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THESIS

CHARACTERIZATION AND IDENTIFICATION OF NOVEL TAXA, PLANT GROWTH PROMOTING PROPERTIES AND NEW COMPOUND FROM ENDOPHYTIC ACTINOMYCETES

CHANTRA INDANANDA

A Thesis Submitted in Partial Fulfillment of
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Chantra Indananda 2013: Characterization and Identification of Novel Taxa, Plant Growth Promoting Properties and New Compound from Endophytic Actinomycetes. Doctor of Philosophy (Genetics), Major Field: Genetics, Department of Genetics. Thesis Advisor: Associate Professor Arinthip Thamchaipenet, Ph.D. 212 pages.

A total of 66 endophytic actinomycetes were obtained from root and leaf tissues of endemic medicinal and agricultural Thai plants. The majority (50%) belonged to genus *Streptomyces* and the remainder belonged to 10 genera including *Microbispora* (n=14), *Micromonospora* (n=7), *Actinomadura* (n=4), *Actinoallomurus* (n=2), *Nocardia* (n=1), *Nocardiopsis* (n=1), *Nonomuraea* (n=1), *Promicromonospora* (n=1), *Saccharopolyspora* (n=1) and a new genus *Actinophytocola* (n=1). Two isolates, GMKU 367 and GMKU 370, were completely identified by polyphasic taxonomy. On the basis of combination of the significant difference data of phylogenetic, chemotaxonomic and morphological analyses, the strain GMKU 367 was assigned as a novel genus in the family *Pseudonocardiaceae*, for which the name *Actinophytocola oryzae* gen. nov., sp. nov. was proposed and the strain GMKU370 represented a novel species of the genus *Actinoallomurus*, for which the name *Actinoallomurus oryzae* sp. nov. was proposed. The properties of plant growth promotion were determined and indicated that 34 strains produced NH_3, 20 strains solubilized phosphate, 33 strains produced siderophores, 13 strains produced IAA and 16 strains produced ACC deaminase. Only *Streptomyces* sp. GMKU 344 showed ability of all five characters. Sixteen strains corresponding to the ACC deaminase producers, showed unique band approximately 650 bp of ACC deaminase gene which highly conserved with other reports at the level of 80-93% identities. For anti-microbial test, most strains were active against bacteria (*Bacillus cereus* ATCC 11778, *Ralstonia solanacearum* ATCC BAA-1114) and fungi (*Aspergillus niger* and *Colletotrichum* sp.). Furthermore, *Microbispora* sp. GMKU 363 isolated from a root of Thai medicinal plant 'Lin Ngu Hao' was determined to produce a new furanone-derived polyketide designated linfuranone A. These results revealed that endophytic actinomycetes isolated from endemic plants of Thailand are a novel valuable source of new taxa, bioactive compound and plant growth promoting agents.

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TABLE OF CONTENTS

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 FIGURES (Continued)

LIST OF FIGURES (Continued)

LIST OF FIGURES (Continued)

LIST OF FIGURES (Continued)

LIST OF ABBREVIATIONS

ACC	=	1-aminocyclopropane-1carboxylate
ACCD	=	1-aminocyclopropane-1carboxylate deaminase
APG	=	PG containing an acyl group
A_2pm	=	diaminopimelic acid
ATCC	=	American Type Culture Collection
BCC	=	BIOTEC *Culture* Collection
BLAST	=	The Basic Local Alignment Search Tool
BSU	=	Bio-Technology Service Unit
$CaCO_3$	=	calcium carbonate
CH_3COOH	=	acetic acid
$C_6H_7NO_3S$	=	sulfanilic acid (4-aminobenzene sulfonic acid)
$C_6H_8O_7{\cdot}nFe{\cdot}nH_3N$	=	ammonium ferric citrate
$C_{10}H_7N(CH_3)_2$	=	N,N-Dimethyl-1-naphthylamine
cm	=	centimeter
$CuSO_4.5H_2O$	=	copper sulphate pentahydrate
°C	=	degree Celsius
DAP	=	diaminopimelic acid
DMSO	=	dimethyl sulfoxide
DNA	=	deoxyribonucleic acid
dNTP	=	deoxynucleotide triphosphate
DDH	=	DNA-DNA hybridization
ddH_2O	=	double distilled water
DW	=	distilled water
EDTA	=	ethylenediamine tetraacetic acid
DEE	=	Diethyl Ether
DPG	=	Diphosphatidylglycerol
EtOH	=	ethanol
ExoI	=	Exonuclease I
$FeSO_4.7H_2O$	=	iron (II) sulfate heptahydrate or Ferrous sulphate
g	=	gram

LIST OF ABBREVIATIONS (Continued)

GLC	=	gas-liquid chromatography
GluNu	=	Phospholipids of unknown structure containing glucosamine
GMKU	=	Genetics Microbiology Kasetsart University
GR	=	Ground roots
GTP	=	guanosine triphosphate
×g	=	times gravity
h	=	hour
H_2O_2	=	hydrogen peroxide
HV	=	Humic acid-salts-vitamin agar medium
I_2	=	Iodine
IAA	=	indole-3-acetic acid
ISP	=	International *Streptomyces* Project
JCM	=	Japan Collection of Microorganisms
kb	=	kilobase pairs
KCl	=	potassium chloride
KCTC	=	*Korean Collection for Type Cultures*
KH_2PO_4	=	potassium dihydrogen phosphate
K_2HPO_4	=	dipotassium phosphate
KI	=	potassium iodide
KNO_3	=	potassium nitrate
λ	=	lambda
L	=	Leaves
l	=	liter
LB	=	Luria-Bertani medium
LPSN	=	List of Prokaryotic names with Standing in Nomenclature
M	=	molar
Mb	=	mega base pairs
MEGA	=	Molecular Evolutionary Genetics Analysis
$MgCl_2$	=	magnesium chloride
$MgSO_4.7H_2O$	=	magnesium sulfate USP

LIST OF ABBREVIATIONS (Continued)

MIDI	=	Microbial Identification Inc.
min	=	minute
MK	=	Menaquinone
MM	=	Minimal medium
ml	=	milliliter
$MnCl_2.4H_2O$	=	manganese chloride tetrahydrate
MS	=	Mannitol Soya agar medium
µg	=	microgram
µl	=	microlitre
µM	=	micromolar
mg	=	milligram
mM	=	millimolar
n	=	number
NA	=	nutrient agar medium
NB	=	nutrient broth medium
$NaHCO_3$	=	sodium hydrogen carbonate
NaCl	=	sodium chloride
NaClO	=	sodium hypochlorite
NaOAc	=	sodium acetate
Na_2HPO_4	=	disodium hydrogen phosphate or disodium phosphate
$Na_2S_2O_3$	=	sodium thiosulfate
NBRC	=	Biological Resource Center
$(NH_4)_2SO_4$	=	ammonium sulfate
$(NH_4)_2HPO_4$	=	ammonium phosphate dibasic (NH4)2HPO4
NRRL	=	Agricultural Research Service (ARS) Culture Collection
nt	=	nucleotides
OD	=	Optical Density
OsO4	=	osmium tetroxide
PCR	=	Polymerase Chain Reaction
PDA	=	potato dextrose agar medium

LIST OF ABBREVIATIONS (Continued)

PB	=	Phosphatidylbutanediol
PC	=	Phosphatidylcholine
PE	=	Phosphatidylethanolamine
PG	=	Phosphatidylglycerol
PGPB	=	Plant growth-promoting bacteria
PI	=	Phosphatidylinositol
PIM	=	Phosphatidylinositol mannosides
PME	=	Phosphatidylmethylethanolamine
ρM	=	picomole
R	=	Roots
rRNA	=	ribosomal ribonucleic acid
rpm	=	revolutions per minute
RT	=	Room Temperature
SAM	=	S-adenosylmethionine
SAP	=	Shrimp Alkaline Phosphatase
SCA	=	starch casein agar medium
SDS	=	sodium dodecyl sulfate
sec	=	second
SEM	=	Scanning Electron Microscopy
TCA	=	Trichloro acetic acid
TAE	=	Tris-acetate-EDTA electrophoresis buffer solution
TE buffer	=	Tris-EDTA buffer
TLC	=	thin-layer-chromatography
TMPD	=	N,N,N',N'-tetramethyl-1,4-phenylenediamine dihydrochloride
U	=	unit
UV	=	ultraviolet
V	=	volume
v	=	various
WA	=	water agar medium
YD	=	yeast extract dextrose medium

LIST OF ABBREVIATIONS (Continued)

YS	=	yeast extract starch agar medium
$ZnSO_4.7H_20$	=	zinc sulphate heptahydrate

CHARACTERIZATION AND IDENTIFICATION OF NOVEL TAXA, PLANT GROWTH PROMOTING PROPERTIES AND NEW COMPOUND FROM ENDOPHYTIC ACTINOMYCETES

INTRODUCTION

Actinomycetes colonizing within living plants are new valuable source of medically and agriculturally useful compounds such as antibiotics, anti-tumor agents and plant growth-promoting agents. Several decades, actinomycetes are known as provider for over two-third of bioactive compounds used these days. However, the development of resistance to multiple drugs in the treatment of infections by pathogenic microorganisms has been increasingly and thus a research strategy to provide novel useful compounds is aimed towards isolation of novel actinomycetes from uncommon habitats. It is highly likely that the novel strains of actinomycetes contain new genes and also hold for the novel bioactive compounds. In general, most members of actinomycetes were isolated from environmental samples such as soil, fresh/sea water and sediments. Recently, some actinomycetes have been reported that they are able to closely associate inside living plants and do not harm the host plants. These microbes, known as endophytic actinomycetes, live in different organs of host plants, mainly in intercellular or intracellular spaces.

