGTGAGCACCTGCCCTAGGCTTTGGGATAACCCCGGGAAAACCGGGGCTAATACCGAATAGGAC
CTGGCACCGCATGGTGTTGGGTGGAAAGTTTTTCGGCCTGGGATGGGCTCGCGGCCTATCAGCTTGTTGGTGGGGT
GATGGCCTACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACACTGGGACTGAGACACGGCCCAG
ACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGGAAGCCTGATGCAGCGACGCCGCGTGAGGGATG
ACGGCCTTCGGGTTGTAACCTCTTTCAGCAGGGACGAAGCGTAAGTGACGGTACCTGCAGAAGAAGCGCCGGCCAA
CTACGTGCCAGCAGCCGCGGTAAGACGTAGGGCGCGAGCGTTGTCCGGATTTATTGGGCGTAAAGAGCTCGTAGGC
GGCTTGTCGCGTCGACTGTGAAAACCCACGGCTCAACCGTGGGCCTGCAGTCGATACGGGCAGGCTAGAGTTCGGT
AGGGGAGACTGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGCGGGTCT
CTGGGCCGATACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCTGTA
AACGTTGGGCGCTAGGTGTGGGGGGCCTCTCCGGTTCCCTGTGCCGCAGCTAACGCATTAAGCGCCCCGCCTGGGG
AGTACGGCCGCAGGCTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGCGGAGCATGCGGATTAATTCGAT
GCAACGCGAAGAACCTTACCTGGGTTTGACATGGCCGCAAAACCTGCAGAGATGTGGGGTCCTTCGGGGGCGGTCA
CAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTCGTTC
GATGTTGCCAGCGCGTTATGGCGGGGACTCATCGAAGACTGCCGGGGTCAACTCGGAGGAAGGTGGGGATGACGTC
AAGTCATCATGCCCCTTATGTCCAGGGCTTCACGCATGCTACAATGGCCGGTACAATGGGCTGCGATACCGTGAGG
TGGAGCGAATCCCAAAAAGCCGGTCTCAGTTCGGATCGGGGTCTGCAACTCGACCCCGTGAAGTCGGAGTCGCTAG
TAATCGCAGATCAGCAACGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACGTCACGAAAGTCG
GCAACACCCGAAGCCGGTGGCCCAACCCTTGTGGAGGGAGCCGTCGAAGGTGGGGCTGGCGATTGGGACGAAGTCG
TAACAAGGTAGCCGTACCGGAAGGTGCGGTGGGA

Figure 22. The PCR amplified 16S rDNA nucleotide sequences of LK2-6

AGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAGCGGAAAGGCCCTTCGGGGTACTCGAGCGGCGAACG
GGTGAGTACACGTGAGCACCTGCCCTAGGCTTTGGGATAACCCCGGGAAAACCGGGGCTAATACCGAATAGGACCT
GGCACCGCATGGTGTTGGGTGGAAAGTTTTTCGGCCTGGGATGGGCTCGCGGCCTATCAGCTTGTTGGTGGGGTGA
TGGCCTACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACACTGGGACTGAGACACGGCCCAGAC
TCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGGAAGCCTGATGCAGCGACGCCGCGTGAGGGATGAC
GGCCTTCGGGTTGTAAACCTCTTTCAGCAGGGACGAAGCGTAAGTGACGGTACCTGCAGAAGAAGCGCCGGCCAAC
TACGTGCCAGCAGCCGCGGTAAGACGTAGGGCGCGAGCGTTGTCCGGATTTATTGGGCGTAAAGAGCTCGTAGGCG
GCTTGTCGCGTCGACTGTGAAAACCCACGGCTCAACCGTGGGCCTGCAGTCGATACGGGCAGGCTAGAGTTCGGTA
GGGGAGACTGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAANGCGGGTCTC
TGGGCCGATACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCTGTAA
CGTTGGGCGCTAGGTGTGGGGGGCCTCTCCGGTTCCTGTGCCGCAGCTAACGCATTAAGCGCCCCGCCTGGGGAGT
ACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGC
AACGCGAAGAACCTTACCTGGGTTTGACATGGCCGCAAAACCTGCAGAGATGTGGGGTCCTTCGGGGGCGGTCACA
GGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTCGTTCGA
TGTTGCCAGCGCGTTATGGCGGGGACTCATCGAAGACTGCCGGGGTCAACTCGGAGGAAGGTGGGGATGACGTCAA
GTCATCATGCCCCTTATGTCCAGGGCTTCACGCATGCTACAATGGCCGGTACAATGGGCTGCGATACCGTGAGGTG
GAGCGAATCCCAAAAAGCCGGTCTCAGTTCGGATCGGGGTCTGCAACTCGACCCCGTGAAGTCGGAGTCGCTAGTA
ATCGCAGATCAGCAACGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACGTCACGAAAGTCGGC
AACACCCGAAGCCGGTGGCCCAACCCTTGTGGAGGGAGCCGTCGAAGGTGGGGCTGGCGATTGGGACGAAGTCGTA
ACAAGGTAGCCGTACCGGAAGGTGCGGTGGATT

Figure 23. The PCR amplified 16S rDNA nucleotide sequences of BTG2-3

AGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAGCGGAAAGGCCCTTCGGGGTACTCGAGCGGCGAACG
GGTGAAGTACACGTGAGCACCTGCCCTAGGCTTTGGGATAACCCCGGGAAAACCGGGGCTAATACCGAATAGGACC
TGGCACCGCATGGTGTTGGGTGGAAAGTTTTTCGGCCTGGGATGGGCTCGCGGCCTATCAGCTTGTTGGTGGGGTG
ATGGCCTACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACACTGGGACTGAGACACGGCCCAGA
CTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGGAAGCCTGATGCAGCGACGCCGCGTGAGGGATGA
CGGCCTTCGGGTTGTAAACCTCTTTCAGCAGGGACGAAGCGTAAGTGACGGTACCTGCAGAAGAAGCGCCGGCCAA
CTACGTGCCAGCAGCCGCGGTAAGACGTAGGGCGCGAGCGTTGTCCGGATTTATTGGGCGTAAAGAGCTCGTAGGC
GGCTTGTCGCGTCGACTGTGAAAACCCACGGCTCAACCGTGGGCCTGCAGTCGATACGGGCAGGCTAGAGTTCGGT
AGGGGAGACTGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGCGGGTCT
CTGGGCCGATACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCTGTA
AACGTTGGGCGCTAGGTGTGGGGGGCCTCTCCGGTTCCCTGTGCCGCAGCTAACGCATTAAAGCGCCCCGCCTGGG
GAGTACGGCCGCAGGCTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGCGGAGCATGCGGATTAATTCGA
TGCAACGCGAAGAACCTTACCTGGGTTTGACATGGCCGCAAAACCTGCAGAGATGTGGGGTCCTTCGGGGGCGGTC
ACAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTCGTT
CGATGTTGCCAGCGCGTTATGGCGGGGACTCATCGAAGACTGCCGGGGTCAACTCGGAGGAAGGTGGGGATGACGT
CAAGTCATCATGCCCCTTATGTCCAGGGCTTCACGCATGCTACAATGGCCGGTACAATGGGCTGCGATACCGTGAG
GTGGAGCGAATCCCAAAAAGCCGGTCTCAGTTCGGATCGGGGTCTGCAACTCGACCCCGTGAAGTCGGAGTCGCTA
GTAATCGCAGATCAGCAACGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACGTCACGAAAGTC
GGCAACACCCGAAGCCGGTGGCCCAACCCTTGTGGAGGGAGCCGTCGAAGGTGGGGCTGGCGATTGGGACGAAGTC
GTAACAAGGTAGCCGTACCGGAAGGTGCGGTGGAATTNNCCCTCCTA

Figure 24. The PCR amplified 16S rDNA nucleotide sequences of BTG3-4

TAGTTTGATCCTGGCTCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAGCGGAAAGGCCCTTCGGGG
TACTCGAGCGGCGAACGGGTGAGTAACACGTGAGCAACCTGCCCTAGGCTTTGGGATAACCCCGGGAAACCGGGGC
TAATACCGAATAGGACCTGGCACCGCATGGTGTTGGGTGGAAAGTTTTTCGGCCTGGGATGGGCTCGCGGCCTATC
AGCTTGTTGGTGGGGTGATGGCCTACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACACTGGGA
CTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGGAAGCCTGATGCAGCGA
CGCCGCGTGAGGGATGACGGCCTTCGGGTTGTAAACCTCTTTCAGCAGGGACGAAGCGTAAGTGACGGTACCTGCA
GAAGAAGCGCCGGCCAACTACGTGCCAGCAGCCGCGGTAAGACGTAGGGCGCGAGCGTTGTCCGGATTTATTGGGC
GTAAAGAGCTCGTAGGCGGCTTGTCGCGTCGACTGTGAAAACCCACGGCTCAACCGTGGGCCTGCAGTCGATACGG
GCAGGCTAGAGTTCGGTAGGGGAGACTGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCG
GTGGCGAAGGCGGGTCTCTGGGCCGATACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCC
TGGTAGTCCACGCTGTAAACGTTGGGCGCTAGGTGTGGGGGGCCTCTCCGGTTCCCTGTGCCGCAGCTAACGCATT
AAGCGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGCGGAG
CATGCGGATTAATTCGATGCAACGCGAAGAACCTTACCTGGGTTTGACATGGCCGCAAAACCTGCAGAGATGTGGG
GTCCTTCGGGGGCGGTCACAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAA
CGAGCGCAACCCTCGTTCGATGTTGCCAGCGCGTTATGGCGGGGACTCATCGAAGACTGCCGGGGTCAACTCGGAG
GAAGGTGGGGATGACGTCAAGTCATCATGCCCCTTATGTCCAGGGCTTCACGCATGCTACAATGGCCGGTACAATG
GGCTGCGATACCGTGAGGTGGAGCGAATCCCAAAAAGCCGGTCTCAGTTCGGATCGGGGTCTGCAACTCGACCCCG
TGAAGTCGGAGTCGCTAGTAATCGCAGATCAGCAACGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCC
GTCACGTCACGAAAGTCGGCAACACCCGAAGCCGGTGGCCCAACCCTTGTGGAGGGAGCCGTCGAAGGTGGGGCTG
GCGATTGGGACGAAGTCGTAACAAGGTAGCCGTACCGGAAGGTGCGGTGGAATCACCTCC

Figure 25. The PCR amplified 16S rDNA nucleotide sequences of BTG1-1

TAGTTTGATCCTGGCTCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAGCGGAAAGGCCCTTCGGGG
TACTCGAGCGGCGAACGGGTGAGTAACACGTGAGCAACCTGCCCTAGGCTTTGGGATAACCCCGGGAAACCGGGGC
TAATACCGAATAGGACCTGGCACCGCATGGTGTTGGGTGGAAAGTTTTTCGGCCTGGGATGGGCTCGCGGCCTATC
AGCTTGTTGGTGGGGTGATGGCCTACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACACTGGGA
CTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGGAAGCCTGATGCAGCGA
CGCCGCGTGAGGGATGACGGCCTTCGGGTTGTAAACCTCTTTCAGCAGGGACGAAGCGTAAGTGACGGTACCTGCA
GAAGAAGCGCCGGCCAACTACGTGCCAGCAGCCGCGGTAAGACGTAGGGCGCGAGCGTTGTCCGGATTTATTGGGC
GTAAAGAGCTCGTAGGCGGCTTGTCGCGTCGACTGTGAAAACCCACGGCTCAACCGTGGGCCTGCAGTCGATACGG
GCAGGCTAGAGTTCGGTAGGGGAGACTGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCG
GTGGCGAAGGCGGGTCTCTGGGCCGATACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCC
TGGTAGTCCACGCTGTAAACGTTGGGCGCTAGGTGTGGGGGGCCTCTCCGGTTCCCTGTGCCGCAGCTAACGCATT
AAGCGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGCGGAG
CATGCGGATTAATTCGATGCAACGCGAAGAACCTTACCTGGGTTTGACATGGCCGCAAAACCTGCAGAGATGTGGG
GTCCTTCGGGGGCGGTCACAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAA
CGAGCGCAACCCTCGTTCGATGTTGCCAGCGCGTTATGGCGGGGACTCATCGAAGACTGCCGGGGTCAACTCGGAG
GAAGGTGGGGATGACGTCAAGTCATCATGCCCCTTATGTCCAGGGCTTCACGCATGCTACAATGGCCGGTACAATG
GGCTGCGATACCGTGAGGTGGAGCGAATCCCAAAAAGCCGGTCTCAGTTCGGATCGGGGTCTGCAACTCGACCCCG
TGAAGTCGGAGTCGCTAGTAATCGCAGATCAGCAACGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCC
GTCACGTCACGAAAGTCGGCAACACCCGAAGCCGGTGGCCCAACCCTTGTGGAGGGAGCCGTCGAAGGTGGGGCTG
GCGATTGGGACGAAGTCGTAACAAGGTAGCCGTACCGGAAGGTGCGGCTGGAATCACCTCCTTA

Figure 26. The PCR amplified 16S rDNA nucleotide sequences of KM1-6

TAGTTTGATCCTGGNTCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAGCGGAAAGGCCCTTCGGGG
TACTCGAGCGGCGAACGGGTGAGTAACACGTGAGCAACCTGCCCTAGGCTTTGGGATAACCCCGGGAAACCGGGGC
TAATACCGAATAGGACCTGGCACCGCATGGTGTTGGGTGGAAAGTTTTTCGGCCTGGGATGGGCTCGCGGCCTATC
AGCTTGTTGGTGGGGTGATGGCCTACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACACTGGGA
CTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGGAAGCCTGATGCAGCGA
CGCCGCGTGAGGGATGACGGCCTTCGGGTTGTAAACCTCTTTCAGCAGGGACGAAGCGTAAGTGACGGTACCTGCA
GAAGAAGCGCCGGCCAACTACGTGCCAGCAGCCGCGGTAAGACGTAGGGCGCGAGCGTTGTCCGGATTTATTGGGC
GTAAAGAGCTCGTAGGCGGCTTGTCGCGTCGACTGTGAAAACCCACGGCTCAACCGTGGGCCTGCAGTCGATACGG
GCAGGCTAGAGTTCGGTAGGGGAGACTGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCG
GTGGCGAAGGCGGGTCTCTGGGCCGATACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCC
TGGTAGTCCACGCTGTAAACGTTGGGCGCTAGGTGTGGGGGGCCTCTCCGGTTCCCTGTGCCGCAGCTAACGCATT
AAGCGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGCGGAG
CATGCGGATTAATTCGATGCAACGCGAAGAACCTTACCTGGGTTTGACATGGCCGCAAAACCTGCAGAGATGTGGG
GTCCTTCGGGGGCGGTCACAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAA
CGAGCGCAACCCTCGTTCGATGTTGCCAGCGCGTTATGGCGGGGACTCATCGAAGACTGCCGGGGTCAACTCGGAG
GAAGGTGGGGATGACGTCAAGTCATCATGCCCCTTATGTCCAGGGCTTCACGCATGCTACAATGGCCGGTACAATG
GGCTGCGATACCGTGAGGTGGAGCGAATCCCAAAAAGCCGGTCTCAGTTCGGATCGGGGTCTGCAACTCGACCCCG
TGAAGTCGGAGTCGCTAGTAATCGCAGATCAGCAACGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCC
GTCACGTCACGAAAGTCGGCAACACCCGAAGCCGGTGGCCCAACCCTTGTGGAGGGAGCCGTCGAAGGTGGGGCTG
GCGATTGGGACGAAGTCGTAACAAGGTAGCCGTACCGGAAGGTGCGGCTGGGATTCACCTCCTTAA

Figure 27. The PCR amplified 16S rDNA nucleotide sequences of BTG1-4

CTAGTTTGATCCTGGCTCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAGCGGAAGGCCCTTCGGGG
TACTCGAGCGGCGAACGGGTGAGTAACACGTGAGCAACCTGCCCTAGGCTTTGGGATAACCCCGGGAAACCGGGGC
TAATACCGAATAGGACCTGGCACCGCATGGTGTTGGGTGGAAAGTTTTTCGGCCTGGGATGGGCTCGCGGCCTATC
AGCTTGTTGGTGGGGTGATGGCCTACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACACTGGGA
CTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGGAAGCCTGATGCAGCGA
CGCCGCGTGAGGGATGACGGCCTTCGGGTTGTAAACCTCTTTCAGCAGGGACGAAGCGTAAGTGACGGTACCTGCA
GAAGAAGCGCCGGCCAACTACGTGCCAGCAGCCGCGGTAAGACGTAGGGCGCGAGCGTTGTCCGGATTTATTGGGC
GTAAAGAGCTCGTAGGCGGCTTGTCGCGTCGACTGTGAAAACCCACGGCTCAACCGTGGGCCTGCAGTCGATACGG
GCAGGCTAGAGTTCGGTAGGGGAGACTGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCG
GTGGCGAAGGCGGGTCTCTGGGCCGATACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCC
TGGTAGTCCACGCTGTAAACGTTGGGCGCTAGGTGTGGGGGGCCTCTCCGGTTCCCTGTGCCGCAGCTAACGCATT
AAGCGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGCGGAG
CATGCGGATTAATTCGATGCAACGCGAAGAACCTTACCTGGGTTTGACATGGCCGCAAAACCTGCAGAGATGTGGG
GTCCTTCGGGGGCGGTCACAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAA
CGAGCGCAACCCTCGTTCGATGTTGCCAGCGCGTTATGGCGGGGACTCATCGAAGACTGCCGGGGTCAACTCGGAG
GAAGGTGGGGATGACGTCAAGTCATCATGCCCCTTATGTCCAGGGCTTCACGCATGCTACAATGGCCGGTACAATG
GGCTGCGATACCGTGAGGTGGAGCGAATCCCAAAAAGCCGGTCTCAGTTCGGATCGGGGTCTGCAACTCGACCCCG
TGAAGTCGGAGTCGCTAGTAATCGCAGATCAGCAACGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCC
GTCACGTCACGAAAGTCGGCAACACCCGAAGCCGGTGGCCCAACCCTTGTGGAGGGAGCCGTCGAAGGTGGGGCTG
GCGATTGGGACGAAGTCGTAACAAGGTAGCCGTACCGGAAGGTGCGGCTGGATCACCTCCTTAA

Figure 28. The PCR amplified 16S rDNA nucleotide sequences of KM1-9

TAGTTTGATCCTGGCTCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAGCGGAAAGGCCCTTCGGGG
TACTCGAGCGGCGAACGGGTGAGTAACACGTGAGCAACCTGCCCTAGGCTTTGGGATAACCCCGGGAAACCGGGGC
TAATACCGAATAGGACCTGGCACCGCATGGTGTTGGGTGGAAAGTTTTTCGGCCTGGGATGGGCTCGCGGCCTATC
AGCTTGTTGGTGGGGTGATGGCCTACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACACTGGGA
CTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGGAAGCCTGATGCAGCGA
CGCCGCGTGAGGGATGACGGCCTTCGGGTTGTAAACCTCTTTCAGCAGGGACGAAGCGTAAGTGACGGTACCTGCA
GAAGAAGCGCCGGCCAACTACGTGCCAGCAGCCGCGGTAAGACGTAGGGCGCGAGCGTTGTCCGGATTTATTGGGC
GTAAAGAGCTCGTAGGCGGCTTGTCGCGTCGACTGTGAAAACCCACGGCTCAACCGTGGGCCTGCAGTCGATACGG
GCAGGCTAGAGTTCGGTAGGGGAGACTGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCG
GTGGCGAAGGCGGGTCTCTGGGCCGATACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCC
TGGTAGTCCACGCTGTAAACGTTGGGCGCTAGGTGTGGGGGGCCTCTCCGGTTCCCTGTGCCGCAGCTAACGCAT
TAAGCGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGCGGA
GCATGCGGATTAATTCGATGCAACGCGAAGAACCTTACCTGGGTTTGACATGGCCGCAAAACCTGCAGAGATGTGG
GGTCCTTCGGGGGCGGTCACAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCA
ACGAGCGCAACCCTCGTTCGATGTTGCCAGCGCGTTATGGCGGGGACTCATCGAAGACTGCCGGGGTCAACTCGGA
GGAAGGTGGGGATGACGTCAAGTCATCATGCCCCTTATGTCCAGGGCTTCACGCATGCTACAATGGCCGGTACAAT
GGGCTGCGATACCGTGAGGTGGAGCGAATCCCAAAAAGCCGGTCTCAGTTCGGATCGGGGTCTGCAACTCGACCCC
GTGAAGTCGGAGTCGCTAGTAATCGCAGATCAGCAACGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCC
CGTCACGTCACGAAAGTCGGCAACACCCGAAGCCGGTGGCCCAACCCTTGTGGAGGGAGCCGTCGAAGGTGGGGCT
GGCGATTGGGACGAAGTCGTAACAAGGTAGCCGTACCGGAAGGTGCGGCTGGAATCACCTCCTTAA