Endophytes confer profoundly enhanced fitness to the host plants by preventing the proliferation of pathogenic organisms as well as stimulating the growth of plants. As for the preventing, endophytic actinomycetes especially from medicinal plants in tropical rainforest possess inhibiting a wide variety of pathogens. For example, endophytic *Streptomyces munumbi* NRRL 30562 isolated from native medicinal snake-vine produces broad-spectrum antibiotic munumbicins which showed activity against several pathogens. For stimulation of plant growth, endophytes show ability for production of plant growth-promoting agents such as auxins and cytokinins, siderophores (to bind Fe^{3+} from environments and help to improve nutrient uptake), and supply of plant nutrients such as phosphate, nitrogen

and other mineral nutrients or lower plant ethylene level through the action of the enzyme 1-aminocyclopropane-1-carboxlate (ACC) deaminase. The bacterial enzyme ACC deaminase is able to hydrolyze ACC, the intermediate biosynthetic precursors of the stress hormone ethylene, to ammonia and ketobutyrate resulting in decreased ethylene in plant tissues. However, a limited number of works have been done to elucidate the aforesaid abilities of endophytic actinomycetes especially the ability to stimulate plant growth.

This research demonstrated that actinomycetes colonized inside endemic plants of Thailand are a novel valuable source of medically and agriculturally useful compounds and plant growth-promoting agents. In this study endophytic actinomycetes were isolated from medicinal and agricultural important plants were characterized their taxonomy position. Some of them were identified at genus/species level by polyphasic taxonomy and new genus and new species were reported. Furthermore, their activities were detected as plant growth promoting agents. Anti-microbial activities were observed and a novel bioactive compound was characterized.

OBJECTIVES

1. To identify novel taxa of endophytic actinomycetes isolated from medicinally and agriculturally important plants of Thailand.

2. To characterize plant growth promoting properties from endophytic actinomycetes.

3. To determine novel bioactive compounds produced by the endophytic actinomycetes.

LITERATURE REVIEW

1. Actinomycetes

Actinomycetes (order Actinomycetales) are Gram-positive bacteria (Williams *et al*., 1989) with a DNA base composition generally above 50 mol% G+C (Stackebrandt *et al*., 1997). They are filamentous bacteria which produce aerial and substrate mycelium. The aerial mycelium is able to become spores (Takizawa *et al*., 1993). Most of actinomycetes widely distribute in nature especially in soil where exist as saprophytes (Takizawa *et al.*, 1993). In the nature habitats, genus *Streptomyces* is commonly a major component of the total actinomycetes population while other genera are rare actinomycetes (Hayakawa, 2008). Actinomycetes, particularly *Stretomyces*, are best known for their ability to produce secondary metabolites especially antibiotics (Okami and Hotta, 1988).

2. Identification and classification of actinomycetes

In a new era of bacterial taxonomy, organisms proposed as members of the class Actinobacteria has its origin in three sources: firstly, the determination of 16S ribosomal ribonucleic acids (16S rRNA) sequence similarities, which reveals the extent of sequence variation among strains at all levels of relatedness (Stackebrandt, *et al.,* 1980); secondly, the establishment of chemotaxonomy; and thirdly, the introduction of DNA-DNA reassociation experiments that measures the gross similarities between single-stranded DNA of strains of closely related species (Stackebrandt and Kandler, 1979). Each of these approaches has contributed to the success of a classification strategy which has been termed polyphasic taxonomy. The term “polyphasic taxonomy” was called by Colwell (Vandamme *et al*., 1996) and is used for the delineation of taxa at all levels (Murray *et al*., 1990). The terms “polyphasic classification” and “polyphasic identification” can also be validly used. Development of bacterial, polyphasic taxonomy was aiming at the integration of different kinds of data and information namely, phenotypic and genotypic on microorganisms and essentially indicates a consensus type of taxonomy. Taxonomy

is generally taken as a synonym of systematics and is traditionally divided into three parts: (1) classification, i.e., the orderly arrangement of organisms into taxonomic groups on the basis of similarity; (2) nomenclature and (3) identification of unknown organisms (Vandamme *et al*., 1996). The major categories of taxonomic method are divided into phenotypic and genotypic methods (Vandamme *et al*., 1996; Tindall *et al*., 2010).

2.1 Phenotypic methods

Phenotypic methods comprise all those that are not directed towards DNA or RNA therefore, they also include the chemotaxonomic techniques. The term "chemotaxonomy" refers to the application of analytical methods to collect information on various chemical constituents of the cell, such as cell wall amino acids, lipids, proteins, isoprenoid quinones and sugars, amongst members of microbial taxa and the use of such information for classification and identification of bacteria (Cummins and Harris, 1956; Vandamme *et al.*, 1996). The most commonly used chemical characters in actinomycete systematics are cellular fatty acids, menaquinones, mycolic acid, muramic acid types, phospholipids, whole-organism amino acids and sugars (Williams *et al.*, 1989). Besides, morphological, physiological, and biochemical characters are also used for identification. These methods were described in the identification key by Nonomura (1974) and Bergey's Manual of Determinative Bacteriology (2012).

2.1.1 Morphological characters

Actinomycetes can be characterized as differentiating prokaryotes which exhibit strain specific types of morphological differentiation including germination of spores, elongation and branching of vegetative mycelium, formation of aerial mycelium, septation of hyphae and spore maturation. This morphological variation was previously used to characterize the actinomycetes (Vobis and Miyadop, 1997).

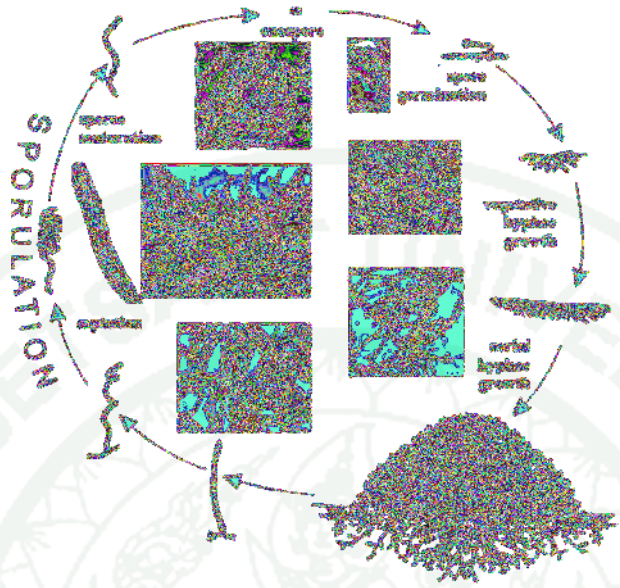


Figure 1 Life cycle of *Streptomyces* that grows as branching of vegetative hyphae mycelium which draw nutrients from the soil environment. When the nutrients are depleted, the *Streptomyces* colonies undergo differentiation, formation of aerial mycelium, septation of hyphae and spore maturation.

Source: Institute of Immunology and Experimental Therapy (2002)

Morphological characters have been an important characteristic to identify actinomycete isolates. It was used in the first descriptions. This study is best made by using a variety of standard culture media, including those recommended for the International *Streptomyces* Project (ISP) (Shirling and Gottlieb, 1966). Morphological observations including germination of spores, elongation and branching of vegetative mycelium, formation of aerial mycelium, color of aerial and substrate mycelium and pigment production are used to identify actinomycetes. These colors were determined by comparing its color with the color chips from Color Harmony Manual (Jacobson *et al*., 1958). The spore surface and spore structure are observed by scanning electron microcopy (SEM).