Figure 29. The PCR amplified 16S rDNA nucleotide sequences of BTG4-1

TAGTTTGATCCTGGNTCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAGCGGAAAGGCCCTTCGGGG
TACTCGAGCGGCGAACGGGTGAGTAACACGTGAGCAACCTGCCCTAGGCTTTGGGATAACCCCGGGAAACCGGGGC
TAATACCGAATAGGACCTGGCACCGCATGGTGTTGGGTGGAAAGTTTTTCGGCCTGGGATGGGCTCGCGGCCTATC
AGCTTGTTGGTGGGGTGATGGCCTACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACACTGGGA
CTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGGAAGCCTGATGCAGCGA
CGCCGCGTGAGGGATGACGGCCTTCGGGTTGTAAACCTCTTTCAGCAGGGACGAAGCGTAAGTGACGGTACCTGCA
GAAGAAGCGCCGGCCAACTACGTGCCAGCAGCCGCGGTAAGACGTAGGGCGCGAGCGTTGTCCGGATTTATTGGGC
GTAAAGAGCTCGTAGGCGGCTTGTCGCGTCGACTGTGAAAACCCACGGCTCAACCGTGGGCCTGCAGTCGATACGG
GCAGGCTAGAGTTCGGTAGGGGAGACTGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCG
GTGGCGAAGGCGGGTCTCTGGGCCGATACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCC
TGGTAGTCCACGCTGTAAACGTTGGGCGCTAGGTGTGGGGGGCCTCTCCGGTTCCCTGTGCCGCAGCTAACGCATT
AAGCGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGCGGAG
CATGCGGATTAATTCGATGCAACGCGAAGAACCTTACCTGGGTTTGACATGGCCGCAAAACCTGCAGAGATGTGGG
GTCCTTCGGGGGCGGTCACAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAA
CGAGCGCAACCCTCGTTCGATGTTGCCAGCGCGTTATGGCGGGGACTCATCGAAGACTGCCGGGGTCAACTCGGAG
GAAGGTGGGGATGACGTCAAGTCATCATGCCCCTTATGTCCAGGGCTTCACGCATGCTACAATGGCCGGTACAATG
GGCTGCGATACCGTGAGGTGGAGCGAATCCCAAAAAGCCGGTCTCAGTTCGGATCGGGGTCTGCAACTCGACCCCG
TGAAGTCGGAGTCGCTAGTAATCGCAGATCAGCAACGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCC
GTCACGTCACGAAAGTCGGCAACACCCGAAGCCGGTGGCCCAACCCTTGTGGAGGGAGCCGTCGAAGGTGGGGCTG
GCGATTGGGACGAAGTCGTAACAAGGTAGCCGTACCGGAAGGTGCGGCTGGAATCACCTCCTTAA

Figure 30. The PCR amplified 16S rDNA nucleotide sequences of LK2-12

TAGTTTGATCCCTGGGCCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAGCGGAAAGGCCCTTCGGG
GTACTCGAGCGGCGAACGGGTGAGTACACGTGAGCACCTGCCCTAGGCTTTGGGATAACCCCGGGAAAACCGGGGC
TAATACCGAATAGGACCTCCTGTCGCATGGTGGGGGGTGGAAAGTTTTTCGGCCTGGGATGGGCTCGCGGCCTATC
AGCTTGTTGGTGGGGTGATGGCCTACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACACTGGGA
CTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGGAAGCCTGATGCAGCGA
CGCCGCGTGAGGGATGAACGGCCTTCGGGTTGTAAACCTCTTTCAGCAGGGACGAAGCGTAAGTGACGGTACCTGC
AGAAGAAGCGCCGGCCAACTACGTGCCAGCAGCCGCGGTAAGACGTAGGGCGCGAGCGTTGTCCGGATTTATTGGG
CGTAAAGAGCTCGTAGGCGGCTTGTCGCGTCGACTGTGAAAACCCGCGGCTCAACCGCGGGCCTGCAGTCGATACG
GGCAGGCTAGAGTTCGGTAGGGGAGACTGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACC
GGTGGCGAAGGCGGGTCTCTGGGCCGATACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACC
CTGGTAGTCCACGCTGTAAACGTTGGGCGCTAGGTGTGGGGGGCCTCTCCGGTTCCTGTGCCGCACTAACGCATTA
GCGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGCGGAGCA
TGCGGATTAATTCGATGCAACGCGAAGAACCTTACCTGGGTTTGACATGGCCGCAAAACCTGCAGAGATGTGGGGT
CCTTCGGGGGCGGTCACAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACG
AGCGCAACCCTCGTTCGATGTTGCCAGCGCGTTATGGCGGGGACTCATCGAAGACTGCCGGGGTCAACTCGGAGGA
AGGTGGGGATGACGTCAAGTCATCATGCCCCTTATGTCCAGGGCTTCACGCATGCTACAATGGCCGGTACAATGGG
CTGCGATACCGTGAGGTGGAGCGAATCCCAAAAAGCCGGTCTCAGTTCGGATCGGGGTCTGCAACTCGACCCCGTG
AAGTCGGAGTCGCTAGTAATCGCAGATCAGCAACGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGT
CACGTCACGAAAGTCGGCAACACCCGAAGCCGGTGGCCCAACCCTTGTGGAGGGAGCCGTCGAAGGTGGGGCTGGC
GATTGGGACGAAGTCGTAACAAGGTAGCCGTACCGGAAGGTGCGGCTGGATT

Figure 31. The PCR amplified 16S rDNA nucleotide sequences of LK2-10

TCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAGCGGAAAGGCCCTTCGGGGTACTCGAGCGGCGAA
CGGGTGAGTAACACGTGAGCAACCTGCCCTAGGCTTTGGGATAACCCCGGGAAACCGGGGCTAATACCGAATAGGA
CCTCCTGTCGCATGGTGGGGGGTGGAAAGTTTTTCGGCCTGGGATGGGCTCGCGGCCTATCAGCTTGTTGGTGGGG
TGATGGCCTACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACACTGGGACTGAGACACGGCCCA
GACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGGAAGCCTGATGCAGCGACGCCGCGTGAGGGAT
GACGGCCTTCGGGTTGTAAACCTCTTTCAGCAGGGACGAAGCGTAAGTGACGGTACCTGCAGAAGAAGCGCCGGCC
AACTACGTGCCAGCAGCCGCGGTAAGACGTAGGGCGCGAGCGTTGTCCGGATTTATTGGGCGTAAAGAGCTCGTAG
GCGGCTTGTCGCGTCGACTGTGAAAACCCGCGGCTCAACCGCGGGCCTGCAGTCGATACGGGCAGGCTAGAGTTCG
GTAGGGGAGACTGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGCGGGT
CTCTGGGCCGATACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCTG
TAAACGTTGGGCGCTAGGTGTGGGGGGCCTCTCCGGTTCCCTGTGCCGCAGCTAACGCATTAAGCGCCCCGCCTGG
GGAGTACGGCCGCAAGGCTAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGCGGAGCATGCGGATTAATTCG
ATGCAACGCGAAGACCCTTACCTGGGTTTGACATGGCCGCAAAACCTGCAGAGATGTGGGGTCCTTCGGGGGCGGT
CACAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTCGT
TCGATGTTGCCAGCGCGTTATGGCGGGGACTCATCGAAGACTGCCGGGGTCAACTCGGAGGAAGGTGGGGATGACG
TCAAGTCATCATGCCCCTTATGTCCAGGGCTTCACGCATGCTACAATGGCCGGTACAATGGGCTGCGATACCGTGA
GGTGGAGCGAATCCCAAAAAGCCGGTCTCAGTTCGGATCGGGGTCTGCAACTCGACCCCGTGAAGTCGGAGTCGCT
AGTAATCGCAGATCAGCAACGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACGTCACGAAAGT
CGGCAACACCCGAAGCCGGTGGCCCAACCCTTGTGGAGGGAGCCGTCGAAGGTGGGGCTGGCGATTGGGACGAAGT
CGTAACAAGGTAGCCGTACCGGAAGGTGCGGTGGATCCACCTCCCTTA

Figure 32. The PCR amplified 16S rDNA nucleotide sequences of LK2-5

TCGAGCGGAAAGGCCCTTCGGGGTACTCGAGCGGCGAACGGGTGAGTACACGTGAGCACCTGCCCTAGGCTTTGGG
ATAACCCCGGGAAACCGGGGCTAATACCGAATATGACCTTTCCTCGCATGAGGTTTGGTGGAAAGTTTTTCGGCCT
GGGATGGGCTCGCGGCCTATCAGCTTGTTGGTGGGGTGATGGCCTACCAAGGCGACGACGGGTAGCCGGCCTGAGA
GGGCGACCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATG
GGCGGAAGCCTGATGCAGCGACGCCGCGTGAGGGATGACGGCCTTCGGGTTGTAAACCTCTTTCAGCAGGGACGAA
GCGTAAGTGACGGTACCTGCAGAAGAAGCACCGGCCAACTACGTGCCAGCAGCCGCGGTAAGACGTAGGGTGCGAG
CGTTGTCCGGATTTATTGGGCGTAAAGAGCTCGTAGGCGGCTTGTCGCGTCGACCGTGAAAACTTGGGGCTCAACT
CCAAGCCTGCGGTCGATACGGGCAGGCTAGAGTTCGGTAGGGGAGACTGGAATTCCTGGTGTAGCGGTGAAATGCG
CAGATATCAGGAGGAACACCGGTGGCGAAGGCGGGTCTCTGGGCCGATACTGACGCTGAGGAGCGAAAGCGTGGGG
AGCGAACAGGATTAGATACCCTGGTAGTCCACGCTGTAAACGTTGGGCGCTAGGTGTGGGGGGCCTCTCCGGTTCC
CTGTGCCGCAGCTAACGCATTAAGCGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGG
GGGCCCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTTACCTGGGTTTGACATGGCCG
CAAAACCTCCAGAGATGGGGGGTCCTTCGGGGGCGGTCACAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAG
ATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTCGTTCGATGTTGCCAGCGCGTTATGGCGGGGACTCATCGAAG
ACTGCCGGGGTCAACTCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGCCCCTTATGTCCAGGGCTTCACGCAT
GCTACAATGGCCGGTACAATGGGCTGCGATACCGTGAGGTGGAGCGAATCCCAAAAAGCCGGTCTCAGTTCGGATC
GGGGTCTGCAACTCGACCCCGTGAAGTCGGAGTCGCTAGTAATCGCAGATCAGCAACGCTGCGGTGAATACGTTCC
CGGGCCTTGTACACACCGCCCGTCACGTCACGAAAGTCGGCAACACCCGAAGCCGGTGGCCCAACCCTTGTGGAGG
GAGCCGTCGAAGGTGGGGCTGGCGATTGGGACGAAGTCGTAACAAGGTAGCCGTACCGGAAGGTGCGTGGAATTCC

Figure 33. The PCR amplified 16S rDNA nucleotide sequences of TT2-4

TAGTTTGATCCTGGNTCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAGCGGAAAGGCCCTTCGGGG
TACTCGAGCGGCGAAACGGGTGAGTAACACGTGAGCAACCTGCCCTAGGCTTTGGGATAACCCCGGGAAACCGGGG
CTAATACCGAATATGACCACATGTCGCATGGTGTGTGGTGGAAAGTTTTTCGGCCTGGGATGGGCTCGCGGCCTAT
CAGCTTGTTGGTGGGGTGATGGCCTACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACACTGGG
ACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGGAAGCCTGATGCAGCG
ACGCCGCGTGAGGGATGACGGCCTTCGGGTTGTAAACCTCTTTCAGCAGGGACGAAGCGTAAGTGACGGTACCTGC
AGAAGAAGCGCCGGCCAACTACGTGCCAGCAGCCGCGGTAAGACGTAGGGCGCGAGCGTTGTCCGGATTTATTGGG
CGTAAAGAGCTCGTAGGCGGCTTGTCGCGTCGACCGTGAAAACCTGGGGCTCAACCCCAGGCCTGCGGTCGATACG
GGCAGGCTAGAGTTCGGTAGGGGAGACTGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACC
GGTGGCGAAGGCGGGTCTCTGGGCCGATACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACC
CTGGTAGTCCACGCTGTAAACGTTGGGCGCTAGGTGTGGGGGGCCTCTCCGGTTCCCTGTGCCGCAGCTAACGCAT
TAAGCGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGCGGA
GCATGCGGATTAATTCGATGCAACGCGAAGAACCTTACCTGGGTTTGACATGGCCGCAAAACTTGCAGAGATGTAA
GGTCCTTCGGGGGCGGTCACAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCA
ACGAGCGCAACCCTCGTTCGATGTTGCCAGCGCGTTATGGCGGGGACTCATCGAAGACTGCCGGGGTCAACTCGGA
GGAAGGTGGGGATGACGTCAAGTCATCATGCCCCTTATGTCCAGGGCTTCACGCATGCTACAATGGCCGGTACAAT
GGGCTGCGATACCGTGAGGTGGAGCGAATCCCAAAAAGCCGGTCTCAGTTCGGATCGGGGTCTGCAACTCGACCCC
GTGAAGTCGGAGTCGCTAGTAATCGCAGATCAGCAACGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCC
CGTCACGTCACGAAAGTCGGCAACACCCGAAGCCGGTGGCCCAACCCTTGTGGAGGGAGCCGTCNAAGGTGGGGCT
GGCGATTGGGACGAAGTCGTAACAAGGTAGCCGTACCGGAAGGTGCGGCTGGAATCACCTCCTTA

Figure 34. The PCR amplified 16S rDNA nucleotide sequences of KM4-24

TAGTTTGATCCTGGCTCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAGCGGAAAGGCCCTTCGGGG
TACTCGAGCGGCGAACGGGTGAGTAACACGTGAGCAACCTGCCCTAGGCTTTGGGATAACCCCGGGAAACCGGGGC
TAATACCGAATATGACCACATGTCGCATGGTGTGTGGTGGAAAGTTTTTCGGCCTGGGATGGGCTCGCGGCCTATC
AGCTTGTTGGTGGGGTGATGGCCTACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACACTGGGA
CTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGGAAGCCTGATGCAGCGA
CGCCGCGTGAGGGATGACGGCCTTCGGGTTGTAAACCTCTTTCAGCAGGGACGAAGCGTAAGTGACGGTACCTGCA
GAAGAAGCGCCGGCCAACTACGTGCCAGCAGCCGCGGTAAGACGTAGGGCGCGAGCGTTGTCCGGATTTATTGGGC
GTAAAGAGCTCGTAGGCGGCTTGTCGCGTCGACCGTGAAAACCTGGGGCTCAACCCCAGGCCTGCGGTCGATACGG
GCAGGCTAGAGTTCGGTAGGGGAGACTGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCG
GTGGCGAAGGCGGGTCTCTGGGCCGATACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCC
TGGTAGTCCACGCTGTAAACGTTGGGCGCTAGGTGTGGGGGGCCTCTCCGGTTCCCTGTGCCGCAGCTAACGCATT
AAGCGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGCGGAG
CATGCGGATTAATTCGATGCAACGCGAAGAACCTTACCTGGGTTTGACATGGCCGCAAAACTTGCAGAGATGTAAG
GTCCTTCGGGGGCGGTCACAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAA
CGAGCGCAACCCTCGTTCGATGTTGCCAGCGCGTTATGGCGGGGACTCATCGAAGACTGCCGGGGTCAACTCGGAG
GAAGGTGGGGATGACGTCAAGTCATCATGCCCCTTATGTCCAGGGCTTCACGCATGCTACAATGGCCGGTACAATG
GGCTGCGATACCGTGAGGTGGAGCGAATCCCAAAAAGCCGGTCTCAGTTCGGATCGGGGTCTGCAACTCGACCCCG
TGAAGTCGGAGTCGCTAGTAATCGCAGATCAGCAACGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCC
GTCACGTCACGAAAGTCGGCAACACCCGAAGCCGGTGGCCCAACCCTTGTGGAGGGAGCCGTCGAAGGTGGGGCTG
GCGATTGGGACGAAGTCGTAACAAGGTAGCCGTACCGGAAGGTGCGGCTGGATTCACCTCCTTAAA

Figure 35. The PCR amplified 16S rDNA nucleotide sequences of KM4-29

TAGTTTGATCCNTGGCTCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAGCGGAAAGGCCCTTCGGG
GTACTCGAGCGGCGAACGGGTGAGTAACACGTGAGCAACCTGCCCTAGGCTTTGGGATAACCCCGGGAAAACCGGG
GCTAATACCGAATATGACCTCGCATCGCATGGTGTGTGGTGGAAAGTTTTTCGGCCTGGGATGGGCTCGCGGCCTA
TCAGCTTGTTGGTGGGGTGATGGCCTACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACACTGG
GACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGGAAGCCTGATGCAGC
GACGCCGCGTGAGGGATGACGGCCTTCGGGTTGTAAACCTCTTTCAGCAGGGACGAAAGCGTAAGTGACGGTACCT
GCAGAAGAAGCGCCGGCCAACTACGTGCCAGCAGCCGCGGTAAGACGTAGGGCGCGAGCGTTGTCCGGATTTATTG
GGCGTAAAGAGCTCGTAGGCGGCTTGTCGCGTCGACCGTGAAAACCTGGGGCTCAACCCCAGGCCTGCGGTCGATA
CGGGCAGGCTAGAGTTCGGTAGGGGAGACTGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACA
CCGGTGGCGAAGGCGGGTCTCTGGGCCGATACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATA
CCCTGGTAGTCCACGCTGTAAACGTTGGGCGCTAGGTGTGGGGGGCCTCTCCGGTTCCCTGTGCCGCAGCTAACGC
ATTAAGCGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGCG
GAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTTACCTGGGTTTGACATGGCCGCAAAACTTGCAGAGATGT
AAGGTCCTTCGGGGGCGGTCACAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCG
CAACGAGCGCAACCCTCGTTCGATGTTGCCAGCGCGTTATGGCGGGGACTCATCGAAGACTGCCGGGGTCAACTCG
GAGGAAGGTGGGGATGACGTCAAGTCATCATGCCCCTTATGTCCAGGGCTTCACGCATGCTACAATGGCCGGTACA
ATGGGCTGCGATACCGTGAGGTGGAGCGAATCCCAAAAAGCCGGTCTCAGTTCGGATCGGGGTCTGCAACTCGACC
CCGTGAAGTCGGAGTCGCTAGTAATCGCAGATCAGCAACGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCG
CCCGTCACGTCACGAAAGTCGGCAACACCCGAAGCCGGTGGCCCAACCCTTGTGGAGGGAGCCGTCGAAGGTGGGG
CTGGCGATTGGGACGAAGTCGTAACAAGGTAGCCGTACCGGAAGGTGCGGTGGATTTCCNCTTCCTTA