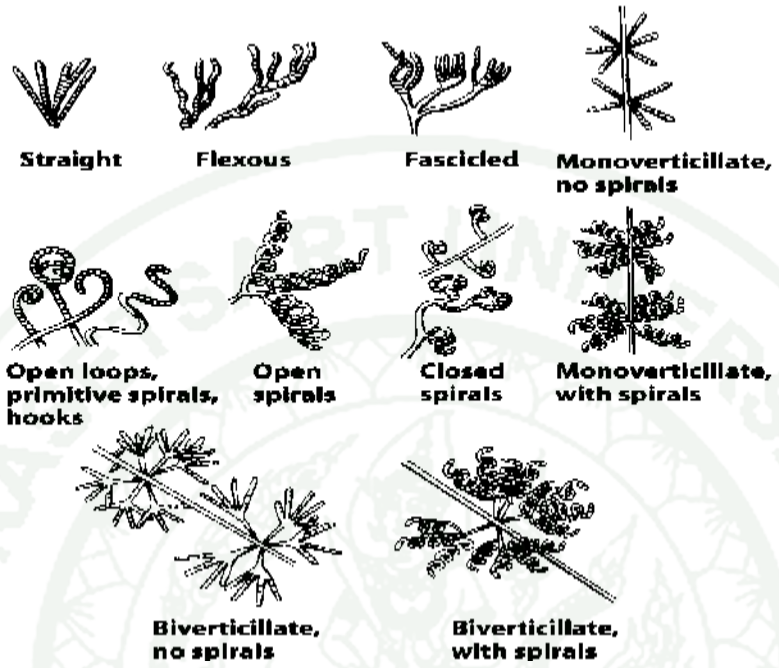


Figure 2 Various type of spore-bearing structure in *Streptomyces*.

Source: Madigan and Martinko (2006)

2.1.2 Chemotaxonomic characters

2.1.2.1 Peptidoglycan

Peptidoglycans provide the basic structure of the bacterial cell wall. Although there is considerable inter-species variation in the detailed structure of peptidoglycan, its chemical architecture remains constant, that is, it consists of β-1,4 linked disaccharides of N-acetylglucosamine and N-acetylmuramic acid. Glycan chain length varies from 10-65 disaccharide units. Tetrapeptide side chains are linked to carboxyl group of muramic acid (Figure 3). L-alanine is usually linked to muramic acid while most variable occurs at position 3 (Figure 4) where a diamino acid usually occurs and the most wildly distributed is diaminopimelic acid (DAP or A_2pm). In Gram-positive bacteria, primary structure of two peptide subunits are usually cross-linked through interpeptide bridges composed of 1-6 amino acid

units. These cross-linkages are normally found between amino acids at position 3 and 4 (Suzuki *et al.*, 1993). Peptidoglycans are usually classified according to the system proposed by Schleifer and Kandler (1972). Most actinomycetes have the type A peptidoglycan (crosss-linkage between positions 3 and 4 of two peptide subunits) though members of the family *Microbacteriaceae* exhibit the distinctive type B form (crosss-linkage between positions 2 and 4 of two peptide subunits). Variation in peptidoglycans provides useful information for the classification of actinomycetes at and above the genus level (Schleifer & Kandler, 1972).

The muramic acids of actinomycete peptidoglycans can be either N-acetylated or N-glycolated (Uchida and Aida, 1977; 1984). Most actinomycetes contain N-acetylated muramic acids while the genera *Micromonospora*, *Mycobacterium*, and *Nocardia* are characterized by the presence of N-glycolated muramic acid residues (Uchida and Aida, 1977). A simple colorimetric method is available for the determination of glycolyl or acetyl residues in bacterial cells (Suzuki, 2003).

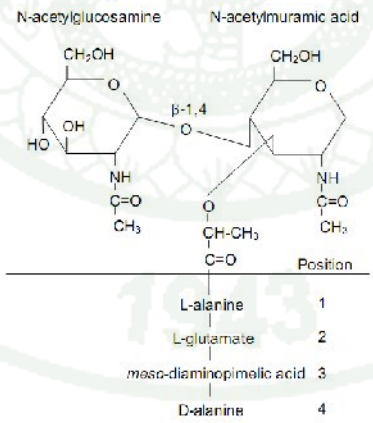


Figure 3 Fragments of the primary structure of typical peptidoglycan. Complete structure of a single subunit show the linkage between the two amino-sugar that make up the glycan strand and tetrapeptide side chains are linked to carboxyl group of muramic acid.

Source: Suzuki *et al.* (1993)

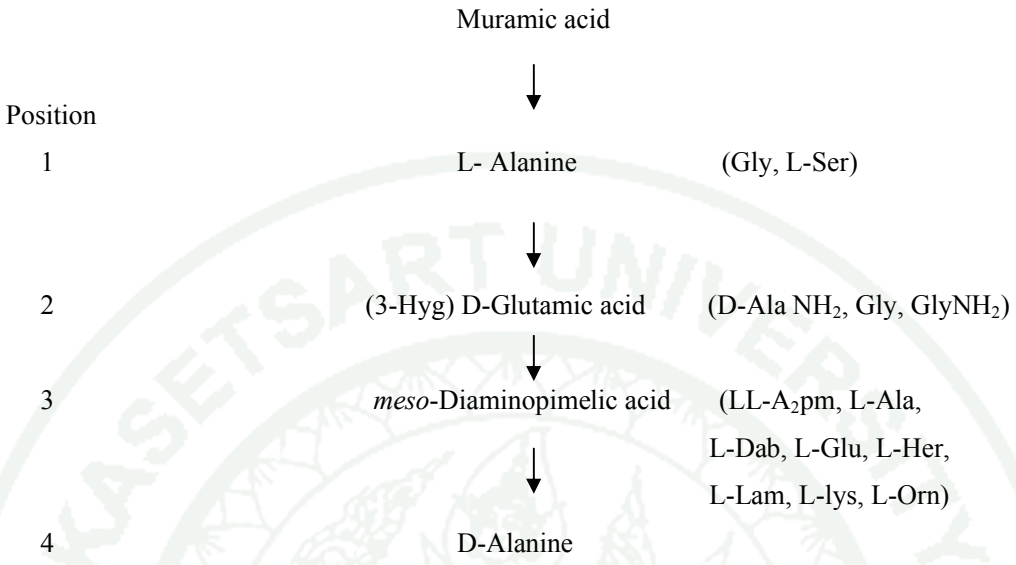


Figure 4 Some of the variations found in peptide subunits of Gram-positive bacteria. The amino acids in parentheses may replace the corresponding amino acid.

Source: Suzuki *et al.* (1993)

2.1.2.2 Cellular fatty acids

Cellular fatty acids can be defined as carboxylic acid derivatives of long-chain aliphatic molecules. In bacteria, they range in chain length from two (C_2) to over ninety (C_{90}) carbon atoms. Fatty acids in the range C_{10} to C_{24} are of the greatest taxonomic value. Cellular fatty acid composition can be quantitatively analyzed by using gas-liquid-chromatography. For gram positive bacteria, there are three main groups of cellular fatty acids, namely straight-chain, branched-chain and complex fatty acid types. Analysis of cellular fatty acids, the MIDI system (Newark, Delaware, USA) is a commercially available identification procedure for bacteria based on gas-liquid chromatography (GLC) analysis (Suzuki *et al.*, 1993).

2.1.2.3 Menaquinones

Various kinds of isoprenoid quinones occur in the cytoplasmic membranes of most prokaryotes as electron carriers in electron transport systems that menaquinones (MK) and ubiquinone (Q) are the two most widely distributed (Suzuki *et al.*, 1993). For actinomycetes, menaquinones are the most common type found in envelopes (Kroppenstedt, 1985; Suzuki *et al.*, 1993). The structures of isoprenoid quinones are generally described by the aberration of the quinone nucleus and by the number of isoprene units present in the side chain. In the case of isoprenoid quinones with partially hydrogenated side chains, the number of hydrogen atom is shown, e.g. MK-m (H_{2n}) (Suzuki *et al.*, 1993). Menaquinones have a chemical structure analogous to that of vitamin K2 (MK-7; unsaturated menaquinone with seven isoprene units and are classified according to the number of isoprene units, which can vary from one up to fifteen, and the degree of saturation or hydrogenation. The structure and composition of bacterial menaquinones are determined either semi-quantitatively by mass spectrometry or quantitatively by high-performance liquid chromatography (Collins, 1977; Kroppenstedt, 1985). The position or point of hydrogenation in isoprenoid side-chains can be very specific and hence of taxonomic value (Collins, 1977).

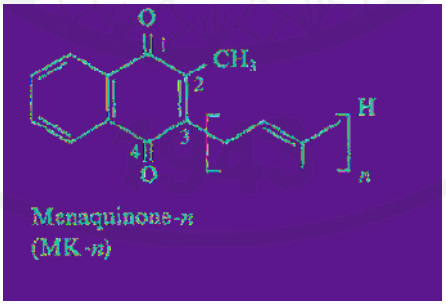


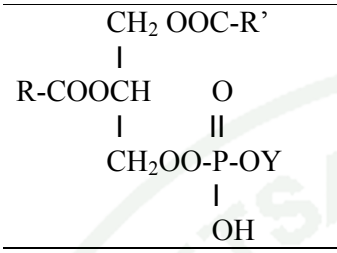
Figure 5 Structure of unsaturated menaquinones.