Figure 36. The PCR amplified 16S rDNA nucleotide sequences of BTG10-2

TAGTTTGATCCTGGCTCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAGCGGAAAGGCCCTTCGGGG
TACTCGAGCGGCGAACGGGTGAGTAACACGTGAGCAACCTGCCCTAGGCTTTGGGATAACCCCGGGAAACCGGGGC
TAATACCGAATATGACCTCGCATCGCATGGTGTGTGGTGGAAAGTTTTTCGGCCTGGGATGGGCTCGCGGCCTATC
AGCTTGTTGGTGGGGTGATGGCCTACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACACTGGGA
CTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGGAAGCCTGATGCAGCGA
CGCCGCGTGAGGGATGACGGCCTTCGGGTTGTAAACCTCTTTCAGCAGGGACGAAGCGTAAGTGACGGTACCTGCA
GAAGAAGCGCCGGCCAACTACGTGCCAGCAGCCGCGGTAAGACGTAGGGCGCGAGCGTTGTCCGGATTTATTGGGC
GTAAAGAGCTCGTAGGCGGCTTGTCGCGTCGACCGTGAAAACCTGGGGCTCAACCCCAGGCCTGCGGTCGATACGG
GCAGGCTAGAGTTCGGTAGGGGAGACTGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCG
GTGGCGAAGGCGGGTCTCTGGGCCGATACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCC
TGGTAGTCCACGCTGTAAACGTTGGGCGCTAGGTGTGGGGGGCCTCTCCGGTTCCCTGTGCCGCAGCTAACGCATT
AAGCGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGCGGAG
CATGCGGATTAATTCGATGCAACGCGAAGAACCTTACCTGGGTTTGACATGGCCGCAAAACTTGCAGAGATGTAAG
GTCCTTCGGGGGCGGTCACAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAA
CGAGCGCAACCCTCGTTCGATGTTGCCAGCGCGTTATGGCGGGGACTCATCGAAGACTGCCGGGGTCAACTCGGAG
GAAGGTGGGGATGACGTCAAGTCATCATGCCCCTTATGTCCAGGGCTTCACGCATGCTACAATGGCCGGTACAATG
GGCTGCGATACCGTGAGGTGGAGCGAATCCCAAAAAGCCGGTCTCAGTTCGGATCGGGGTCTGCAACTCGACCCCG
TGAAGTCGGAGTCGCTAGTAATCGCAGATCAGCAACGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCC
GTCACGTCACGAAAGTCGGCAACACCCGAAGCCGGTGGCCCAACCCTTGTGGAGGGAGCCGTCGAAGGTGGGGCTG
GCGATTGGGACGAAGTCGTAACAAGGTAGCCGTACCGGAAGGTGCGGTGGANTCACCTCCTT

Figure 37. The PCR amplified 16S rDNA nucleotide sequences of BTG7-3

TAGTTTGATCCCTTGGCCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAGCGGAAAGGCCCTTCGGG
GTACTCGAGCGGCGAACGGGTGAGTAACACGTGAGCAACCTGCCCCAGGCTTTGGGATAACCCCGGGAAACCGGGG
CTAATACCGAATATGACCTCCGATCGCATGGTCGGTGGTGGAAAGTTTTTCGGCTTGGGATGGGCTCGCGGCCTAT
CAGCTTGTTGGTGGGGTGATGGCCTACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACACTGGG
ACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGGAAGCCTGATGCAGCG
ACGCCGCGTGAGGGATGACGGCCTTCGGGTTGTAAACCTCTTTCAGCAGGGACGAAGCGTAAGTGACGGTACCTGC
AGAAGAAGCGCCGGCCAACTACGTGCCAGCAGCCGCGGTAAGACGTAGGGCGCGAGCGTTGTCCGGATTTATTGGG
CGTAAAGAGCTCGTAGGCGGCTTGTCGCGTCGACCGTGAAAACCTGGGGCTCAACCCCAGGCCTGCGGTCGATACG
GGCAGGCTAGAGTTCGGTAGGGGAGACTGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACC
GGTGGCGAAGGCGGGTCTCTGGGCCGATACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACC
CTGGTAGTCCACGCTGTAAACGTTGGGCGCTAGGTGTGGGGGGGCCTCTCCGGTTCCCTGTGCCGCAGCTAACGCA
TTAAGCGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGCGG
AGCATGCGGATTAATTCGATGCAACGCGAAGAACCTTACCTGGGTTTGACATGGCCGCAAAACTGTCAGAGATGGC
AGGTCCTTCGGGGGCGGTCACAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGC
AACGAGCGCAACCCTCGTTCGATGTTGCCAGCGCGTTATGGCGGGGACTCATCGAAGACTGCCGGGGTCAACTCGG
AGGAAGGTGGGGATGACGTCAAGTCATCATGCCCCTTATGTCCAGGGCTTCACGCATGCTACAATGGCCGGTACAA
TGGGCTGCGATACCGTGAGGTGGAGCGAATCCCAAAAAGCCGGTCTCAGTTCGGATCGGGGTCTGCAACTCGACCC
CGTGAAGTCGGAGTCGCTAGTAATCGCAGATCAGCAACGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGC
CCGTCACGTCACGAAAGTCGGCAACACCCGAAGCCGGTGGCCCAACCCTTGTGGAGGGAGCCGTCGAAGGTGGGGC
TGGCGATTGGGACGAAGTCGTAACAAGGTAGCCGTACCGGAAGGTGCGNGGAATNCCCCCTCCTAAA

Figure 38. The PCR amplified 16S rDNA nucleotide sequences of KM3-1

CGAGCGGAAAGGCCCTTCGGGGTACTCGAGCGGCGAACGGGTGAGTAACACGTGAGCAACCTGCCCCAGGCTTTGG
GATAACCCCGGGAAACCGGGGCTAATACCGAATATGACCTTGCACCGCATGGTGTGTGGTGGAAAGTTTTTCGGCT
TGGGATGGGCTCGCGGCCTATCAGCTTGTTGGTGGGGTGATGGCCTACCAAGGCGACGACGGGTAGCCGGCCTGAG
AGGGCGACCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAAT
GGGCGGAAGCCTGATGCAGCGACGCCGCGTGAGGGATGACGGCCTTCGGGTTGTAAACCTCTTTCAGCAGGGACGA
AGCGTAAGTGACGGTACCTGCAGAAGAAGCGCCGGCCAACTACGTGCCAGCAGCCGCGGTAAGACGTAGGGCGCGA
GCGTTGTCCGGATTTATTGGGCGTAAAGAGCTCGTAGGCGGCTTGTCGCGTCGACCGTGAAAACCTGGGGCTCAAC
CCCAGGCCTGCGGTCGATACGGGCAGGCTAGAGTTCGGTAGGGGAGACTGGAATTCCTGGTGTAGCGGTGAAATGC
GCAGATATCAGGAGGAACACCGGTGGCGAAGGCGGGTCTCTGGGCCGATACTGACGCTGAGGAGCGAAAGCGTGGG
GAGCGAACAGGATTAGATACCCTGGTAGTCCACGCTGTAAACGTTGGGCGCTAGGTGTGGGGGGCCTCTCCGGTTC
CCTGTGCCGCAGCTAACGCATTAAGCGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACG
GGGGCCCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTTACCTGGGTTTGACATGGCC
GCAAAACTGTCAGAGATGGCAGGTCCTTCGGGGGCGGTCACAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGA
GATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTCGTTCGATGTTGCCAGCGCGTTATGGCGGGGACTCATCGAA
GACTGCCGGGGTCAACTCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGCCCCTTATGTCCAGGGCTTCACGCA
TGCTACAATGGCCGGTACAATGGGCTGCGATACCGTGAGGTGGAGCGAATCCCAAAAAGCCGGTCTCAGTTCGGAT
CGGGGTCTGCAACTCGACCCCGTGAAGTCGGAGTCGCTAGTAATCGCAGATCAGCAACGCTGCGGTGAATACGTTC
CCGGGCCTTGTACACACCGCCCGTCACGTCACGAAAGTCGGCAACACCCGAAGCCGGTGGCCCAACCCTTGTGGAG
GGAGCCGTCGAAGGTGGGGCTGGCGATTGGGACGAAGTCGTAACAAGGTAGCCGTACCGGAAGGTGCGGCTGGATT

Figure 39. The PCR amplified 16S rDNA nucleotide sequences of LK5-4

AGAGTTTGATCATGGCTCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAGCGGAAAGGCCCTTCGGG
GTACTCGAGCGGCGAACGGGTGAGTAACACGTGAGCAACCTGCCCTAGGCTTTGGGATAACCCTCGGAAACGGGGG
CTAATACCGAATATGACTACTGATCGCATGGTTGGTGGTGGAAAGTTTTTCGGCCTGGGATGGGCTCGCGGCCTAT
CAGCTTGTTGGTGGGGTGATGGCCTACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACACTGGG
ACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGGAAGCCTGATGCAGCG
ACGCCGCGTGAGGGATGACGGCCTTCGGGTTGTAAACCTCTTTCAGCAGGGACGAAGCGAAAGTGACGGTACCTGC
AGAAGAAGCGCCGGCCAACTACGTGCCAGCAGCCGCGGTAAGACGTAGGGCGCGAGCGTTGTCCGGATTTATTGGG
CGTAAAGAGCTCGTAGGCGGCTTGTCGCGTCGACTGTGAAAACCCGCAGCTCAACTGCGGGCCTGCAGTCGATACG
GGCAGGCTAGAGTTCGGTAGGGGAGACTGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATNAGGAGGAACACC
GGTGGCGAAGGCGGGTCTCTGGGCCGATACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACC
CTGGTAGTCCACGCTGTAAACGTTGGGCGCTAGGTGTGGGGGGCCTCTCCGGTTCCCTGTGCCGCAGCTAACGCAT
TAAGCGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGCGGA
GCATGCGGATTAATTCGATGCAACGCGAAGAACCTTACCTGGGTTTGACATGGCCGCAAAACTCGCAGAGATGTGA
GGTCCTTCGGGGGCGGTCACAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCA
ACGAGCGCAACCCTCGTTCGATGTTGCCAGCGCGTTATGGCGGGGACTCATCGAAGACTGCCGGGGTCAACTCGGA
GGAAGGTGGGGATGACGTCAAGTCATCATGCCCCTTATGTCCAGGGCTTCACGCATGCTACAATGGCCGGTACAAT
GGGCTGCGATACCGTGAGGTGGAGCGAATCCCAAAAAGCCGGTCTCAGTTCGGATCGGGGTCTGCAACTCGACCCC
GTGAAGTCGGAGTCGCTAGTAATCGCAGATCAGCAACGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCC
CGTCACGTCACGAAAGTCGGCAACACCCGAAGCCGGTGGCCCAACCCTTGTGGAGGGAGCCGTCGAAGGTGGGGCT
GGCGATTGGGACGAAGTCGTAACAAGGTAGCCGTAAAT

Figure 40. The PCR amplified 16S rDNA nucleotide sequences of TT1-11

TAGTTGATCCCTGGCTAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAGCGGAAAGGCCCTTCGGGGT
ACTCGAGCGGCGAACGGGTGAGTAACACGTGAGCAACCTGCCCCAGGCTTTGGGATAACCCCGGGAAAACCGGGGC
TAATACCGAATATGACCTTGCATCGCATGATGTCTTGGTGGAAAGTTTTTCGGCTTGGGATGGGCTCGCGGCCTAT
CAGCTTGTTGGTGGGGTGATGGCCTACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACACTGGG
ACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGGAAGCCTGATGCAGCG
ACGCCGCGTGAGGGATGACGGCCTTCGGGTTGTAAACCTCTTTCAGCAGGGACGAAGCGCAAGTGACGGTACCTGC
AGAAGAAGCACCGGCCAACTACGTGCCAGCAGCCGCGGTAAGACGTAGGGTGCGAGCGTTGTCCGGATTTATTGGG
CGTAAAGAGCTCGTAGGCGGCTTGTCGCGTCGACCGTGAAAACTTGGGGCTCAACTCCAAGCCTGCGGTCGATACG
GGCAGGCTAGAGTTCGGTAGGGGAGACTGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCANGAGGAACACC
GGTGGCGAAGGCGGGTCTCTGGGCCGATACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACC
CTGGTAGTCCACGCTGTAAACGTTGGGCGCTAGGTGTGGGGGGCCTCTCCGGTTCCCTGTGCCGCAGCTAACGCAT
TAAGCGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGCGGA
GCATGCGGATTAATTCGATGCAACGCGAAGAACCTTACCTGGGTTTGACATGGCCGCAAAACTGTCAGAGATGGCA
GGTCCTTCGGGGGCGGTCACAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCA
ACGAGCGCAACCCTCGTTCGATGTTGCCAGCGCGTTATGGCGGGGACTCATCGAAGACTGCCGGGGTCAACTCGGA
GGAAGGTGGGGATGACGTCAAGTCATCATGCCCCTTATGTCCAGGGCTTCACGCATGCTACAATGGTCGGTACAAT
GGGCTGCGATACCGTGAGGTGGAGCGAATCCCAAAAAGCCGGTCTCAGTTCGGATCGGGGTCTGCAACTCGACCCC
GTGAAGTCGGAGTCGCTAGTAATCGCAGATCAGCAACGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCC
CGTCACGTCACGAAAGTCGGCAACACCCGAAGCCGGTGGCCCAACCCTTGTGGAGGGAGCCGTCGAAGGTGGGGCT
GGCGATTGGGACGAAGTCGTAACAAGGTAGCCGTACCGGAAGGTGCGGTGGATTCNNCCTCCTAAA

Figure 41. The PCR amplified 16S rDNA nucleotide sequences of LK6-12

TAGTTGATCCCTTGGNTCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAGCGGAAAGGCCCTTCGGG
GTACTCGAGCGGCGAACGGGTGAGTACACGTGAGCACCTGCCCCAGGCTTTGGGATAACCCCGGGAAACCGGGGCT
AATACCGAATATGACCTCTGACCGCATGGTTGGTGGTGGAAAGTTTTTCGGCTTGGGATGGGCTCGCGGCCTATCA
GCTTGTTGGTGGGGTGATGGCCTACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACACTGGGAC
TGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGGAAGCCTGATGCANCGAC
NCCGCGTGAGGGATGACGGCCTTCGGGTTGTAAACCTCTTTCAGCAGGGACGAAGCGTAAGTGACGGTACCTGCAG
AAGAAGCGCCGGCCAACTACGTGCCAGCAGCCGCGGTAAGACGTAGGGCGCGAGCGTTGTCCGGATTTATTGGGCG
TAAAGAGCTCGTAGGCGGCTTGTCGCGTCGACCGTGAAAACTTGGGGCTCAACCCCAAGCCTGCGGTCGATACGGG
CAGGCTAGAGTTCGGTAGGGGAGACTGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGT
GGCGAAGGCGGGTCTNTGGGCCGATACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTG
GTAGTCCACGCTGTAAACGTTGGGCGCTAGGTGTGGGGGGCCTCTCCGGTTCCCTGTGCCGCAGCTAACGCATTAA
GCGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGCGGAGCA
TGCGGATTAATTCGATGCAACGCGAAGAACCTTACCTGGGTTTGACATGGCCGCAAAACTGTCAGAGATGGCAGGT
CCTTCGGGGGCGGTCACAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACG
AGCGCAACCCTCGTTCGATGTTGCCAGCGCGTTATGGCGGGGACTCATCGAAGACTGCCGGGGTCAACTCGGAGGA
AGGTGGGGATGACGTCAAGTCATCATGCCCCTTATGTCCAGGGCTTCACGCATGCTACAATGGCCGGTACAATGGG
CTGCGATACCGTGAGGTGGAGCGAATCCCAAAAAGCCGGTCTCAGTTCGGATCGGGGTCTGCAACTCGACCCCGTG
AAGTCGGAGTCGCTAGTAATCGCAGATCAGCAACGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGT
CACGTCACGAAAGTCGGCAACACCCGAAGCCGGTGGCCCAACCCTTGTGGAGGGAGCCGTCNAAGGTGGGGCTGGC
GATTGGGACGAAGTCGTAACAAGGTAGCCGTACCGGAAGGTGCGGCTGGATTCNCCTCCTNAAA

Figure 42. The PCR amplified 16S rDNA nucleotide sequences of BTG3-2

TCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAGCGGAAAGGCCCTTCGGGGTACTCGAGCGGCGAA
CGGGTGAGTAACACGTGAGCAACCTGCCCCAGGCTTTGGGATAACCCCGGGAAACCGGGGCTAATACCGAATATGA
CCTCTGACCGCATGGTTGGTGGTGGAAAGTTTTTCGGCTTGGGATGGGCTCGCGGCCTATCAGCTTGTTGGTGGGG
TGATGGCCTACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACACTGGGACTGAGACACGGCCCA
GACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGGAAGCCTGATGCAGCGACGCCGCGTGAGGGAT
GACGGCCTTCGGGTTGTAAACCTCTTTCAGCAGGGACGAAGCGTAAGTGACGGTACCTGCAGAAGAAGCGCCGGCC
AACTACGTGCCAGCAGCCGCGGTAAGACGTAGGGCGCGAGCGTTGTCCGGATTTATTGGGCGTAAAGAGCTCGTAG
GCGGCTTGTCGCGTCGACCGTGAAAACTTGGGGCTCAACCCCAAGCCTGCGGTCGATACGGGCAGGCTAGAGTTCG
GTAGGGGAGACTGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGCGGGT
CTCTGGGCCGATACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCTG
TAANCGTTGGGCGCTAGGTGTGGGGGGCCTCTCCGGTTCCCTGTGCCGCAGCTAACGCATTAAGCGCCCCGCCTGG
GGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGCGGAGCATGCGGATTAATTC
GATGCAACGCGAAGAACCTTACCTGGGTTTGACATGGCCGCAAAACTGTCAGAGATGGCAGGTCCTTCGGGGGCGG
TCACAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTCG
TTCGATGTTGCCAGCGCGTTATGGCGGGGACTCATCGAAGACTGCCGGGGTCAACTCGGAGGAAGGTGGGGATGAC
GTCAAGTCATCATGCCCCTTATGTCCAGGGCTTCACGCATGCTACAATGGCCGGTACAATGGGCTGCGATACCGTG
AGGTGGAGCGAATCCCAAAAAGCCGGTCTCAGTTCGGATCGGGGTCTGCAACTCGACCCCGTGAAGTCGGAGTCGC
TAGTAATCGCAGATCAGCAACGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACGTCACGAAAG
TCGGCAACACCCGAAGCCGGTGGCCCAACCCTTGTGGAGGGAGCCGTCGAAGGTGGGGCTGGCGATTGGGACGAAG
TCGTAACAAGGTAGCCGTACCGGAAGGTGCGGCTGGGATCACCTCCTTAA

Figure 43. The PCR amplified 16S rDNA nucleotide sequences of KM3-14

TAGTTTGATCCTTGGNTCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAGCGGAAAGGCCCTTCGGG
GTACTCGAGCGGCGAACGGGTGAGTAACACGTGAGTAACCTGCCCTGCGCTTTGGGATAACCCTCGGAAACGGGGG
CTAATACCGGATATGATCTCCTGCCGCATGGTGGGGGGTGGAAAGTTTTTCGGCGTGGGATGGGCTCGCGGCCTAT
CAGCTTGTTGGTGGGGTGATGGCCTACCAAGGCGGTGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACACTGGG
ACTGAGACACGNCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGGAAGCCTGATGCAGCG
ACGCCGCGTGAGGGATGACGGCCTTCGGGTTGTAAACCTCTTTCAGTAGGGACGAAGCGGAAGTGACGGTACCTAC
AGAAGAAGCGCCGGCCAACTACGTGCCAGCAGCCGCGGTAAGACGTAGGGCGCGAGCGTTGTCCGGATTTATTGGG
CGTAAAGAGCTCGTAGGCGGCTTGTCGCGTCGACTGTGAAAACCCGCGGCTCAACTGCGGGCTTGCAGTCNATACG
GGCAGGCTAGAGTTCGGTAGGGGAGACTGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACC
GGTGGCGAAGGCGGGTCTCTGGGCCGATACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACC
CTGGTAGTCCACGCTGTAAACGTTGGGCGCTAGGTGTGGGGGGCCTCTCCGGTTCTCTGTGCCGCAGCTAACGCAT
TAAGCGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGCGGA
GCATGCGGATTAATTCGATGCAACGCGAAGAACCTTACCTGGGTTTGACATGGCCGCAAAACCTCCAGAGATGGGG
GGTCCTTCGGGGGCGGTCACAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCA
ACGAGCGCAACCCTCGTTCGATGTTGCCAGCGCGTTATGGCGGGGACTCATCGAAGACTGCCGGGGTCAACTCGGA
GGAAGGTGGGGATGACGTCAAGTCATCATGCCCCTTATGTCCAGGGCTTCACGCATGCTACAATGGCCGGTACAAT
GGGCTGCGATACCGTGAGGTGGAGCGAATCCCAAAAAGCCGGTCTCAGTTCGGATCGGGGTCTGCAACTCGACCCC
GTGAAGTCGGAGTCGCTAGTAATCGCAGATCAGCAACGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCC
CGTCACGTCACGAAAGTCGGCAACACCCGAAGCCCATGGCCTAACCGGTTTTCCGGGGGGAGTGGTCGAAGGTGGG
GCTGGCGATTGGGACGAANTCNTAACAAGGTAGCCGTACCGGAAGGTGCGGCTGGANTTCNCCTACTTAA

Figure 44. The PCR amplified 16S rDNA nucleotide sequences of KM1-7

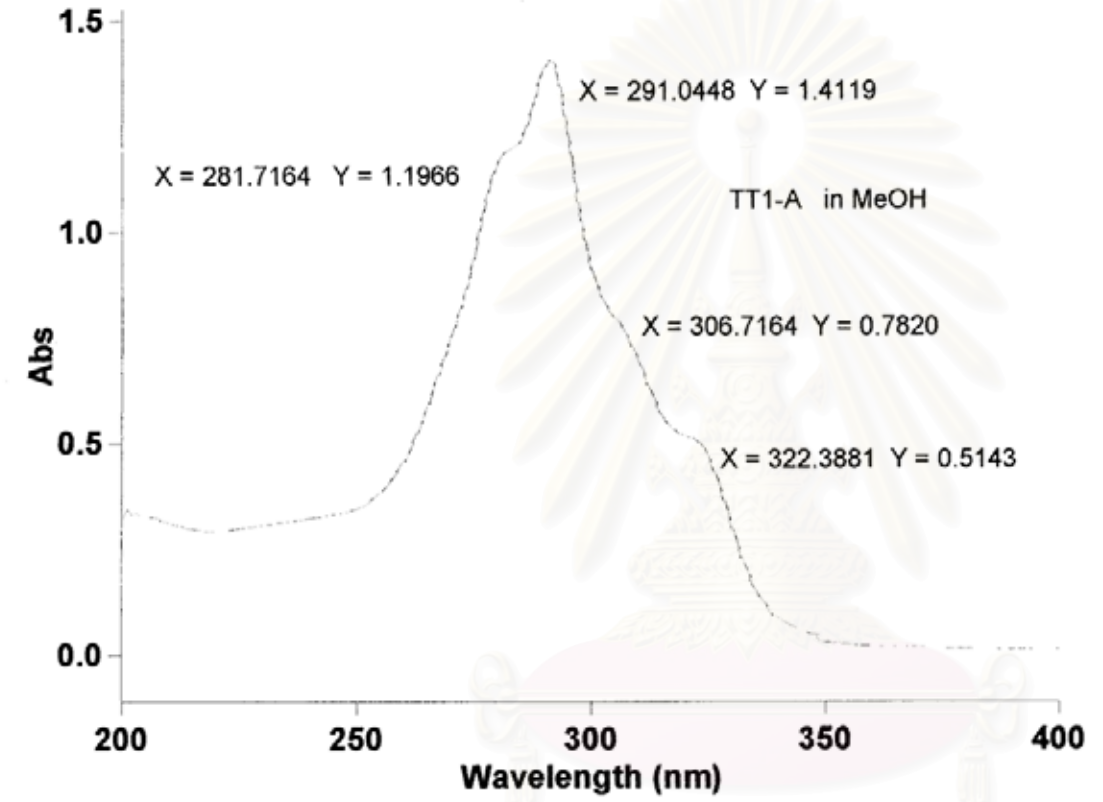

Figure 45. The UV spectrum of micromonosporin A.

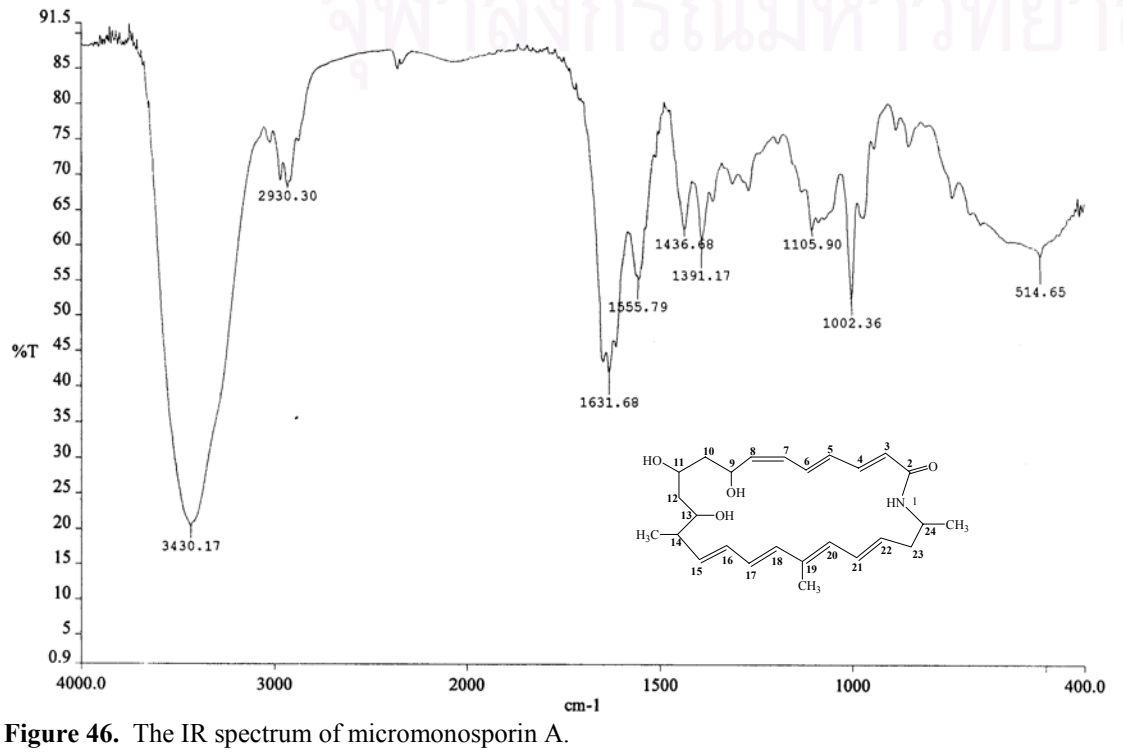

Figure 46. The IR spectrum of micromonosporin A.

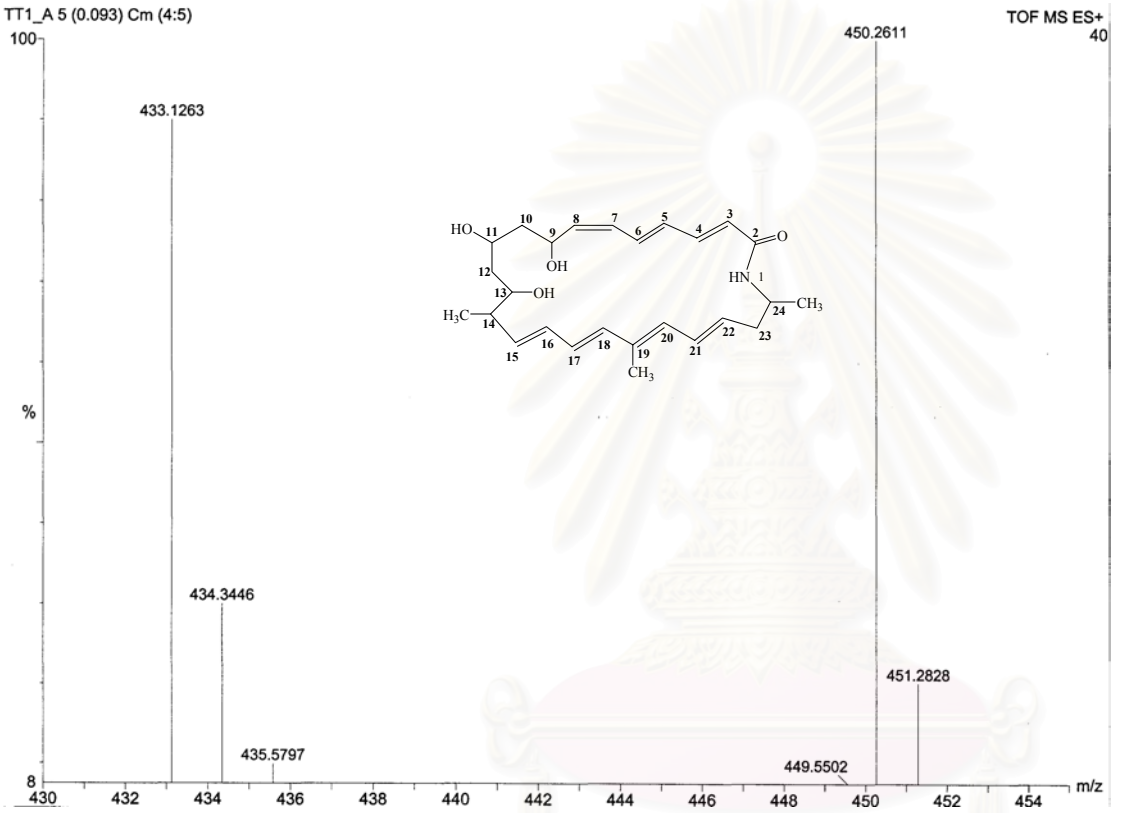

Figure 47. The ESI-TOF mass spectrum of micromonosporin A.

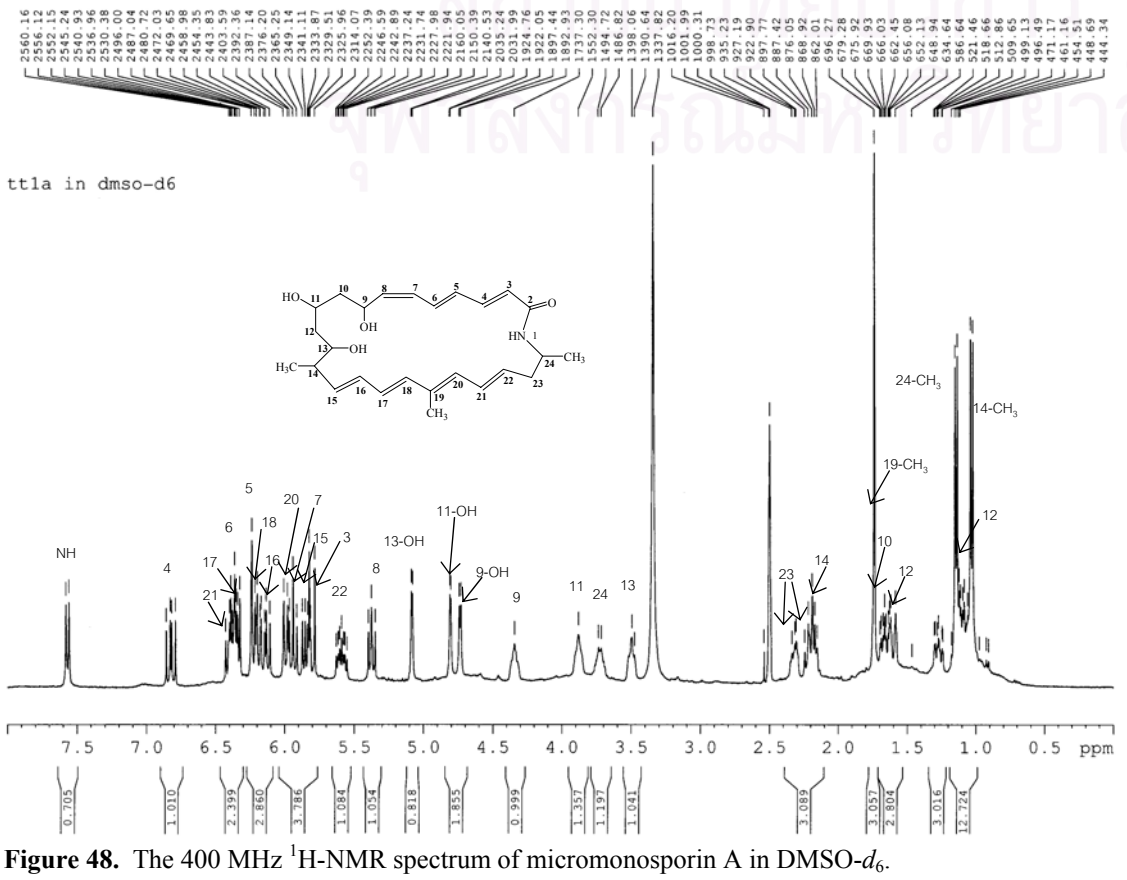

Figure 48. The 400 MHz ^{1}H-NMR spectrum of micromonosporin A in DMSO-d_6.

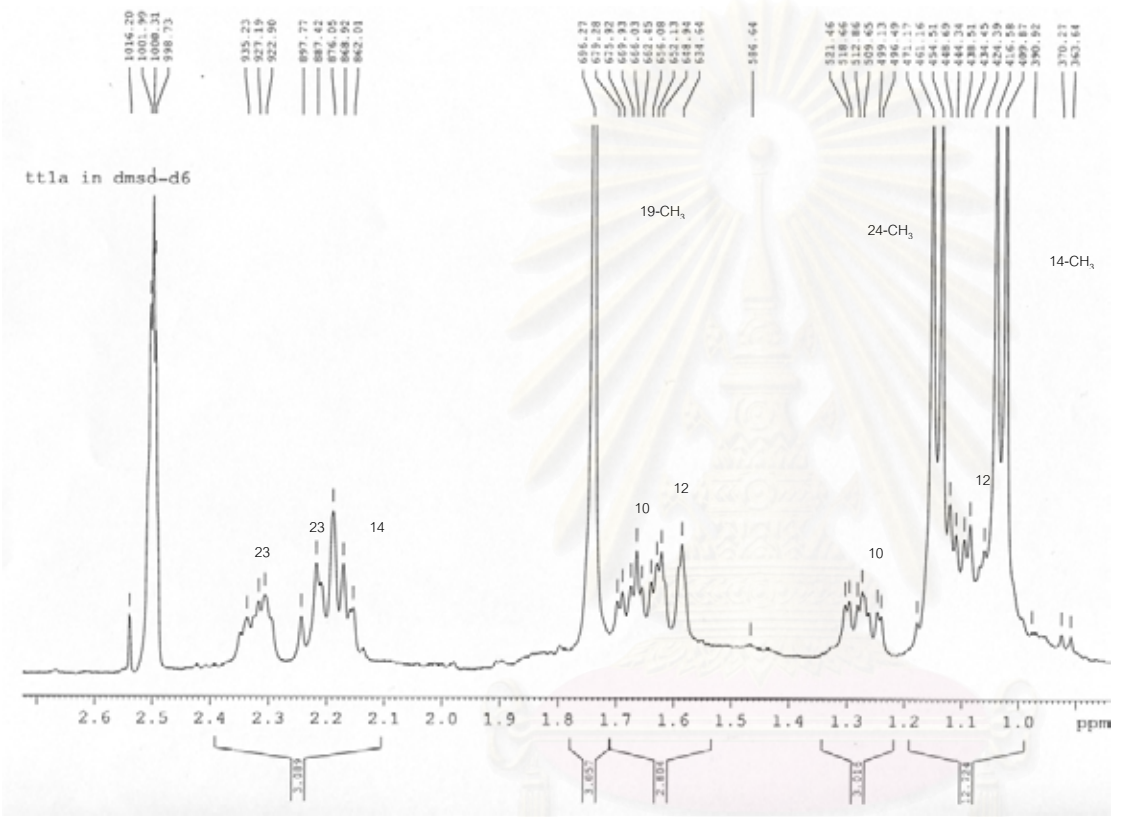

Figure 49. The 400 MHz ^{1}H-NMR spectrum of micromonosporin A in DMSO-d_6. (expanded from δ_H 0.9-2.7)

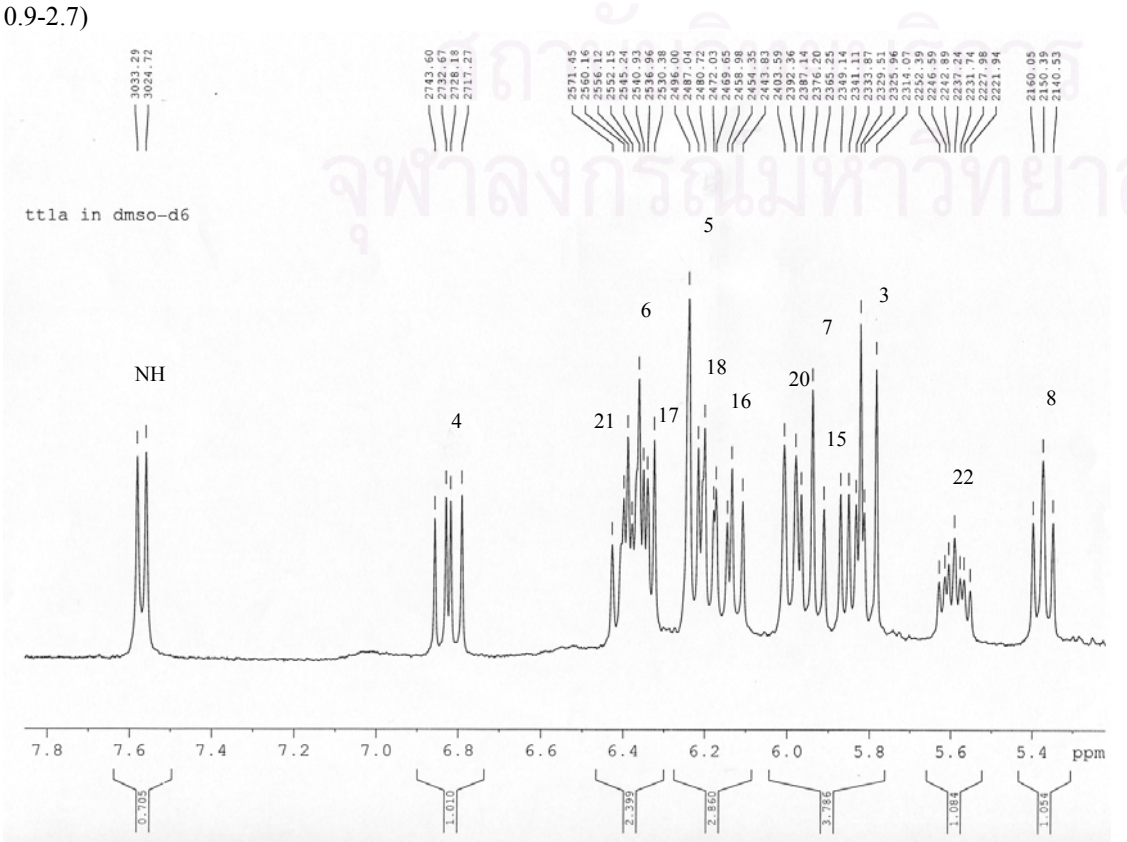

Figure 50. The 400 MHz ^{1}H-NMR spectrum of micromonosporin A in DMSO-d_6. (expanded from δ_H 5.4-7.8)