Source: Suzuki *et al.* (1993)

2.1.2.4 Phospholipids

Phospholipids are the most common polar lipids found in bacterial cytoplasmic membranes (Suzuki *et al.*, 1993). The structural variations found in bacterial phospholipids are illustrated in Table 1. The different types of phospholipids are discontinuously distributed in actinomycetes and hence provide useful taxonomic information (Minnikin and O'Donnell, 1984). Lechevalier *et al.* (1981) classified actinomycetes into five phospholipid groups based on major phospholipids markers found in whole-organism extracts. Phospholipids extracted from actinomycetes by using organic solvent systems (Minnikin *et al.*, 1984) can be separated by thin-layer-chromatography (TLC) and detected using non-specific (5%, w/v, ethanolic molybdophosphoric acid) (Suzuki *et al.*, 1993) or specific spray reagents. The latter can be used to detect a-glycols (periodate-Schiff) (Shaw, 1968), amino groups (0.2% ninhydrin, w/v, in water-saturated butanol) (Consden and Gordon, 1948), choline (Dragendorff reagent; Wagner *et al.*, 1961), lipid phosphates (Dittmer and Lester, 1964) and sugars (α-naphthol; Jacin & Mishkin, 1965). Actinomycetes can be assigned to established phospholipid patterns according to the presence or absence of specific or combinations of specific phospholipid markers (Table 2). In general, members of the same actinomycete genus have the same phospholipid type. Phospholipid patterns can be important for the recognition of actinomycete genera (Goodfellow, 1989; Williams *et al.*, 1989).

Table 1 Actinomycete phospholipids.



R, R’ = long-chain alkyl

Overall charge	Polar head group substituent (Y)	Name and abbreviation
1+	Glycerol	Phosphatidylglycerol (PG)
2+	Phosphatidylglycerol	Diphosphatidylglycerol (DPG)
1+	Butane-2,3-diol	Phosphatidylbutanediol (PB)
1+	Inositol	Phosphatidylinositol (PI)
1+	Acylated mannosylinositols	Phosphatidylinositol mannosides (PIM)
0	Ethanolamine	Phosphatidylethanolamine (PE)
0	Choline	Phosphatidylcholine (PC)
0	Methylethanolamine	Phosphatidylmethylethanolamine (PME)

Source: Minikin and O’Donnell (1984)

Table 2 Classification of actinomycete phospholipids

Type	**Characteristics**								
	PIM	**PI**	**PC**	**PG**	**PE**	**PME**	**GluNu**	**APG**	**DPG**
PI	+	+	–*	various	–*	–	–*	various	various
PII	+	+	–*	various	+*	–	–*	various	+
PIII	various	+	+*	various	various *	+	–*	various	various
PIV	?	+	–*	–*	various *	various	+*	–	+
PV	?	+	–*	various *	various *	–	+*	various	+

*diagnostic phospholipid

Source: Lechevalier *et al*. (1977) and Lechevalier *et al*. (1981)

2.1.2.5 Sugar composition

Neutral sugars, which are major components of actinomycete cell envelopes, are useful taxonomic markers at the suprageneric level. Sugar composition can be determined by simple paper chromatography (Schaal, 1985) or by using gas-liquid chromatography (Saddler *et al.*, 1991). Actinomycetes can be assigned to five groups on the basis of the discontinuous distribution of major diagnostic sugars, namely: group A, arabinose plus galactose; group B, madurose (3-O-methyl-D-galactose); C, no diagnostic sugars, D, arabinose plus xylose; and E, galactose plus rhamnose (Lechevalier and Lechevalier, 1970). A few rare sugars have also been reported to be diagnostic for members of some actinomycete taxa, notably the occurrence of 3-O-methyl-rhamnose in *Catellatospora* (Asano *et al.*, 1989) and tyvelose in *Agromyces* (Maltsev *et al.*, 1992).

2.1.2.6 Mycolic acids

Mycolic acids are especially long chain 3-hydroxy fatty acids with an alkyl branch at position 2. These molecules are only present in members of the genera *Corynebacterium*, *Dietzia*, *Gordona*, *Mycobacterium*, *Nocardia*, *Rhodococcus* and *Tsukamurella* (Suzuki *et al.*, 1993).

2.2 Genotypic methods

Genotypic methods are those that are directed towards DNA or RNA molecules.

2.2.1 Determination of the DNA base ratio (moles percent G+C)

Determination of the moles percent guanosine plus cytosine is one of the classical genotypic methods and is considered part of the standard description of bacterial taxa. Generally, the range observed is not more than 3% within a well-

defined species and not more than 10% within a well-defined genus. Actinomycetes contain DNA within the range 51- 78 mol % G+C (Stackebrandt and Liesack, 1993).

2.1.2 Ribosomal ribonucleic acid (rRNA) gene

Bacterial systematics is increasingly being based on phylogenetic information, which derived from DNA and RNA molecules (Woese, 1987; Ludwig *et al.*, 1993; Ludwig and Schleifer, 1994). Woese (1987) focused on the small and large subunit of rRNA sequences that provide the most useful and suitable chronometer for phylogenetic analyses. They: (1) show a high degree of functional constancy which assures relatively good clockwise behavior; (2) occur in all organisms; (3) show different rates of mutation along different parts of the macromolecule thereby allowing distant phylogenetic comparisons to be made; and (4) can be sequenced directly. For these reason, the rRNA sequences were shown to be a very useful molecular marker for phylogenetic analyses (Luwig and Schleifer, 1994). There are three rRNAs in bacteria which are classified by their sedimentation (S) rates in ultracentrifugation, 23S, 16S and 5S which are 3300, 1650 and 120 bases, respectively. Most bacterial species, genes that encode for the three rRNA types are closely linked in gene sets in the order 16S-23S-5S rDNA (Rossello-Mora and Amann, 2001). Among the three rRNA molecules, 16S rRNA is the most commonly housekeeping genetic marker that used for bacterial phylogeny and taxonomy because (1) its presence in almost all bacteria, often existing as a multigene family, or operons; (2) the function of the 16S rRNA gene over time has not changed, suggesting that random sequence changes are a more accurate measure of time (evolution); and (3) the 16S rRNA gene is large enough for informatics purposes. Thus, significance of phylogenetic studies based on 16S rRNA gene is increasing in the systematics of bacteria. Phylogenetic tree based on sequences of 16S ribosomal DNA allows the investigation of evolution of bacteria also provides the basis for identification (Woese, 1987; Yokota, 1997). For 5S rRNA, it is not usually considered to be a suitable molecule for phylogenetic analyses because of its small size that does not allow statistically significant sampling (Woese, 1987). Relatively little work has been done on the more complex 23S rRNA which consists of approximately 3300 nucleotides.

Reasonably good agreement has been found between evolutionary trees based on 16S and 23S rRNA sequence data (Ludwig *et al.*, 1992).

The analysis of 16S rRNA sequence produces numerical values of 16S rRNA similarities that can be used as circumscription of a species. Strains that show ≤ 97% 16S rRNA sequence similarity to all known taxa are considered to belong to a new species and isolates that have ≥ 97% identity is not as clear. This latter value can represent a new species or, alternatively, indicate clustering within a previously defined taxon (Amann *et al*., 1992; Stackebrandt and Goebel, 1994; Vandamme *et al*., 1996; Stackebrandt *et al.,* 2002; Gevers *et al*., 2005 and Janda and Abbott, 2007). On the basis of phylogenetic analysis using particular 16S rRNA gene sequences, actinomycetes (order Actinomycetales) is known to be composed of 13 suborder (Zhi *et al*., 2009), as shown in Figure 6, with a recent proposed suborder Jiangellineae (Tang, 2011).