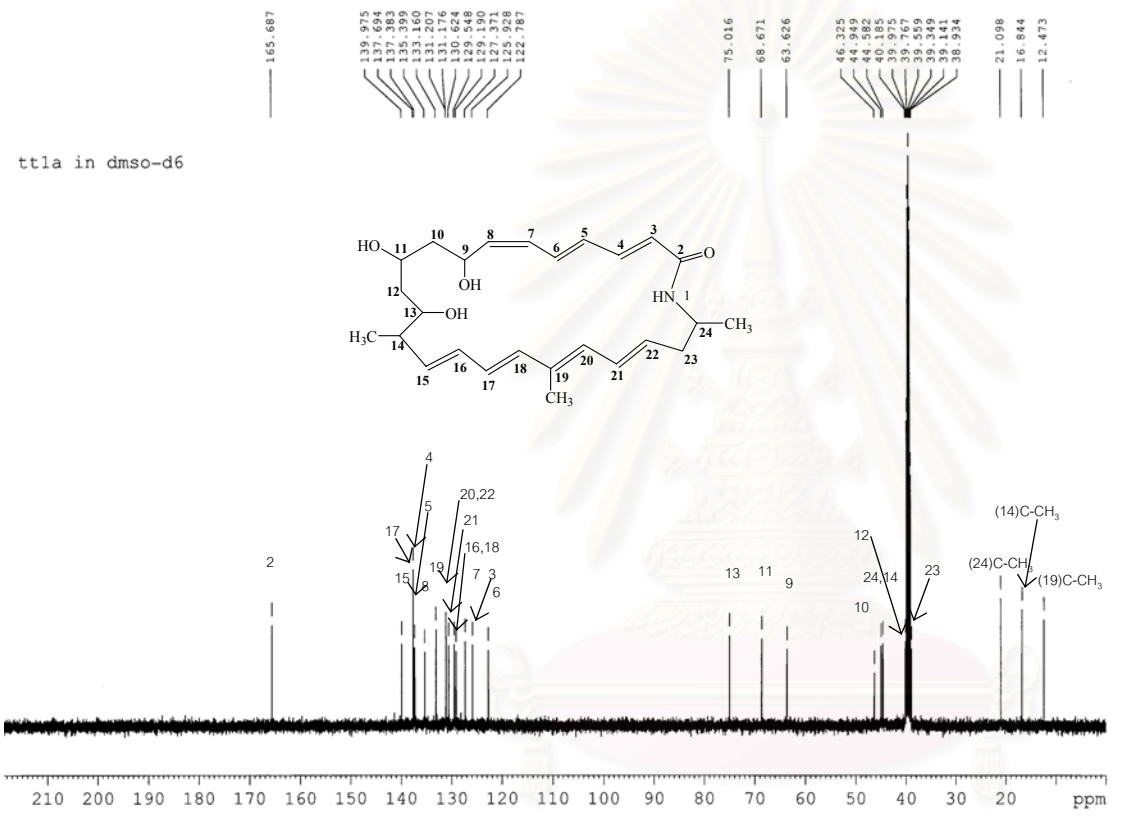

Figure 51. The 100 MHz ^{13}C-NMR spectrum of micromonosporin A in DMSO-d_6.

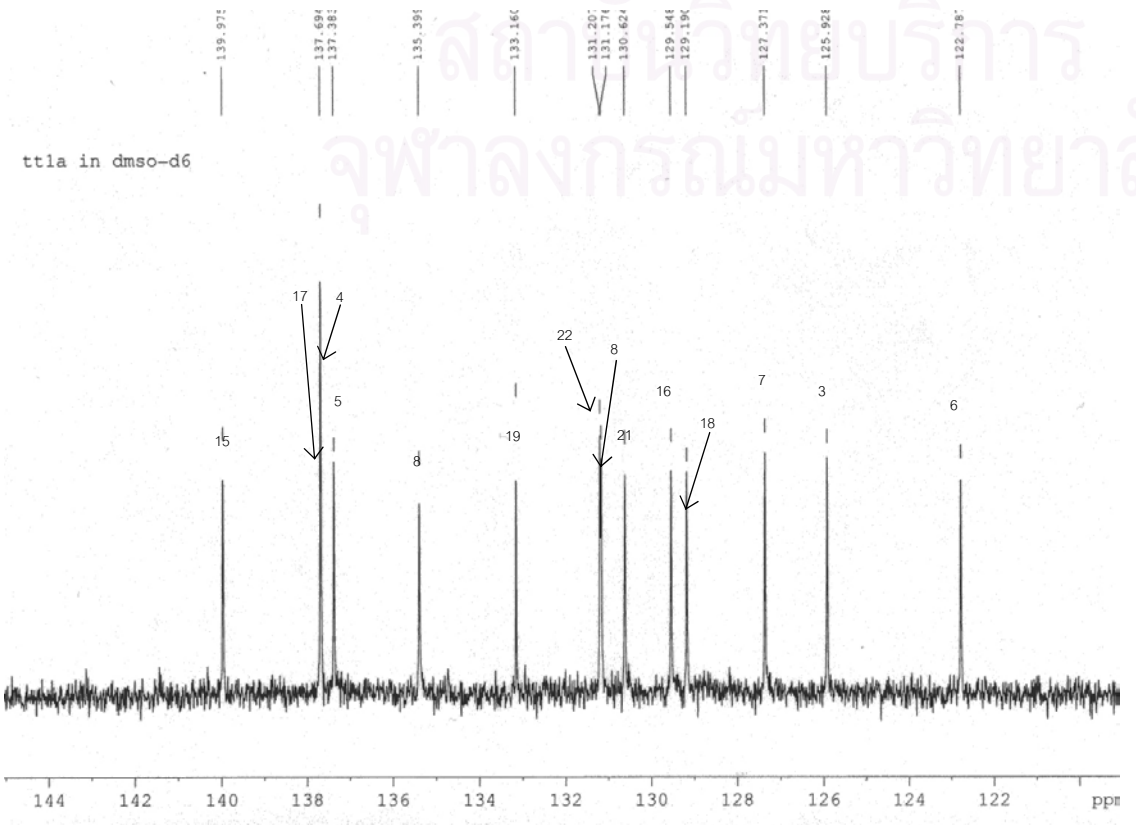

Figure 52. The 100 MHz ^{13}C-NMR spectrum of micromonosporin A in DMSO-d_6. (expand from δ_C120-144)

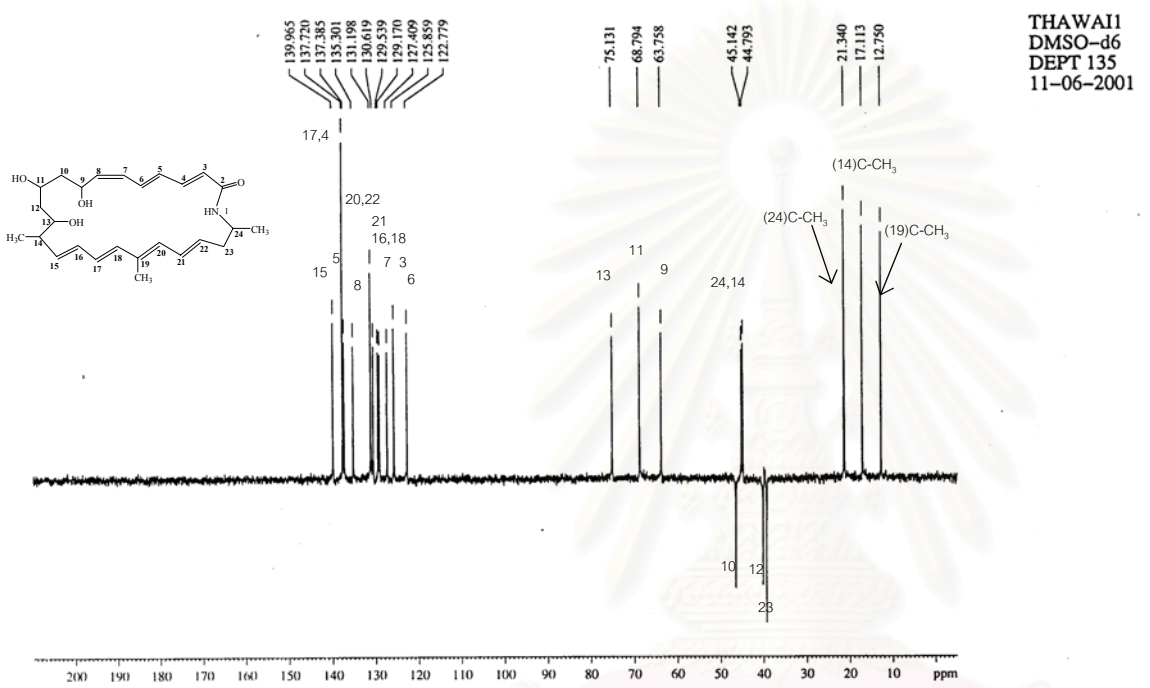

Figure 53. The 100 MHz DEPT 135 spectrum of micromonosporin A in DMSO-d_6.

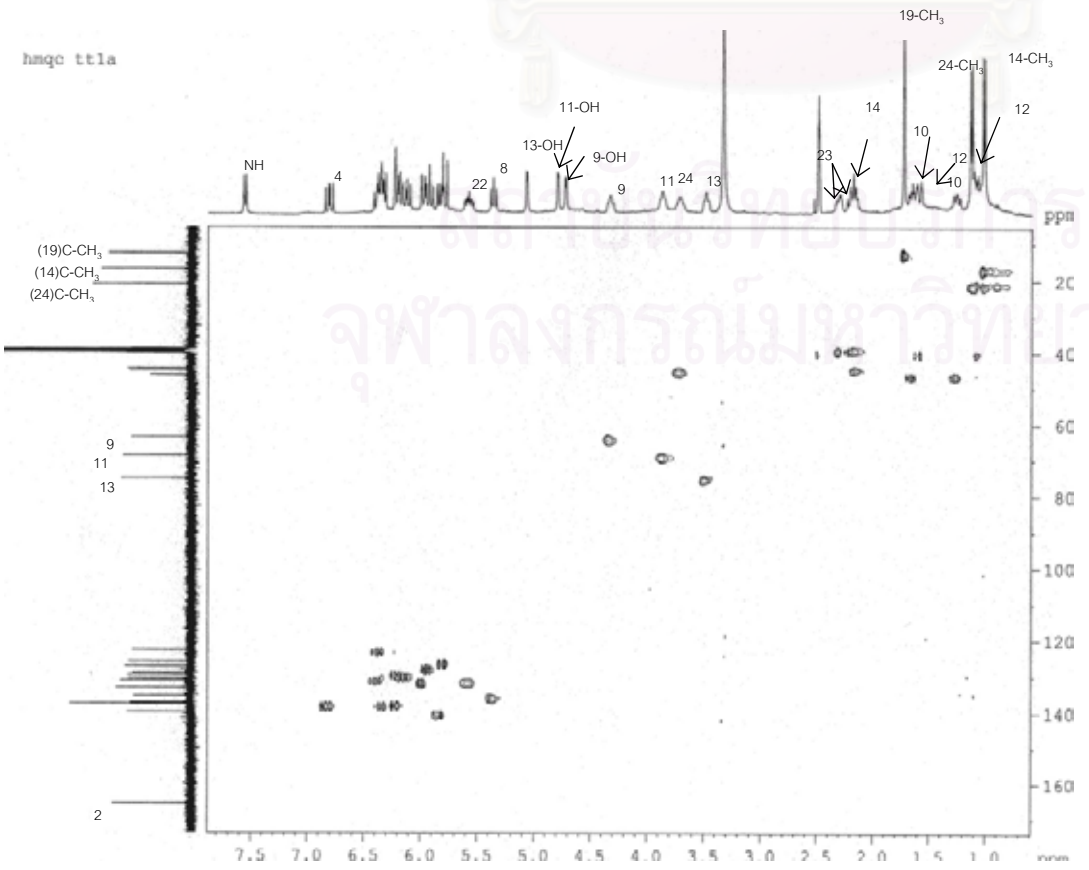

Figure 54. The 400 MHz HMQC spectrum of micromonosporin A in DMSO-d_6.

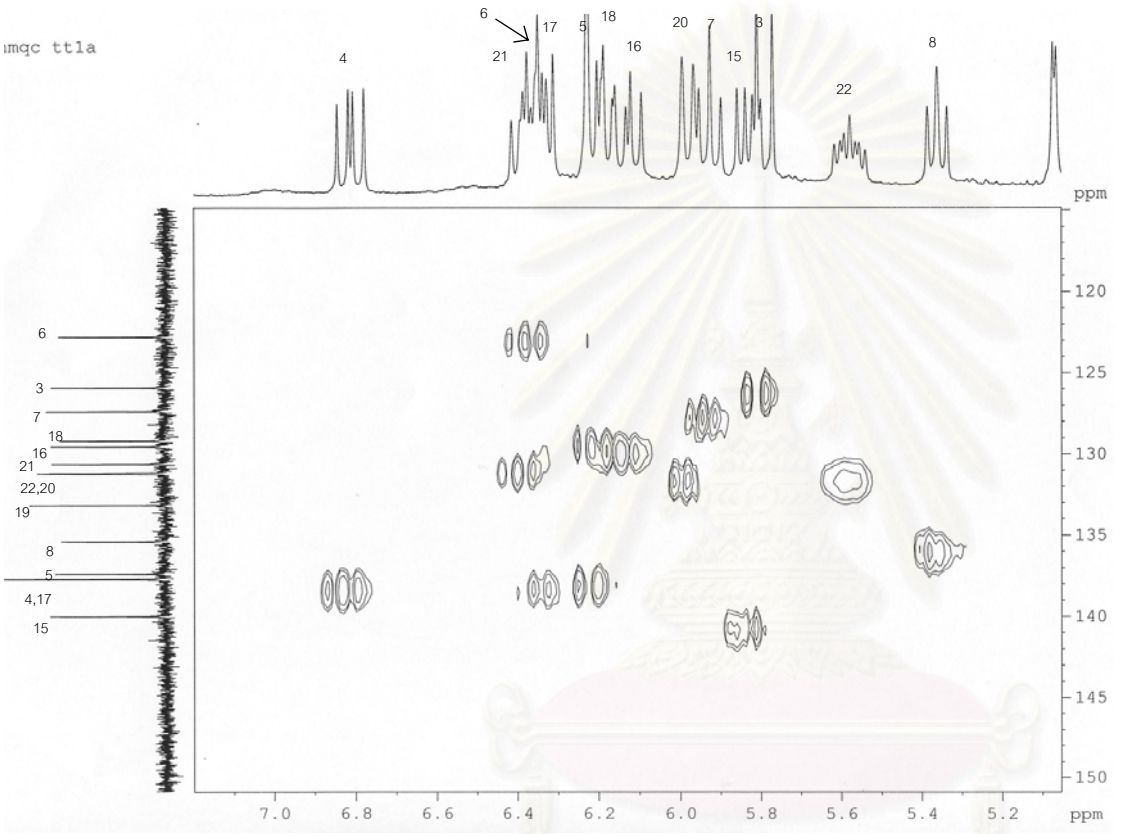

Figure 55. The 400 MHz HMQC spectrum of micromonosporin A in DMSO-d_6. (expanded from δ_H 5.0-7.2)

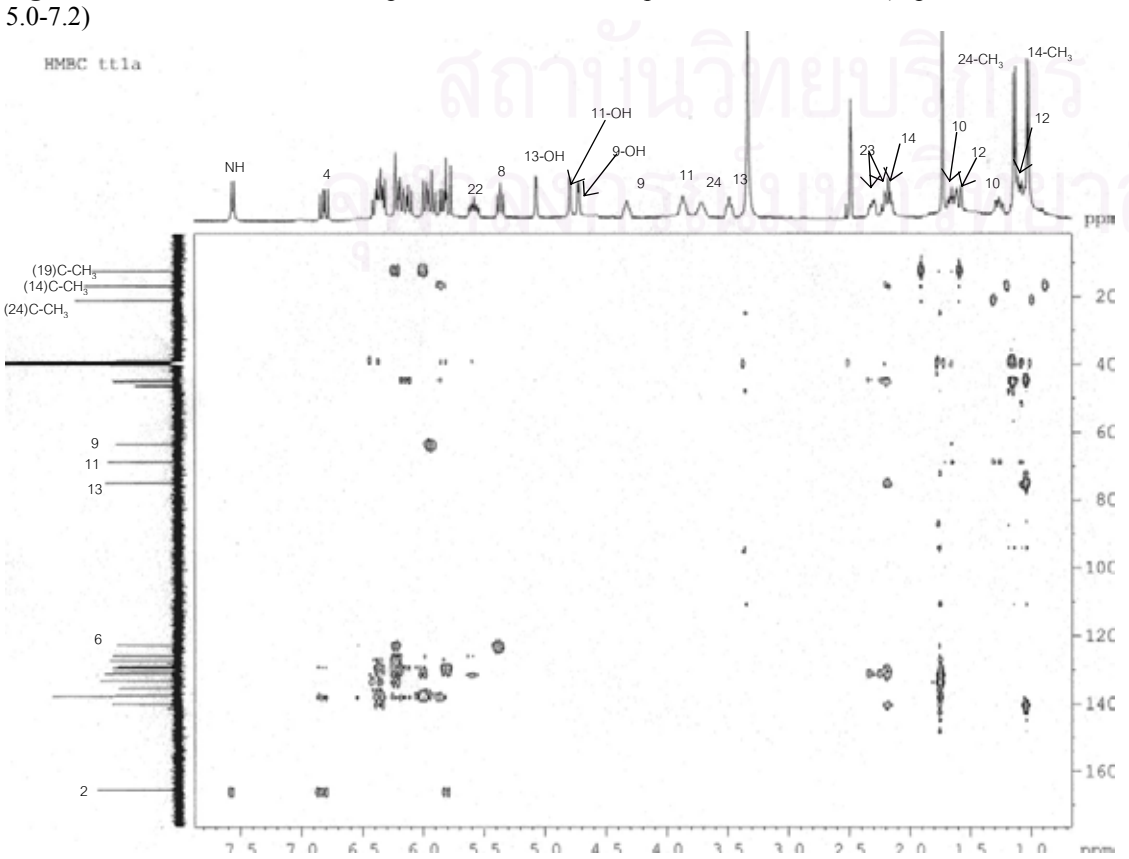

Figure 56. The 400 MHz HMBC spectrum ($^nJ_{HC}$ = 8 Hz) of micromonosporin A in DMSO-d_6.