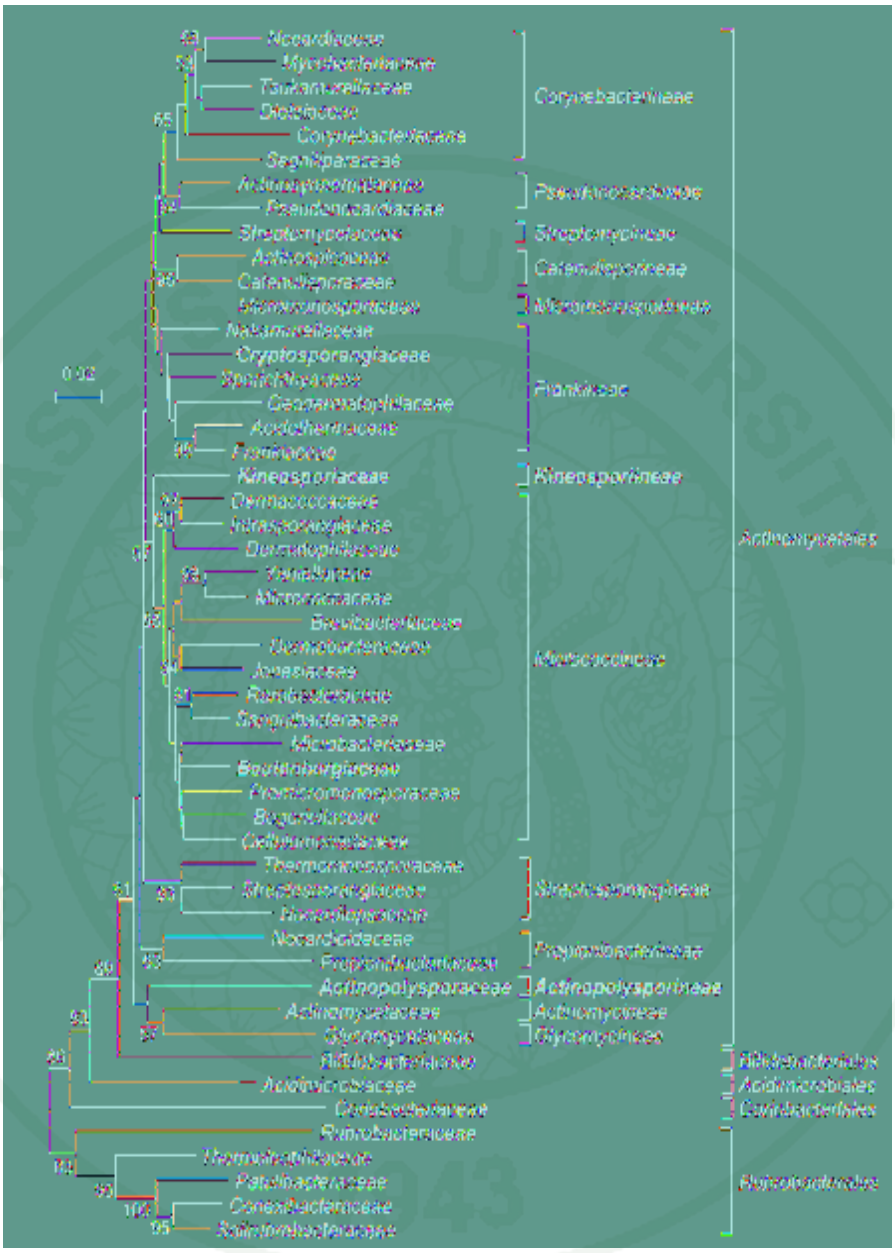


Figure 6 Intraclass relatedness of the class Actinobacteria showing the presence of five orders (Actinomycetales, Bifidobacteriales, Acidimicrobiales, Coriobacteriales and Rubrobacterales) based on 16S rRNA gene sequence comparison.

Source: Zhi *et al*. (2009)

2.2.3 DNA-DNA hybridization (DDH)

The percent DDH is unequivocally the gold standard for proposed new species (Wayne *et al*., 1987). The percent DNA binding or the DDH value (percentage of whole-genome sequence similarity) or the relative binding ratio is an indirect parameter of the sequence similarity between two entire genomes (Grimont *et al*., 1980). In this approach, the overall genetic similarity among isolates is assessed by the degree to which their genomes hybridize under standardized conditions. Isolates show more than 70% DNA-DNA-binding values and less than 5% difference in their melting temperature (ΔTm) are considered to belong to the same species (although this threshold value might be adjusted by expert practitioners that are familiar with the vagaries of individual taxonomic groups) (Vandamme *et al*., 1996; Stackebrandt *et al.,* 2002; Gevers *et al*., 2005). Comparison of 16S rRNA gene sequence homology and DNA-DNA reassociation values, strains that show < 97% 16S rRNA sequence similarity usually share < 70% DDH, isolates that have ≥ 97% identity might or might not meet the 70% DDH criterion for inclusion in the same species as shown in Figure 7 (Stackebrandt and Goebel, 1994; Gevers *et al*., 2005).

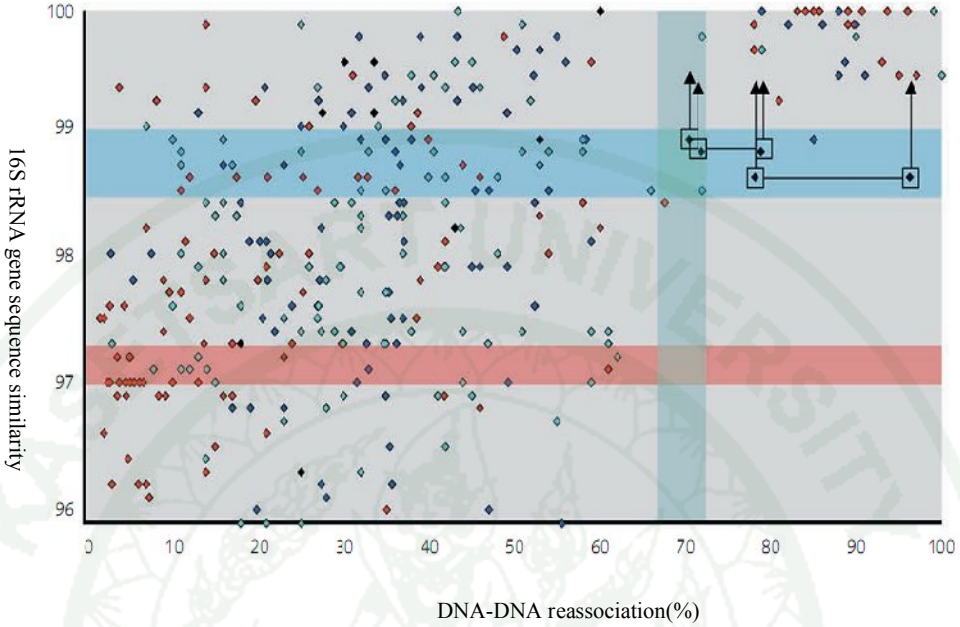


Figure 7 Comparison of 16S rRNA gene sequence homology and DNA-DNA reassociation values. The different colours refer to broad categories of reassociation methods: red, microtitre plate technique, dark blue, spectrophotometric technique, light-blue, membrane filter method; black, other methods, e.g. dot hybridization, or not defined. Horizontal rules between squares indicate data obtained by two different reassociation methods. Arrows point to the position of *in silico*-recalculated binary 16S rRNA gene sequence similarity values of sequences. The horizontal blue bar indicates the threshold range above which it is now recommended to perform DNA–DNA reassociation experiments; the horizontal red bar indicates the threshold values published previously (Stackebrandt and Goebel, 1994).

Source: Stackebrandt and Eber (2006)

3. Endophytic actinomycetes

There are several definitions of endophytes but many reports have followed the definition of Hallmann *et al*. (1997) because this definition includes internal colonies with apparently neutral behavior as symbionts (Hesagawa *et al*., 2006). The endophytes are defined as any microbes (fungi, bacteria, actinomycetes) as an endophyte if it does not visibly harm the plant and it can be isolated from the surface of disinfected plant tissues or extracted from inside the plant (Hallmann *et al*., 1997). As for actinomycetes, the majority of them were once believed to survive in environments such as the soil, rhizosphere, and pond and lake sediments as saprophytes by degrading organic materials for nutrition (Suzuki *et al*., 2005). However, in the 19th century some actinomycetes, proved to be closely associated within living plants and giving beneficial to the host plants, are known as endophytic actinomycetes (Hasegawa *et al*., 2006).

3.1 Distribution and diversity

The first report of endophytic actinomycete was found in member of the genus *Frankia* and was isolated from *Comptonia peregrina* root nodules in 1978 (Callaham, 1978). The *Frankia* species is nitrogen-fixing symbiosis with a large number of woody dicotyledonous (Simonet *et al*., 1994; Provorov *et al*., 2002). Symbiosis of *Frankia* is closest to the legume-rhizobia in terms of evolution, structure and function. While it had been well established that *Frankia* formed a symbiotic relationship with angiosperms it was not identified as endophyte until the first electron micrograph was made (Provorov *et al*., 2002). Since the first isolation of *Frankia*, a number of other biologically active endophytes and root-colonising microorganisms belonging to the order actinomycetales have been isolated or detected. Endophytic actinomycetes have been isolated from variety of plants. The most common endophytic actinomycetes isolated from surface-sterilized of plant tissue belonged to the genus *Streptomyces* (Sardi *et al*., 1992; Taechowisan *et al*., 2003). Sardi *et al*. (1992) isolated 49 endophytic actinobacteria from a range of plant species with approximately 96% of isolates belonging to the genus *Streptomyces.*

The remainder belonged to *Nocardia, Micromonospora* and *Streptosporangium*. Stamford *et al*. (2001) isolated *Nocardiopsis* sp. from tubers of yam bean (*Pachyrhizus erosus* L. Urban) and studied their α-amylase production. Taechowisan *et al*. (2003) isolated 330 actinomycetes from 26 different Thai medicinal plant species and the majority was *Streptomyces* spp. Likewise, Coombs and Franco (2003a; 2003b) isolated 38 strains belonging to *Streptomyces*, *Microbispora*, *Micromonospora* and *Nocardia* from surface-sterilized root tissues of healthy wheat plants and *Streptomyces* was demonstrated by using GFP-expressing. Endophytic streptomycete strains were isolated from roots of tomato (*Lycopersicon esculentum*) and examined their antimicrobial activity (Cao *et al*., 2004). Furthermore, Cao *et al*. (2004) and Tian *et al*. (2004) found that *Streptomyces* spp. were dominant species in banana (*Musa acuminata*) and rice plants. Besides, Valdes *et al*., (2005) have reported actinobacteria in families of Thermomonosporaceae and Micromonosporaceae isolated from surface-sterilized root nodules of *Casuarina equisetifolia* growing in Mexico. Rosenblueth and Martinez-Romero (2006) listed 8 genera of plant-associated actinomycetes including *Arthrobacter, Curtobacterium, Kocuria, Nocardia* and *Streptomyces.*

3.2 As a source of novel genetic resources

Since a new genus of endophytic actinomycetes, *Actinosynnema*, isolated from a grass blade was described by Hasegawa *et al*. in 1978, only distribution and diversity of endophytic actinobacteria were reported in several plants. Until 1998, Kudo *et al*. (1998) have discovered four novel species of genus *Kineosporia* as endophytic actinomycetes isolated from leaves and roots of plant samples in Japan. Since then, several novel species of endophytic actinomycetes have been reported. For example, *Agromyces albus* was isolated from stems and leaves of *Androsace* sp. (Dorofeeva *et al*., 2003). Song *et al*. (2004) have discovered a novel species of genus *Kribbella* as endophytic actinomycetes isolated from potato tuber. *Micromonospora coriariae* was isolated from root nodules of *Coriaria myrtifolia* (Trujillo *et al*., 2006). *Pseudonocardia oroxyli* was isolated from root of surface-sterilized of traditional Chinese medicinal plant (*Oroxylum indicum*) (Gu *et al*., 2006). *Glycomyces*

sambucus was isolated from stem of traditional Chinese medicinal plant, *Sambucus adnata* Wall (Gu *et al*., 2007). Trujillo *et al*. (2007) have reported novel species *Micromonospora lupine* and *M. saelicesensis* that were isolated from root nodules of *Lupinus angustifolius*. And in Thailand, Duangmal *et al*. (2009) and Thachaipenet *et al*. (2010) isolated *Pseudonocardia acacia* and *Actinoallomurus acacia* from *Acacia auriculiformis* A. Cunn. ex Benth. (earpod wattle) and reported novel species. The list of novel species and genus isolated from several plants was shown in Table 3.

Table 3 The novel species and genus of endophytic actinomycetes

Name of the endophytic actinomycetes	Host plants	Closest non-endophytic species and 16S rRNA gene sequences similarity (%)	GenBank accession no.
Actinosynnema mirum	Grass	No report	CP001630
Kineosporia succinea	*Cyperus microiria*	*K. aurantiaca* (96.3-99.8%)	AB003932
Kineosporia rhizophila	*Cyperus microiria*		AB003933
Kineosporia mikuniensis	*Sphagnum* sp.		AB003934
Kineosporia rhamnosa	*Typha latifolia*		AB003935
Agromyces albus	*Androsace* sp.	*A. ramosus* (98.8%)	AF503917
Kribbella solani	*Ipomoea batatas* L.	*K. sandramycini* (98.6%)	AY253862
Pseudonocardia oroxyli	*Oroxylum indicum*	*P. halophobica* (97.8%)	DQ343154
Micromonospora coriariae	*Coriaria myrtifolia*	*M. endolithica* (98.94%)	AJ784008
Glycomyces sambucus	*Sambucus adnata* Wall	*G. lechevalierae* (97.2%)	DQ460469

Table 3 (Continued)

Name of the endophytic actinomycetes	Host plants	Closest non-endophytic species and 16S rRNA gene sequences similarity (%)	GenBank accession no.
Micromonospora lupine	*Lupinus angustifolius*	*M. mirobrigensis* (98.5%)	AJ783996
Micromonospora saelicesensis	*Lupinus angustifolius*	*M. purpureochromogenes* (98.7%)	AJ783993
Leifsonia ginsengi	*Ginseng*	*L. poae* (97.6%)	DQ473536
Rhodococcus cercidiphylli	*Cercidiphyllum japonicum*	*R. fascians* (99.6%)	EU325542
Dietzia schimae	*Schima* sp.	*D. maris* (99.8%)	EU375845
Dietzia cercidiphylli	*Cercidiphyllum japonicum*	*D. natronolimnaea* (99.5%)	EU375846
Saccharopolyspora endophytica	*Maytenus austroyunnanensis*	*S. flava* (97.7%)	EU814512
Glycomyces endophyticus	*Carex baccans* Nees	*G. algeriensis* (99%)	EU200681
Streptomyces mayteni	*Maytenus austroyunnanensis*	*S. phaeopurpureus* (99.5%)	EU200683
Pseudonocardia endophytica	*Lobelia clavata*	*P. kongjuensis* (98.5%)	DQ887489
Micrococcus endophyticus	*Aquilaria sinensis*	*M. luteus* (99.06%)	EU005372
Pseudonocardia acacia	*Acacia auriculiformis* A. Cunn. ex Benth	*P. spinosispora* (96.2%)	EU921261
Phytohabitans suffuscus	Orchid	*M. pattaloongensis* (97.7%)	AB490769
Kineosporia mesophila	*Tripterygium wilfordii*	*K. mikuniensis* (98.2%)	FJ214362

Table 3 (Continued)

Name of the endophytic actinomycetes	Host plants	Closest non-endophytic species and 16S rRNA gene sequences similarity (%)	GenBank accession no.
Saccharopolyspora tripterygii	*Tripterygium hypoglaucum*	*S. flava* (97.6%)	FJ214364
Herbidospora osyris	*Osyris wightiana Wall. ex Wight*	*H. cretacea* (99.9%)	FJ214356
Streptomyces sedi	*Sedum* sp.	*S. specialis* (97.5%)	EU925562
Streptomyces alni	*Alnus nepalensis* D. Don	*S. hebeiensis* (97.6%)	DQ460470
Glycomyces scopariae	*Scoparia dulcis*	*G. algeriensis* (97.4%)	EU200682
Glycomyces mayteni	*Maytenus austroyunnanensis*	*G. algeriensis* (97.1%)	EU814511
Plantactinospora mayteni	*Maytenus austroyunnanensis*	*M. endolithica* (98.1%)	FJ214343
Nonomuraea antimicrobica	*Maytenus austroyunnanensis*	*N. candida* (98.2%)	FJ157184
Actinomadura flavalba	*Maytenus austroyunnanensis*	*A. atramentaria* (97.4%)	FJ157185
Jiangella alba	*Maytenus austroyunnanensis*	*J. alkaliphila* (98.8%)	FJ157186
Pseudonocardia tropica	*Maytenus austroyunnanensis*	*P. alni* (99.5%)	GQ906587
Micrococcus yunnanensis	*Polyspora axillaris*	*M. luteus* (99.7%)	FJ214355
Micromonospora pisi	*Pisum sativum*	M. *pattaloongensis* (98.7%)	AM944497
Pseudonocardia adelaidensis	*Eucalyptus microcarpa*	*P. zijingensis* (98.7%)	FJ805427
Pseudonocardia eucalypti	*Eucalyptus microcarpa*	*P. spinosispora (96.3%)*	FJ805426

Table 3 (Continued)

Name of the endophytic actinomycetes	Host plants	Closest non-endophytic species and 16S rRNA gene sequences similarity (%)	GenBank accession no.
Nocardia callitridis	*Callitris preissii*	*N. nova* (97.4%)	FJ805428
Actinoallomurus oryzae	*Oryza sativa* L. cv. KDML 105	*A. iriomotensis* (99.2%)	EU420071
Actinophytocola oryzae	*Oryza sativa* L. cv. RD6	*Kibdelosporangium aridum* (95.5%)	EU420070
Nonomuraea endophytica	*Artemisia annua* L.	*N. candida* (98.8%)	GU367158
Saccharopolyspora gloriosae	*Gloriosa superba* L.	*S. gregorii* (99.1%)	EU005371
Pseudonocardia sichuanensis	*Jatropha curcas* L	*P. zijingensis* (98.6%)	HM153789
Nocardioides caricicola	*Carex scabrifolia* Steud	*N. pyridinolyticus* (97.0%)	EF466117
Actinoallomurus acaciae	*Acacia auriculiformis* A. Cunn. ex Benth	*A. caesius* (99.3%)	EU429322
Jishengella endophytica	*Acanthus illicifolius*	*M. olivasterospora* (98.7%)	EU560726
Nocardia endophytica	*Jatropha curcas* L.	*N. nova* (97.5%)	HM153801
Pseudonocardia artemisiae	*Artemisia annua* L.	*P. saturnea* (96.6%)	GU227146
Streptomyces artemisiae	*Artemisia annua* L.	*S. armeniacus* (99.9%)	EU200685

Source: Qin *et al*. (2011)

3.3 As a source of plant growth-promoting bacteria (PGPB)

Nowadays, concern about environmental problems has prompted researchers to consider alternatives strategies for facilitating plant growth in agriculture (Glick *et al.,* 2007). Ideally, replacements for the chemicals that are currently in widespread use should not only enhance plant growth, but should also inhibit plant pathogens. One potential alternative may be the use of PGPB. PGPB can bind to either roots (rhizosphere bacteria), leaves (phyllosphere bacteria), or they may exist within plant tissues (bacterial endophytes) (Glick *et al.*, 2007). Among them, endophytes are special interested since they possess many properties that could benefit to plant growth involved in production of biological control agents, or production of plant growth promotion compounds, such as auxins, cytokinins and gibberellins, or producing siderophore to bind Fe^{3+} from the environment and help to improve nutrient uptake, or supply of plant nutrients through nitrogen fixation, ammonia production and phosphate solubilization or suppression of stress ethylene production by 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity (Compant *et al.,* 2005; Sun *et al.,* 2009; Nimnoi *et al.,* 2010; Qin *et al*., 2011).