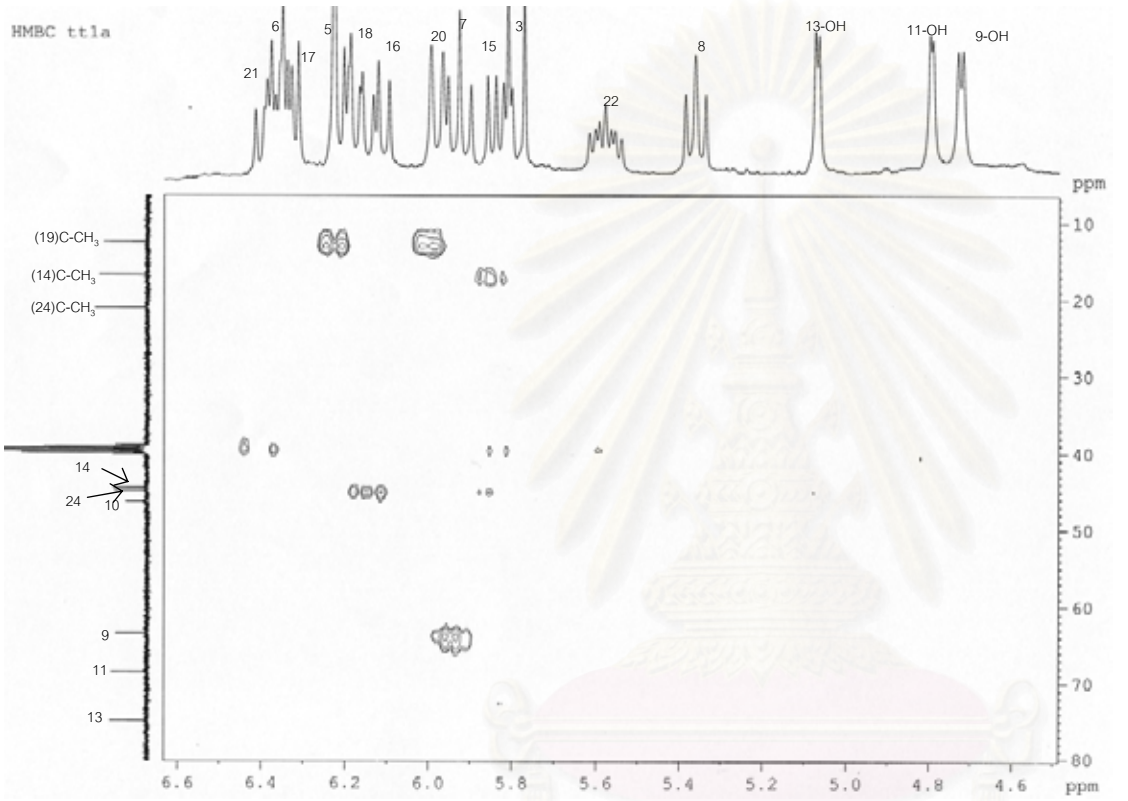

Figure 57. The 400 MHz HMBC spectrum ($^{n}J_{HC}$ = 8 Hz) of micromonosporin A in DMSO-d_6. (expanded from δ_H 4.4-6.6)

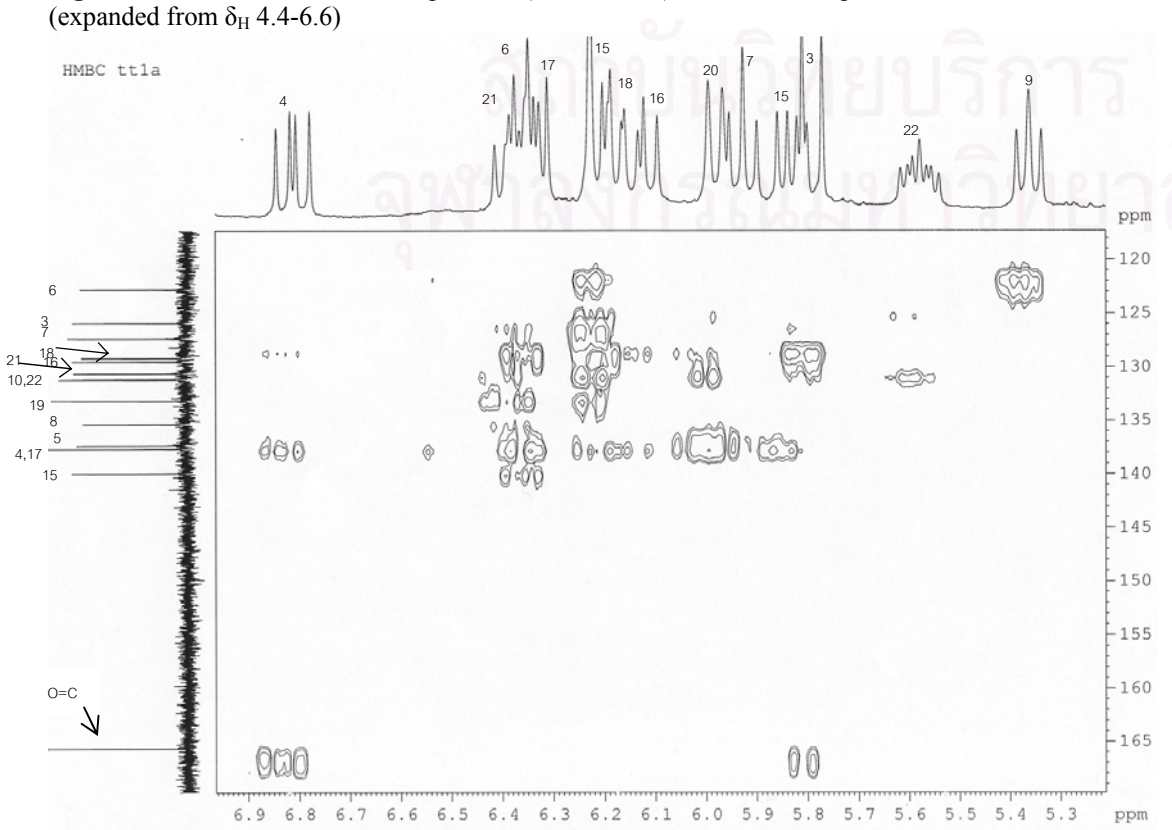

Figure 58. The 400 MHz HMBC spectrum ($^{n}J_{HC}$ = 8 Hz) of micromonosporin A in DMSO-d_6. (expanded from δ_H 5.2-6.9)

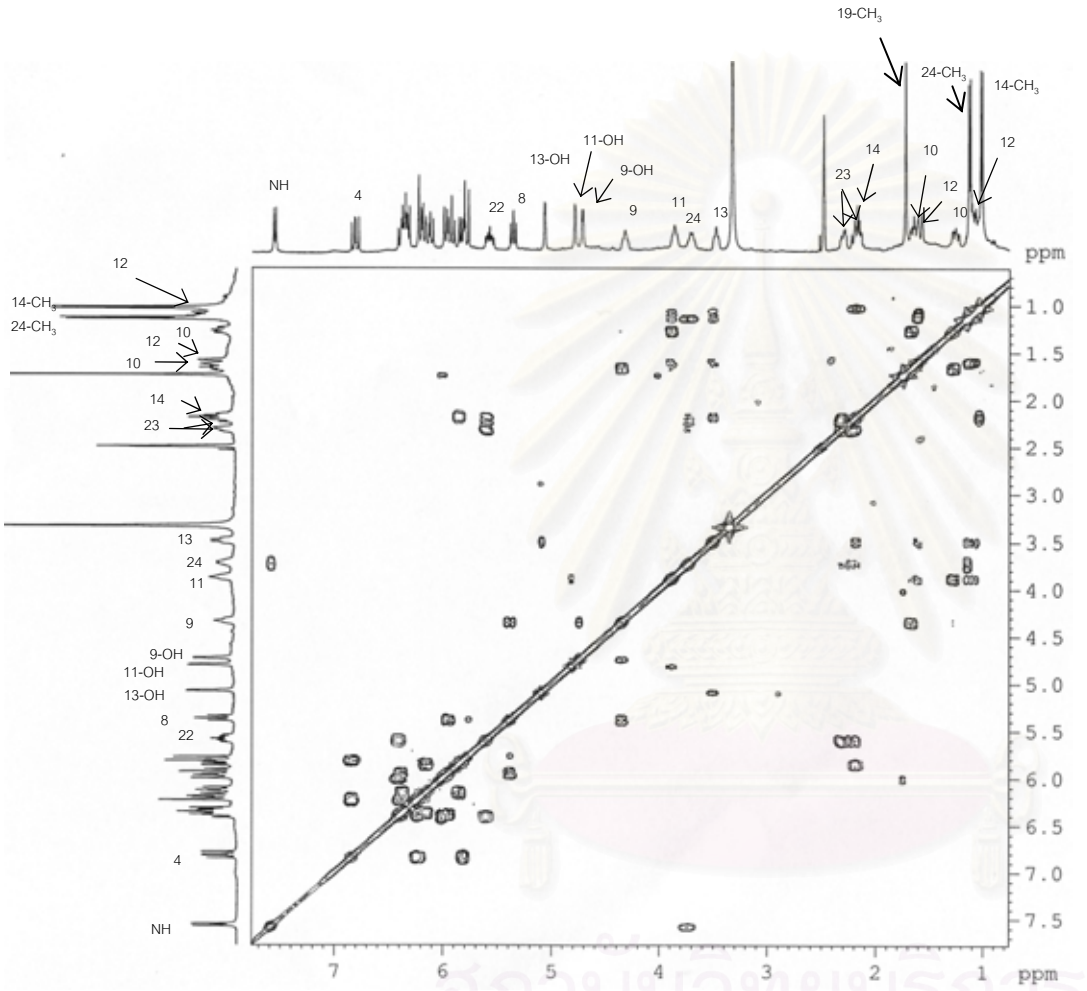

Figure 59. The 400 MHz ^{1}H-^{1}H COSY spectrum of micromonosporin A in DMSO-d_6

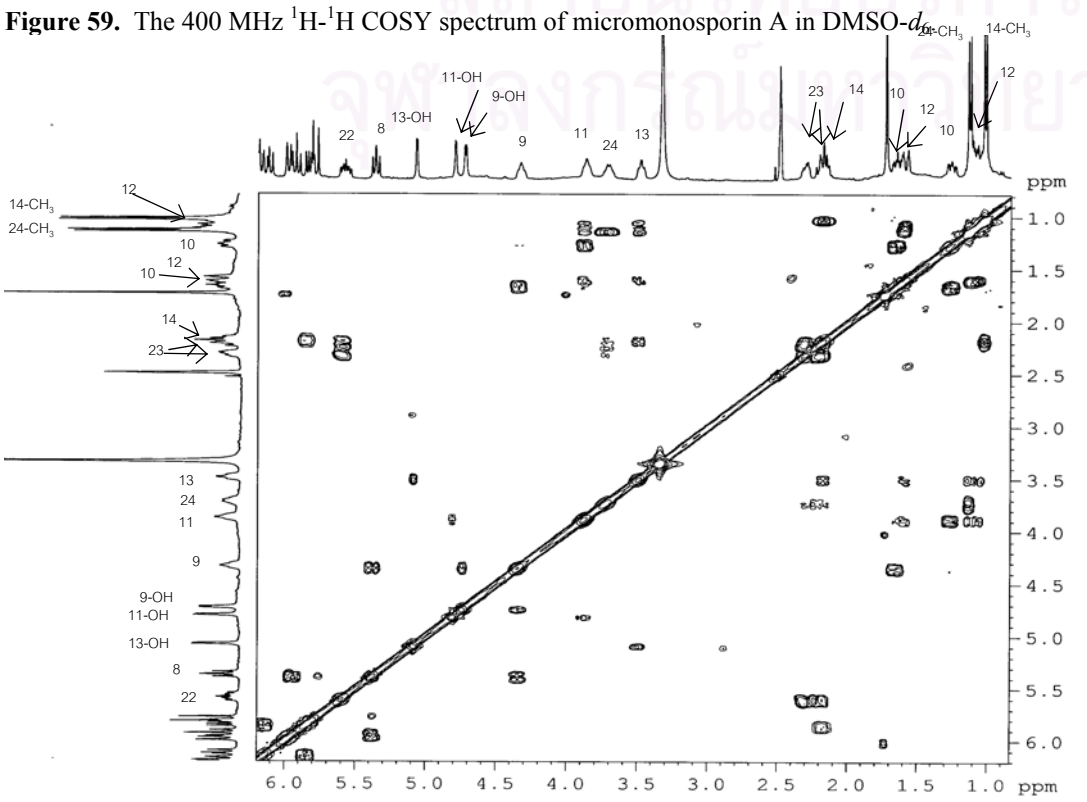

Figure 60. The 400 MHz ^{1}H-^{1}H COSY spectrum of micromonosporin A in DMSO-d_6. (expand from δ_H 1.00-6.00)

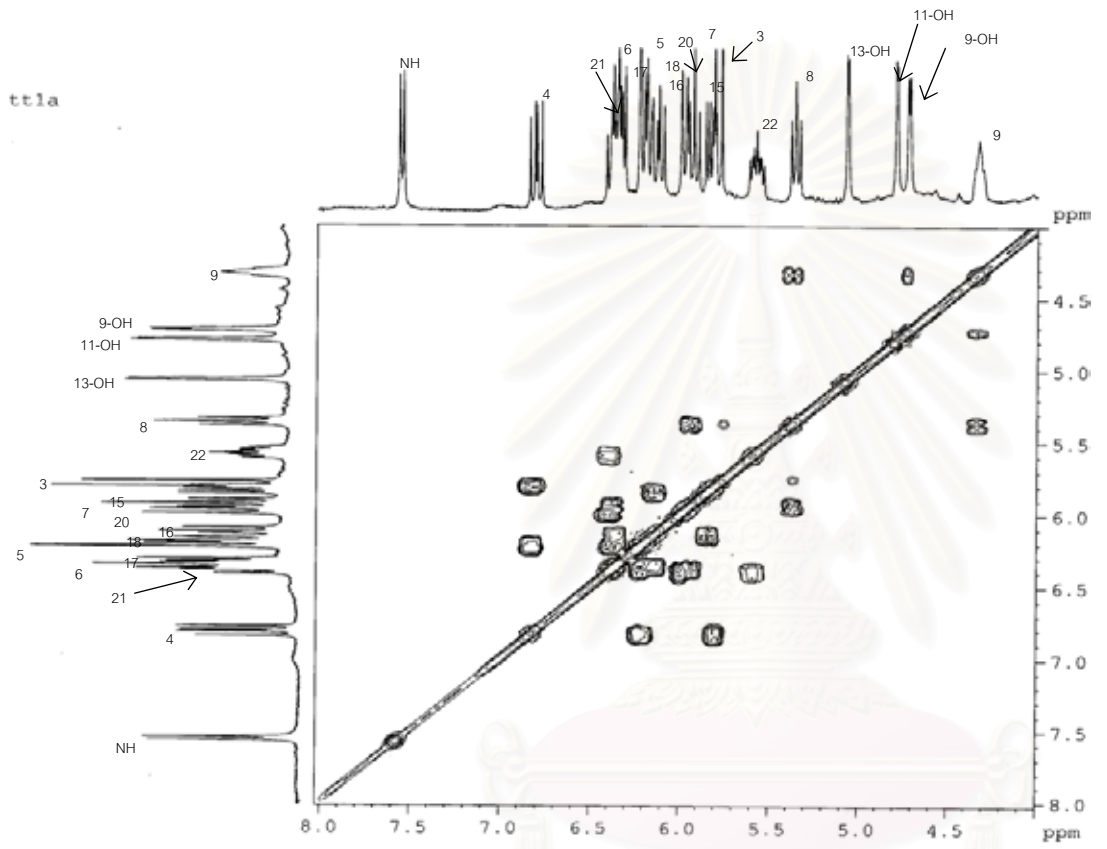

Figure 61. The 400 MHz ^{1}H-^{1}H COSY spectrum of micromonosporin A in DMSO-d_6. (expand from δ_H 4.00-8.00)

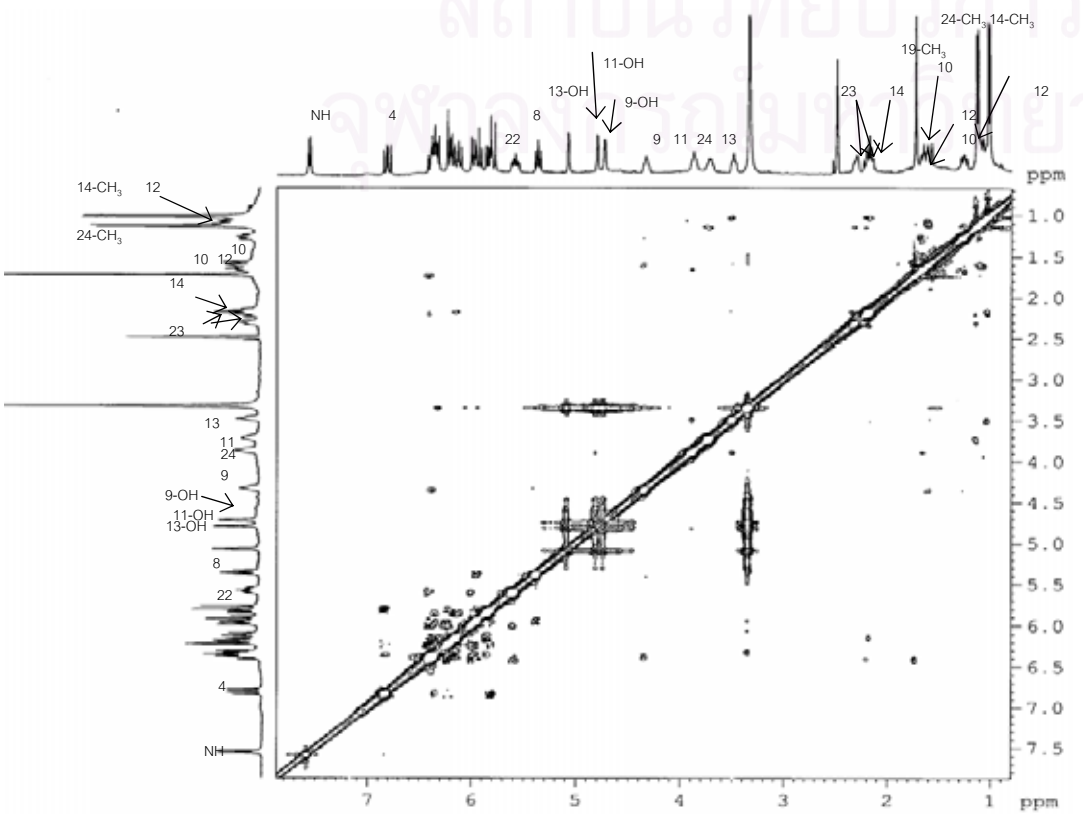

Figure 62. The 400 MHz NOESY spectrum of micromonosporin A in DMSO-d_6.

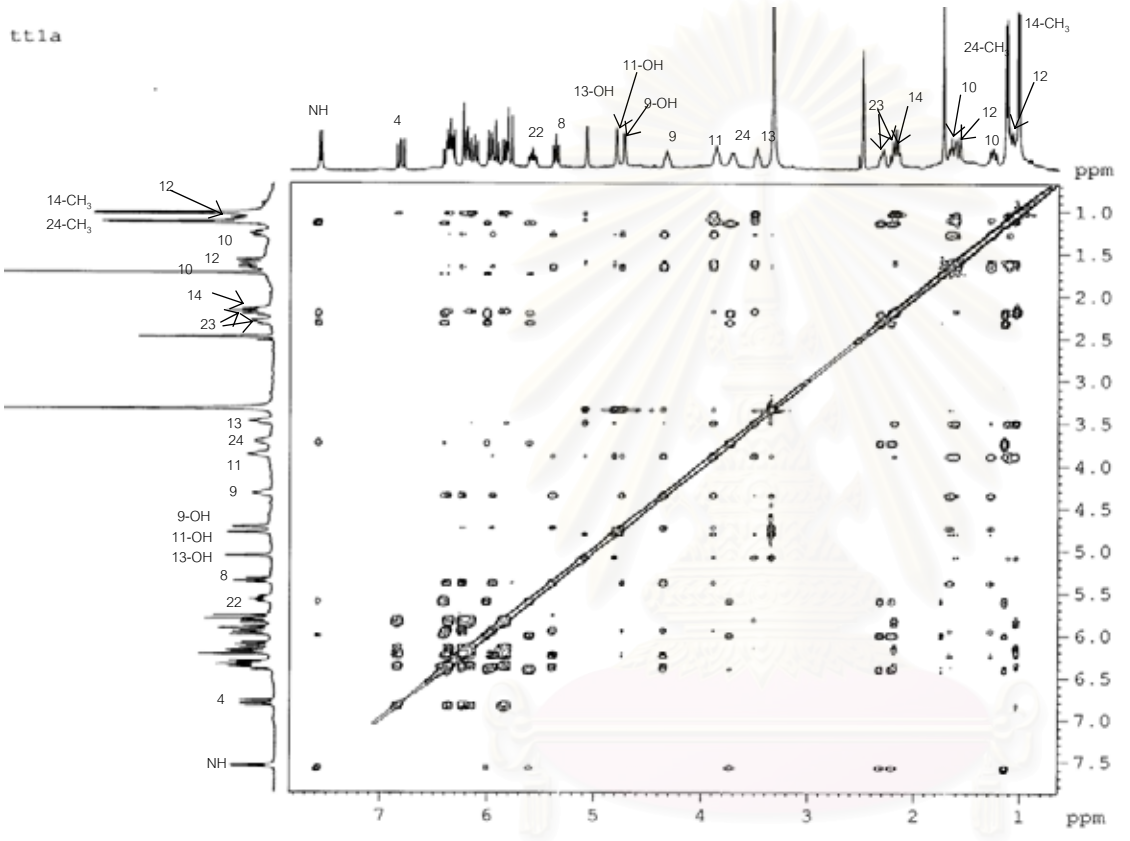

Figure 63. The 400 MHz TOCSY spectrum of micromonosporin A in DMSO-d_6.