3.3.1 Auxin production

The plant auxin hormone has profound effects on growth and differentiation of plants. Auxin (indole-3- acetic acid, IAA) is a key plant hormone which plays important role in cell division, cell elongation and differentiation, and in processes such as meristem maintenance, root elongation, lateral root development and senescence (Moller and Chua, 1999; Tao *et al*., 2002). Not only plants but also bacteria can synthesis auxin. According to Sarwar and Kremer (1995), IAA synthetic bacteria may be useful in increasing the growth of crops. Soil or rhizosphere actinomycetes have been shown ability to produce IAA and promote growth of several cultivated plants (El-Tarabily and Sivasithamparam, 2006). Rhizospheric *Micromonospora*, *Nocardia*, *Actinomadura* and S*treptosporangium* were reported to IAA and most of them could increase dry-weight of corn, cucumbers, tomatoes, sorghums and carrots (Mishra *et al*., 1987; El-Tarabily *et al*., 1997). Besides,

Siddikee *et al.* (2010) reported that rhizospheric actinobacteria including *Arthrobacter*, *Brevibacterium*, *Corynebacterium*, *Micrococcus* and *Zhihengliuella* were able to produce IAA and promote the growth of canola seedling. For endophytic actinomycetes, *Streptomyces hygroscopicus* isolated from *Pteridium aquilinum* was able to produce novel pteridic acids A and B as plant growth promoters with auxin-like activity (Igarashi *et al*., 2002). Three selected endophytic actinomycetes isolated from cucumber seedlings showed ability to produce IAA and the isolates colonized cucumber roots and also promoted plant growth (El-Tarabily *et al*., 2009). Fialho *et al*. (2010) isolated 70 endophytic actinomycetes from tomato plants in southern Brazil of which 72.1% showed a positive reaction for IAA production. Nimnoi *et al*. (2010) showed that endophytic actinomycetes, isolated from *Aquilaria crassna* Pierre ex Lec including *Streptomyces*, *Nonomuraea*, *Actinomadura*, *Pseudonocardia* and *Nocardia* were able to detect of IAA production by using colorimetric assay.

3.3.2 Siderophore production

The term of siderophore was described as a low molecular weight molecule that binds ferric iron (Fe^{3+}) with an extremely high affinity (Lankford, 1973). Siderophore was derived from a Greek term meaning iron carrier (Ishimaru, 1993). Siderophores were produced and secreted under iron-deficient conditions and then bround iron with an extremely high affinity. It is specifically recognized by a corresponding outer membrane receptor protein, which in turn actively transports the complex into the periplasm of the cell. The molecular weights of siderophores range from approximately 600 to 1500 daltons, and passive diffusion does not occur for molecules greater than 600 daltons, so that siderophores must be actively transported across the outer and inner membranes of bacteria and fungi (Ishimaru, 1993). Many bacteria and fungi are capable of producing more than one type of siderophore or have more than one iron-uptake system to take up multiple siderophores (Neilands, 1981).

There are two main classes of siderophores, catechol and hydroxamate, which are classified on the basis of the chemical functional groups that use to chelate iron. Catechol-type siderophores bind Fe^{3+} using adjacent hydroxyl

groups of catechol rings while hydroxamate-type siderophores bind Fe^{3+} via a carbonyl group with adjacent nitrogen. Enterobactin, also known as enterochelin, is produced by a number of bacteria and is the classic example of a catechol-type siderophore (O'Brien and Gibson 1970; Pollack *et al*., 1970; Clark, 2004). Enterobactin production has been demonstrated in some nitrogen-fixing bacteria, including *Klebsiella pneumoniae* and *K. terrigena.* Ferrichrome is the classic hydroxamate-type siderophore. It is produced by a number of fungi. Although produced by fungi, ferrichrome is used by a number of bacterial species with the appropriate receptor protein (Hofte, 1993).

For actinomycetes, *Streptomyces* species are known for the production of hydroxamate type siderophores, which inhibit phytopathogen growth by competing for iron in rhizosphere soils (Khamna *et al*., 2009). Cao *et al*. (2005) indicated that siderophore-producing *Streptomyces* endophytes were effective candidates for the biological control of *Fusarium* wilt disease of banana roots. Endophytic actinobacterial isolated from the root of tomato showed ability to produce siderophore and inhibited phytopathogens that endophytic *Streptomyces* sp. R18(6) inhibited all phytopathogens (Tan *et al*., 2006; Fialho *et al*., 2010). In addition, Nimnoi *et al*. (2010) isolated 10 endophytic actinomycetes including *Streptomyces*, *Nonomuraea*, *Actinomadura*, *Pseudonocardia*, and *Nocardia* from *Aquilaria crassna* Pierre ex Lec. Eight isolates produced hydoxamate type siderophores while only one isolate, *Actinomadura*, produced catechols type siderophores. Xing *et al.* (2011) proposed a new species of endophytic *Kibdelosporangium phytohabitans* isolated from the root of the oil-seed plant *Jatropha curcas* L.collected from Sichuan province, south-west China that could produce siderophore. Besides, 72% of actinobacterial strains isolated from different plants, lentil (*Lens esculentus*), chickpea (*Cicer arietinum* L.), pea (*Pisum sativum*), faba bean (*Vicia faba*) and wheat (*Triticum vulgare*) from Paskerville, south Australia were able to produce siderophores (Misk and Franco, 2011). Recent reports showed that endophytic actinomycetes isolated from rice and wheat were able to produce hydoxamate and catechol type siderophores (Gangwar *et al*., 2012).

3.3.3 Ammonia production

The nitrogen in most plants and animals exists in the form of protein. Most of the nitrogen in soil exists in the form of organic molecules, mostly proteins derived from the decomposition of dead plant and animal tissue. When an organism dies, its proteins are attacked by the proteases of soil bacteria to produce polypeptides (peptones) and amino acids ($C_2H_4NO_2R$). This process is called peptonization. Then, the amino groups on the amino acids are removed by a process called deamination, producing ammonia (NH_3). In most soils, the ammonia dissolves in water to form ammonium ions (NH_4^+). The process of the production of ammonia from organic compounds is called ammonification. In addition to the ammonification of amino acids, other compounds such as nucleic acids, urea, and uric acid go through the ammonification process. The bacteria that accomplish it (*Bacillus*, *Clostridium*, *Proteus*, *Pseudomonas*, and *Streptomyces*) are called ammonifying bacteria (Savant and DeDatta, 1982; Kadlec and Knight, 1996; Rebollido *et al*, 2008). Rhizospheric actinobacteria including *Arthrobacter*, *Brevibacterium*, *Corynebacterium*, *Micrococcus* and *Zhihengliuella* were able to produce ammonia (Siddikee *et al.*, 2010). For endophytic actinomycetes, Nimnoi *et al.* (2010) have reported that 10 endophytic actinomycetes from *Aquilaria crassna* Pierre ex Lec were able to produce ammonia detected by Nessler's reagent.

3.3.4 Phosphate solubilization

Phosphorus (P) is one of the major essential macronutrients for plants and is applied to soil in the form of phosphate fertilizers. However, a large portion of soluble inorganic phosphate which is applied to the soil as chemical fertilizers are rapidly immobilized in the soil or quickly washed away by the raining waters, polluting rivers and ground waters and thus not available for plant growth (Reddy *et al*., 2002; Shigaki *et al*., 2006). Currently, phosphate solubilizing bacteria (PSB) which is a group of beneficial bacteria capable of hydrolysing organic and inorganic phosphorus from insoluble compounds have attracted the attention of agriculturists as soil inoculums to improve plant growth and yield (Young *et al*.,

1998; Goldstein *et al*., 1999; Chen *et al*., 2006). Among these bacteria, actinomycetes are of special interest because they posses many properties (Hamdali *et al*., 2008). *Rhodococcus erythropolis* isolated from soil in central Taiwan showed ability as phosphate solubilizers (Chen *et al*., 2006). Sahu *et al*. (2007) reported that seven strains of actinomycetes isolated from sediment in southeast coast of India showed positive phosphate activity. Among them, *Streptomyces galbus* PS-3 exhibited good activity and further investigated for optimum phosphorus solubilization at different pH and incubation periods.