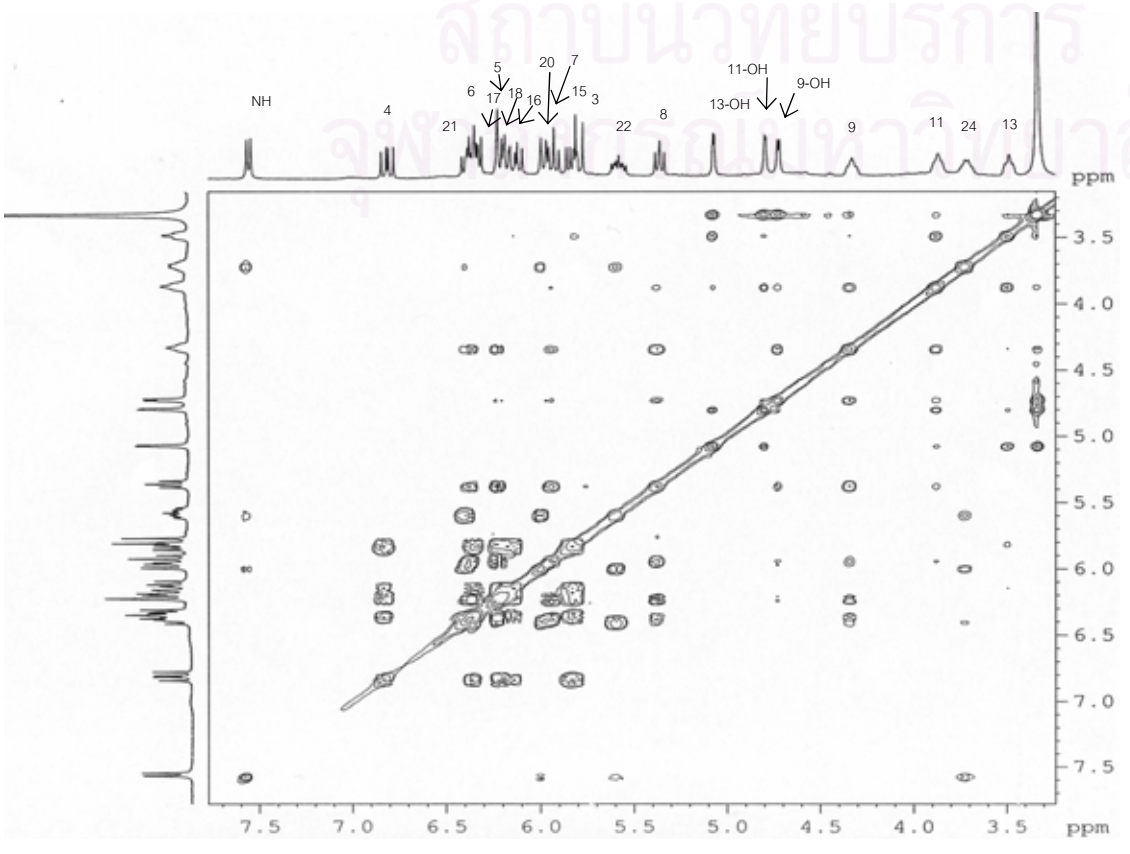

Figure 64. The 400 MHz TOCSY spectrum of micromonosporin A in DMSO-d_6. (expand from δ_H 3.3-7.8)

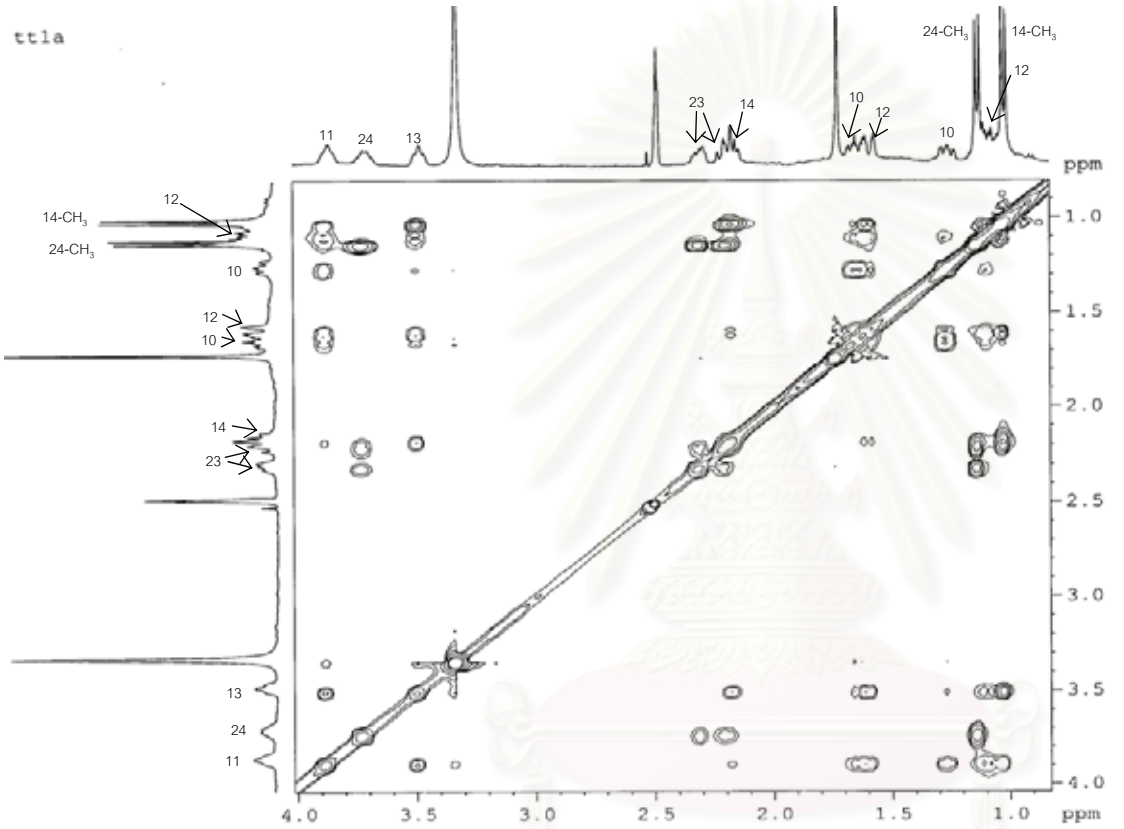

Figure 65. The 400 MHz TOCSY spectrum of micromonosporin A in DMSO-d_6. (expand from δ_H 0.8-4.0)

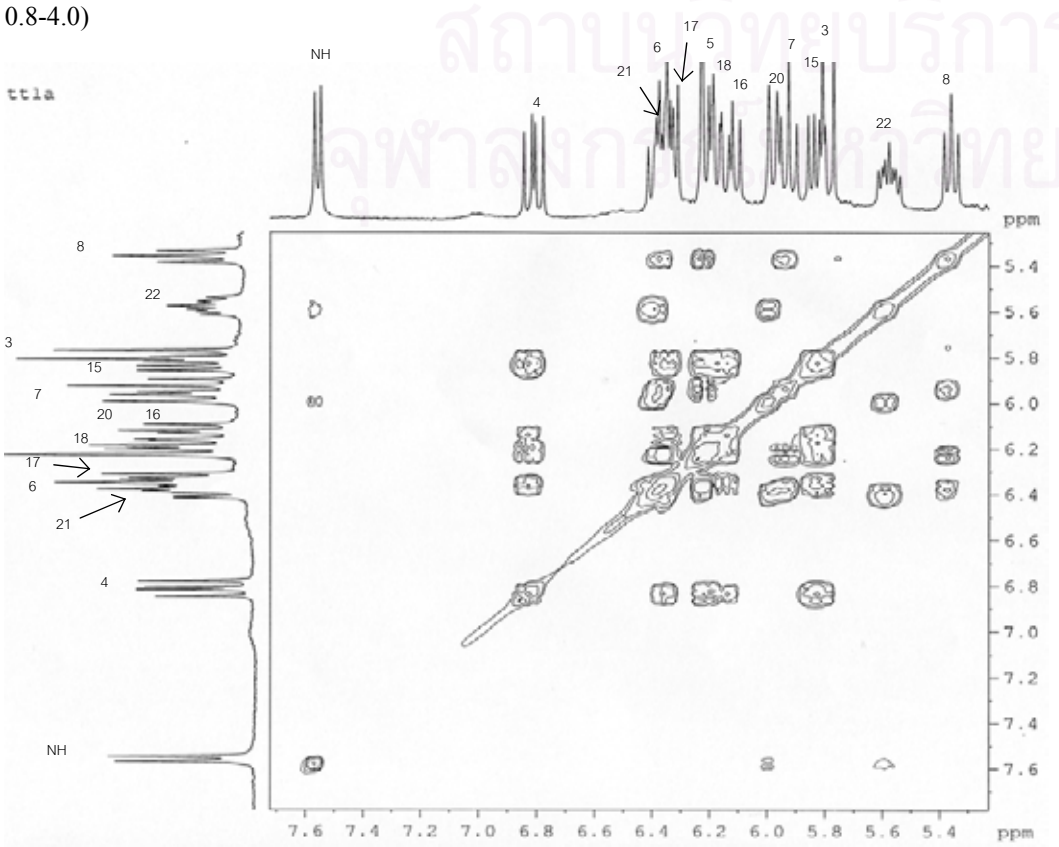

Figure 66. The 400 MHz TOCSY spectrum of micromonosporin A in DMSO-d_6. (expand from δ_H 5.2-7.8)

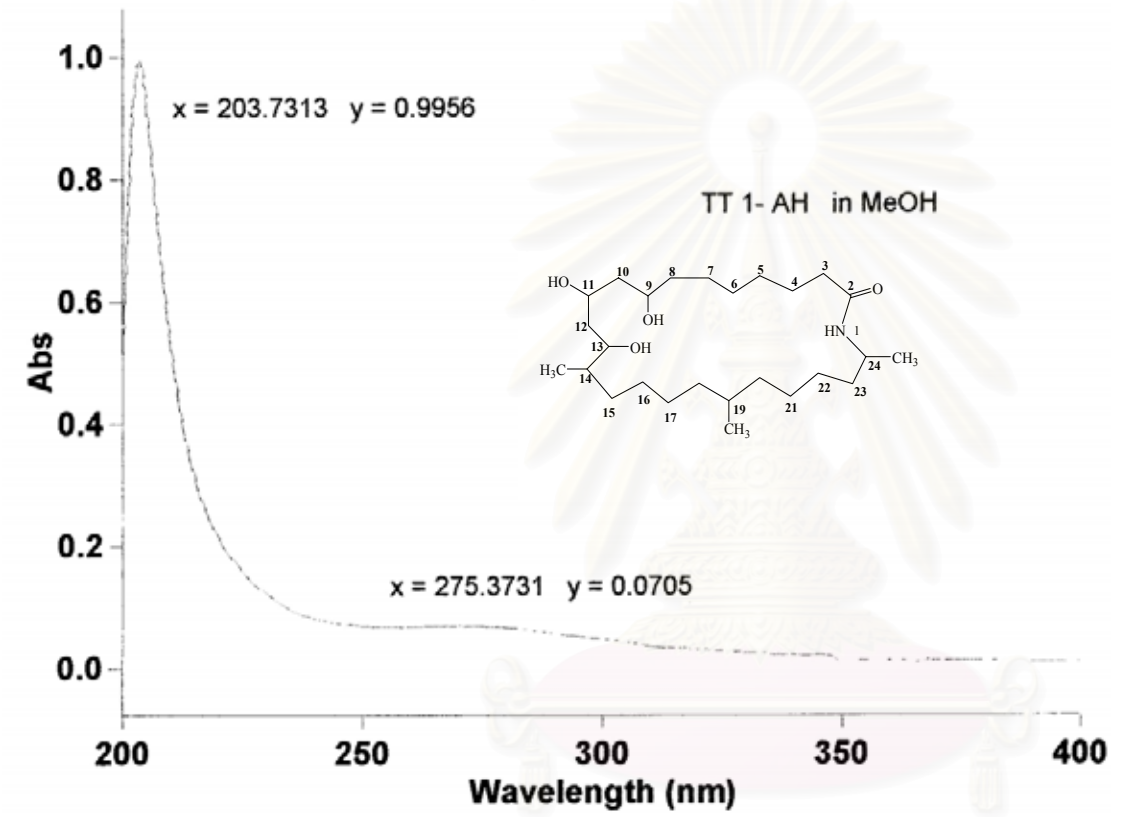

Figure 67. The UV spectrum of compound 2.

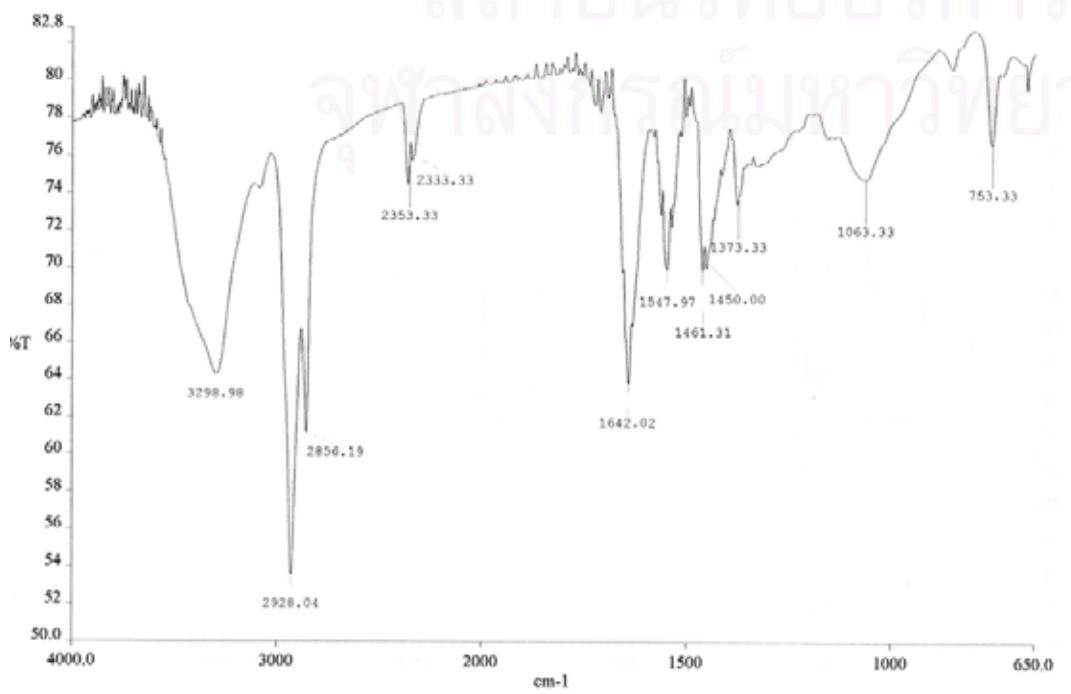

Figure 68. The IR spectrum of compound 2.

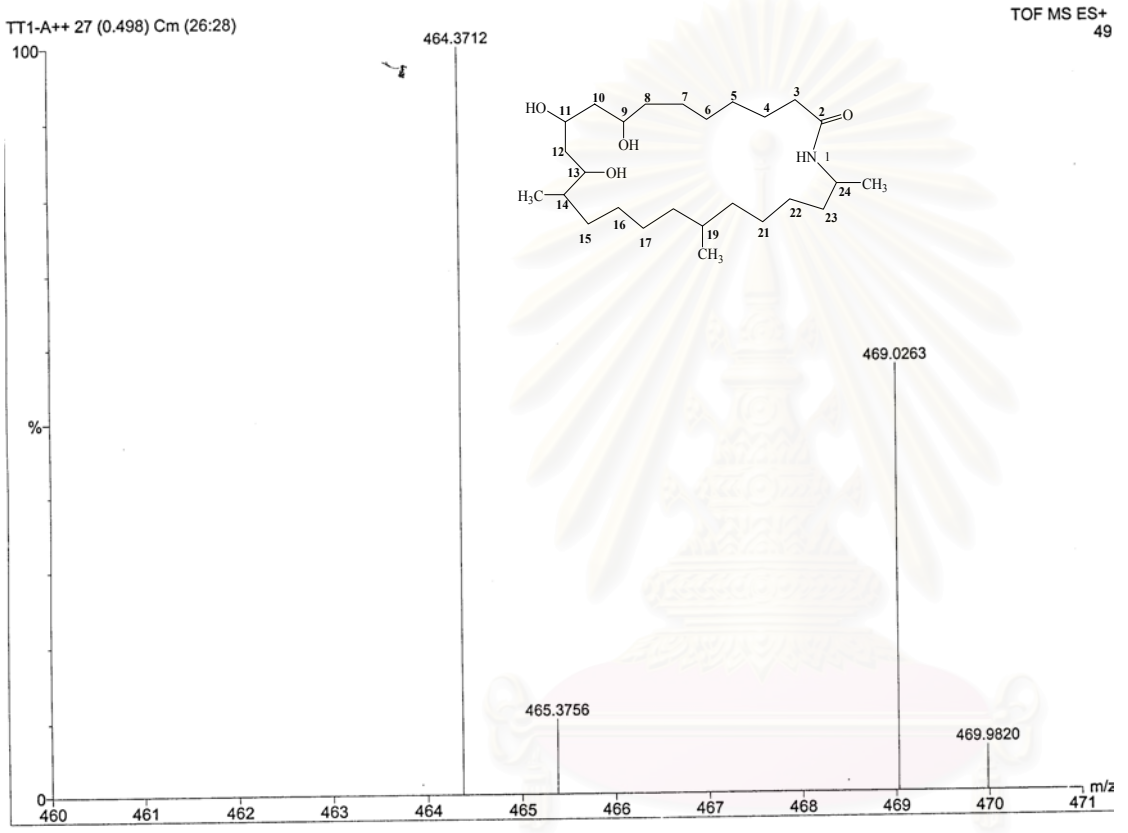

Figure 69. The ESI-TOF mass spectrum of compound 2.

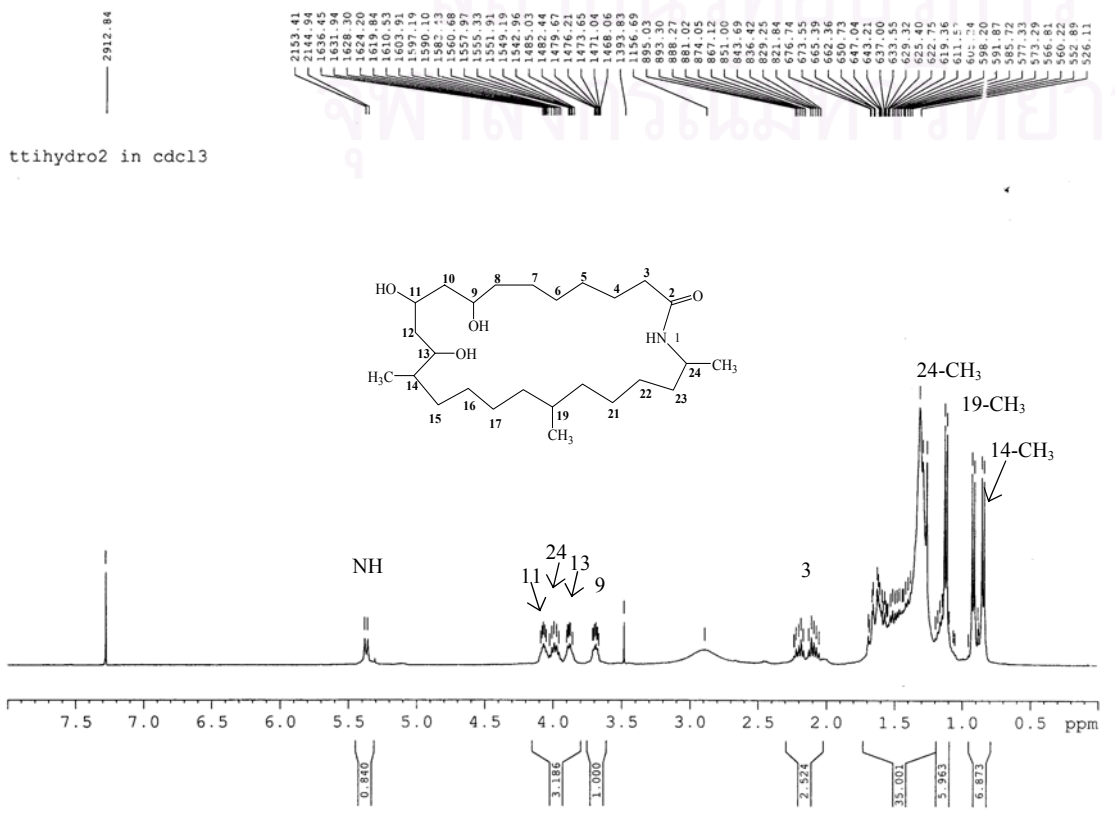

Figure 70. The 400 MHz ^{1}H-NMR spectrum of compound 2 in $CDCl_3$.

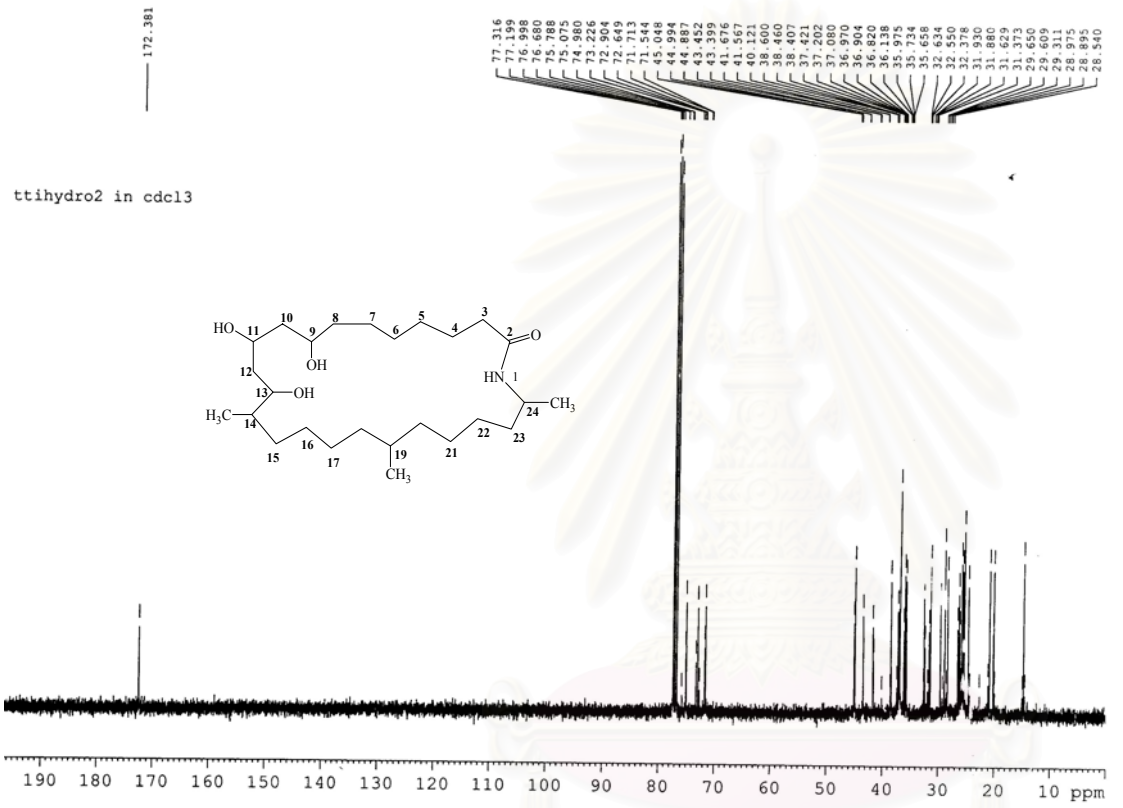

Figure 71. The 100 MHz ^{13}C-NMR spectrum of compound 2 in $CDCl_3$.

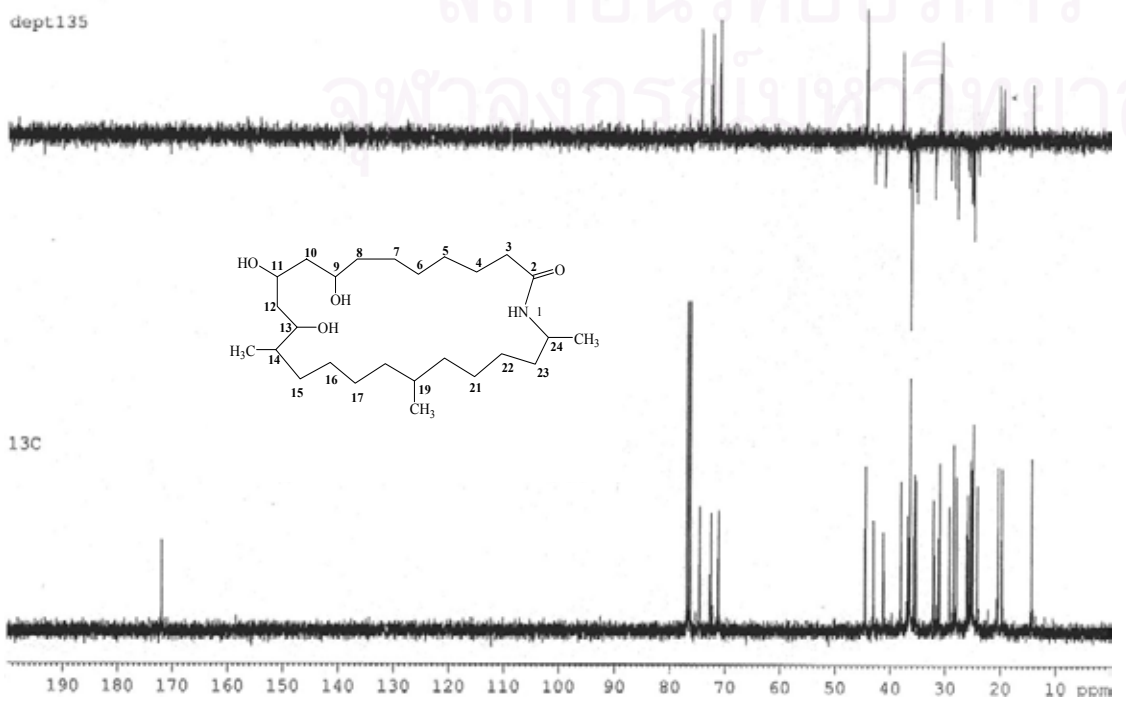

Figure 72. The 100 MHz DEPT 135 spectrum of compound 2 in $CDCl_3$.

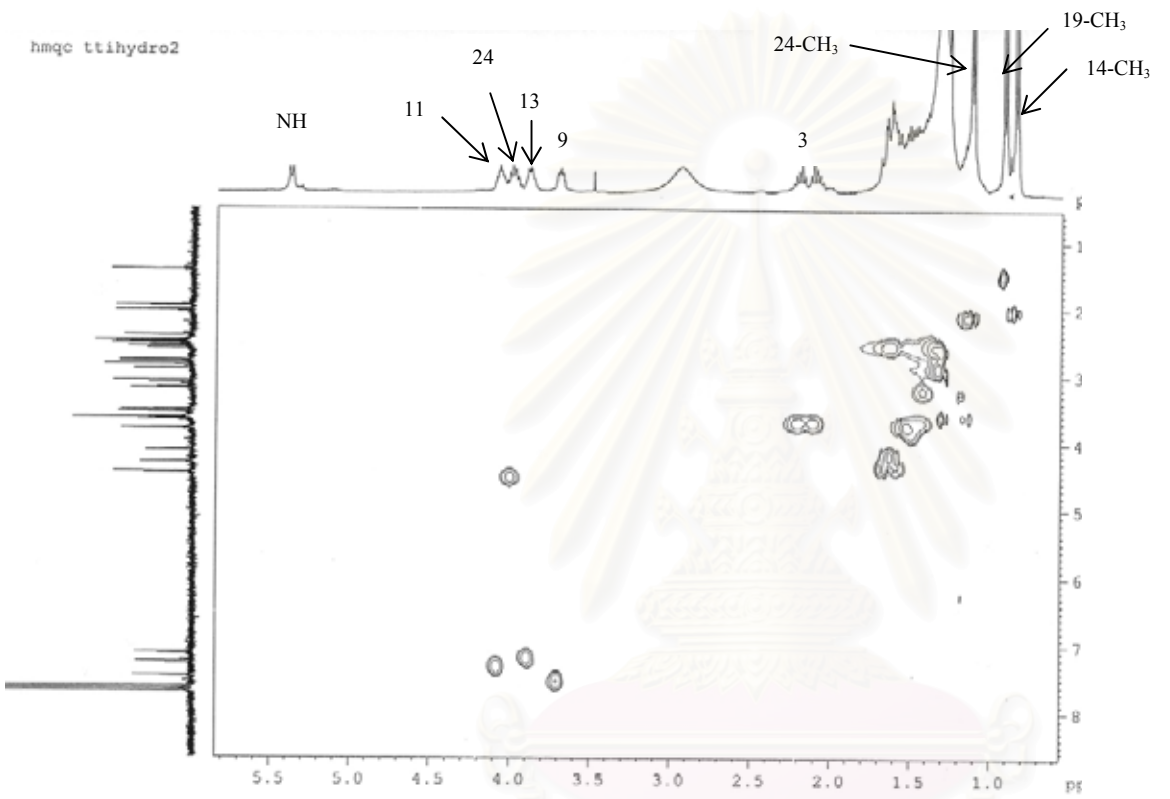

Figure 73. The 400 MHz HMQC spectrum of compound 2 in $CDCl_3$.

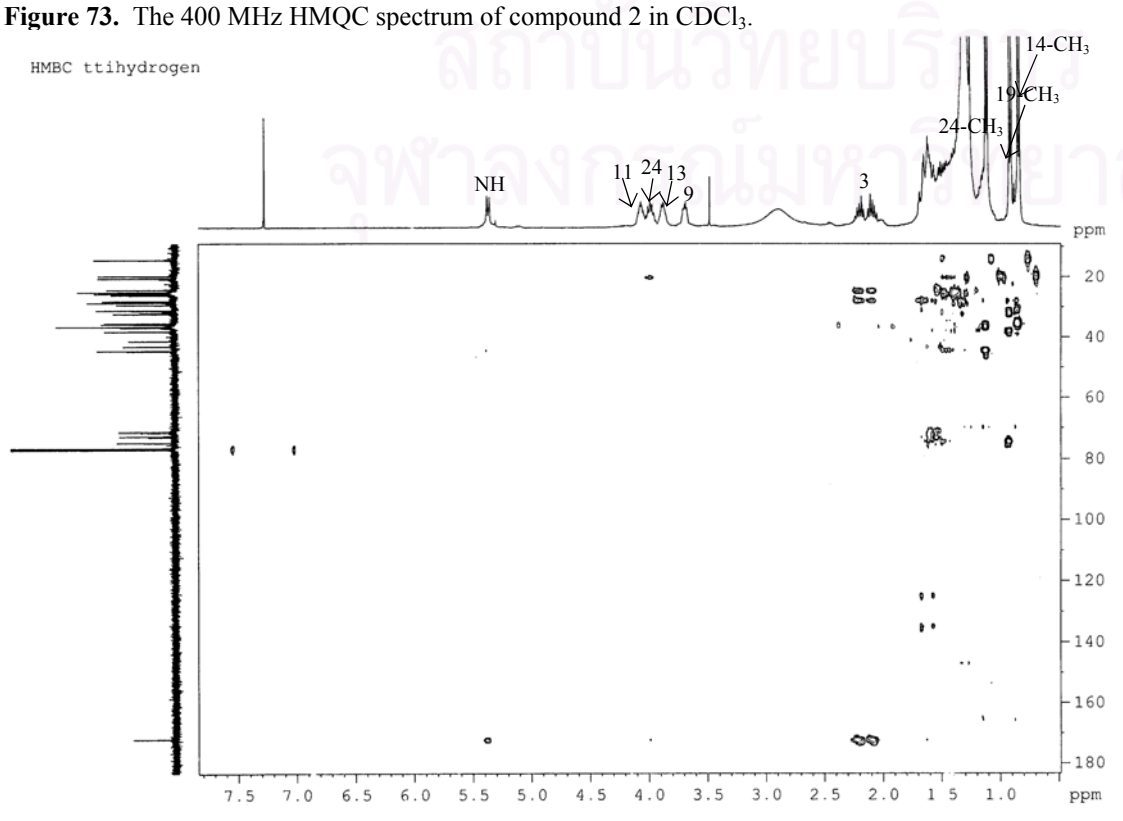

Figure 74. The 400 MHz HMBC spectrum of compound 2 in $CDCl_3$.

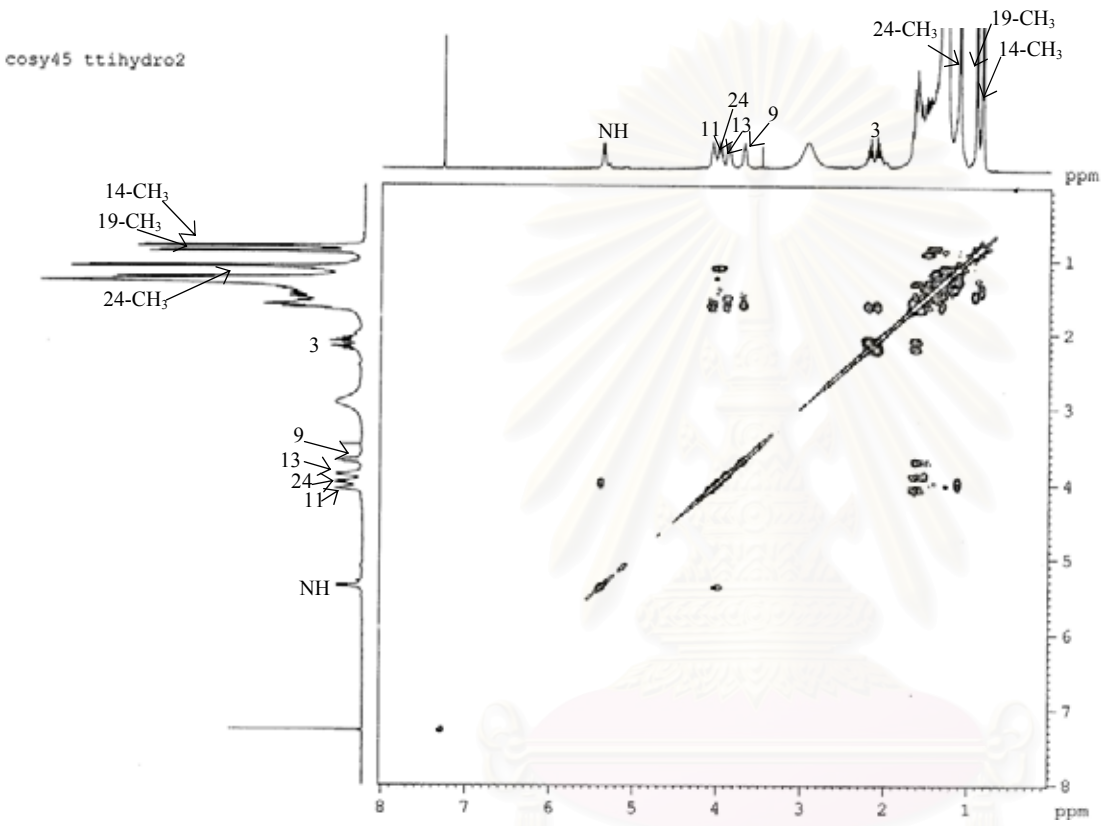

Figure 75. The 400 MHz ^{1}H-^{1}H COSY spectrum of compound 2 in $CDCl_3$.

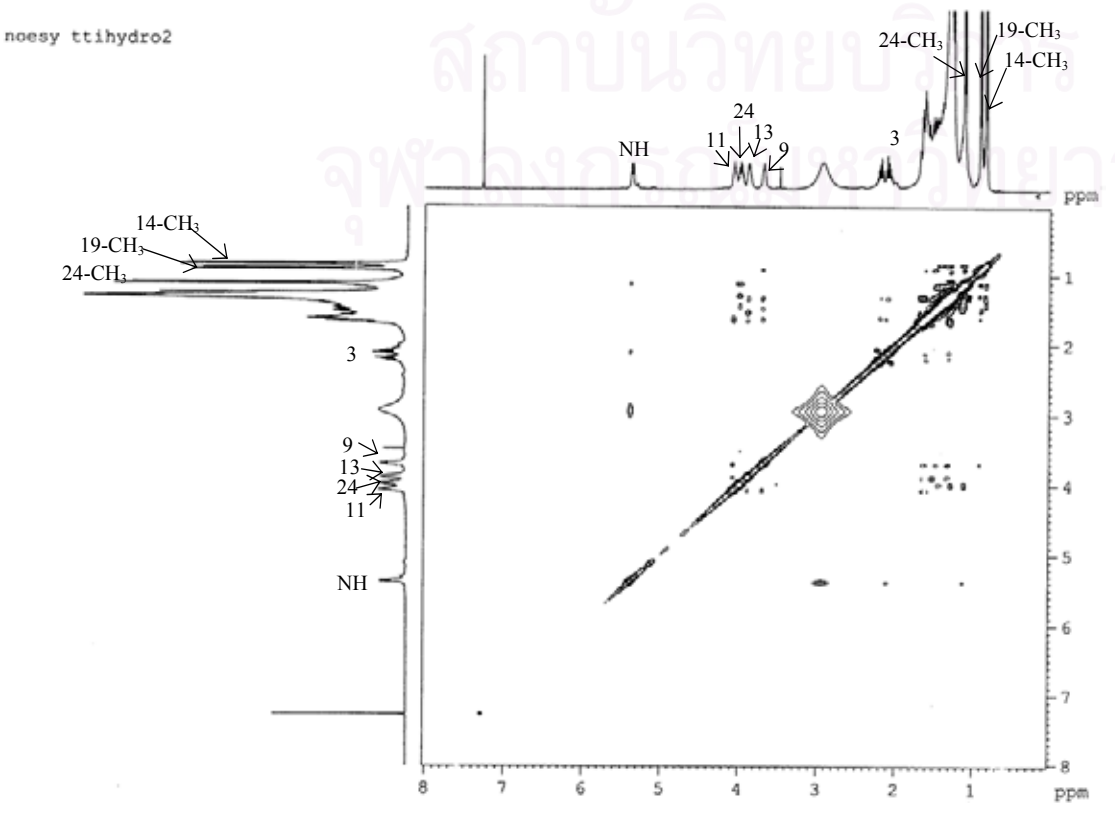

Figure 76. The 400 MHz NOESY spectrum of compound 2 in $CDCl_3$.

VITA

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Publications

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