Hamdali *et al*. (2008a; 2008b; 2008c) reported that actinomycetes isolated from rock phosphate (RP) soil extracts were were able to decompose RP in synthetic minimum medium. Among these, *Streptomyces griseus* BH7 and *Micromonospora aurantiaca* KH7 were selected to investigate the effect on plant growth and fitness. Seeds of wheat plant (*Triticum durum* L. cv Vitron) were coated with mycelia of these strains and then grown in soluble phosphate deficient sterile soil supplemented with insoluble RP. The results showed that inoculation of the soil with *S. griseus* or *M. aurantiaca* promoted growth of shoots and roots (Hamdali *et al*., 2008b). In addition, *S. griseus* BH7 showed colonization inside the root of wheat plants (Hamdali *et al*., 2008c). Kaviyarasi *et al*. (2011) demonstrated that six actinomycetes including *Actinobispora yunnanensis*, *Micromonospora echinospora*, *Sacchraromonospora viridis*, *Sacchraropolyspora hirsute*, *Thermonospora mesophila* and *Streptoverticillum album* isolated from soil in coastal region of India acted as potent phosphate solubilizers.

For endophytic actinomycetes, 16.2% of test strains isolated from roots of tomato plants (*L. esculentum*) in southern Brazil showed positive for phosphate solubility (Fialho *et al*., 2010). Two endophytic *Streptomyces* from wheat also showed positive for phosphate solubility (Misk and Franco, 2011). Recently, seventeen isolates of endophytic actinomycetes isolated from wheat and rice were able to solubilize phosphate. The amount of phosphate solubilized by the isolates ranged from 4.8-42 mg/100 ml (Gangwar *et al*., 2012a; 2012b).

3.3.5 Lower the plant ethylene level by an enzyme ACC deaminase

In plants, ethylene regulates several physiological processes such as seed germination, root hair development, root elongation and organ senescence. Under stresses including salinity, flooding, drought, extremes of temperature, the presence of organic or inorganic toxicants, phytopathogens, ethylene is accumulated in plants and very effective on plant growth and development and also on fruit ripening, the increased concentration of ethylene, especially in dicotyledons, may decrease seed germination and root growth (Belimov *et al*., 2001; Saravana- kumar and Samiyappan, 2007). Ethylene is derived from the amino acid methionine that is converted to S-adenosylmethionine (SAM) by SAM synthetase, which metabolite serves as an intermediate in numerous biosynthetic pathways. After that, SAM is converted into ACC and then ethylene by the activity of ACC synthase and ACC oxidase, respectively. This ethylene production pathway can be controlled by altering endogenous levels of ACC, the intermediate precursor to ethylene, and thereby lower the level of ethylene in a developing or stressed plant (Glick *et al*., 1997; Glick, 2004; Cheng *et al*., 2007; Contesto *et al*., 2007; McDonnell *et al*., 2009). An enzyme ACC deaminase (E.C. 4.1.99.4) hydrolyses ACC into ammonia and α-ketobutyrate instead of its conversion into ethylene (Glick *et al.,* 1994; 1998; Mayak *et al.,*1999; Shaharoona *et al.,* 2006a). It is a multimeric enzyme (homodimer or homotrimer) with a subunit molecular mass of approximately 35-42 kDa (Glick *et al.,* 2007).

For the promotion of plant growth by bacterial ACC deaminase, a model was developed by Glick *et al*. (1998) to explain the role of bacteria that have ACC deaminase activity. In the model, the ACC deaminase-containing plant growth-promoting bacteria bind to the surface or located inside of either seed or root of a developing plant. In response to root exudate, including the amino tryptophan, the bacteria synthesize IAA. Plant cell take up some of the bacterial IAA and together with the endogenous plant IAA, can stimulate plant cell proliferation an elongation as well as induce the synthesis of the enzyme ACC synthase. Some of ACC is exuded and take up by ACC deaminase-containg bacteria. This ACC is cleaved by ACC deaminase into ammonia and α-ketobutyrate, both of which are readily metabolized

by bacteria. As a consequence of lowering the level of ACC within a plant, the amount of endogenous ethylene is reduced (Figure 8). Direct consequence of this interaction are significantly increased plant root and shoot length, an increase biomass, and protection of plant from inhibitory effects of ethylene (Glick *et al*., 2007).

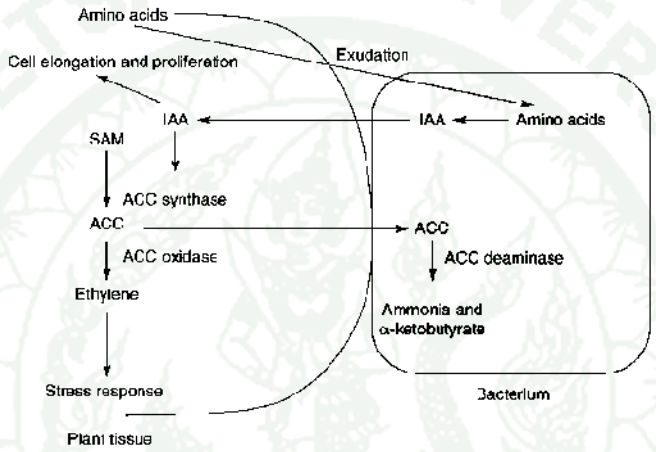


Figure 8 Schematic representation of the interaction between bacteria and plant through ACC deaminase and ethylene.

Source: Glick *et al*. (1998); Arshad *et al*. (2007)

ACC deaminase activity were found in a wide range of bacterial isolates including *Azospirillum*, *Rhizobium*, *Agrobacterium*, *Achromobacter*, *Burkholderia*, *Ralstonia*, *Pseudomona*s and *Enterobacter* (Blaha *et al*., 2006). For actinomycetes, Christopher *et al*. (2008) reported that *Atriplex lentiformis* rhizospheric actinomycetes including *Streptomyces*, *Arthrobacter* and *Rhodococcus* possessed ACC deaminase activity. Production of ACC deaminase by tomato rhizospheric *Streptomyces* isolates was also found and some of isolates decreased the endogenous levels of ACC and increase the growth of plant (El-Tarabily, 2008). Besides, *Microbacterium*, *Brevibacterium*, *Zhihengliuella*, *Micrococcus* and *Corynebacterium* isolated from soil or rhizosphere showed ability of ACC deaminase activity. Inoculation of the isolates to ameliorate salt stress (150 mM NaCl) in canola

plants, could increase root length and dry weight when compared with uninoculated salt stressed canola seedling (Siddikee *et al*., 2010). He *et al*. (2010) reported that Cu-resistant isolates from different rhizosphere including *Microbacterium lactium* YJ7 and *Microbacterium* sp. JYC17 showed ACC deaminase activity. Sgroy *et al*. (2009) isolated 29 endophytic plant growrh-promoting bacteria from roots of *Prosopis strombulifera* and six of which, *Bacillus subtilis*, *B. licheniformis*, *B. pumilus*, *Brevibacterium halotolerans*, *Pseudomonas putida* and *Achromobacter xylosoxidans* were positive for ACC deaminase activity. Tan *et al*. (2011) reported that ACC deaminase producing endophytic *Streptomyces* coated tomato seedlings showed significant resistant from bacterial wilt compared with the control seedlings. Xing *et al.* (2011) proposed a new species of endophytic *Kibdelosporangium phytohabitans* isolated from the root of the oil-seed plant *Jatropha curcas* L. collected from Sichuan Province, south-west China. *K. phytohabitans* had the ability to utilize ACC as sole source of nitrogen and possessed ACC deaminase enzyme.

For the structure of ACC deaminase gene (*acdS*) of actinomycetes, Hontzeas *et al*. (2005) designed degenerate primers Deg ACC5' (5'- GGBGGVAAY AARMYVMGSAAGCTYGA) and Deg ACC3' (5'- TTDCCHKYRTANACBGGRT C) based on stretches of conserved base pair towards the N terminus of the protein around the putative pyridoxal phosphate cofactor binding domain and a conserved region close to the carboxyl terminus of the protein. This allows for the amplification of a fragment of approximately 750 bp. ACC deaminase PCR products were positive for member of class Gammaproteobacteria (*Pseudomonas*, *Enterbacter* and *Serratia*), species of order Burkhoderiales (*Variovorax*, *Acidovorax* and *Achromobacter*), member of class Alphaproteobacteria (*Rhizobium*) and two strains of class Actinobacteria (*Rhodococcus* sp. Fp2 and *Rhodococcus* sp. 4N-4). The Maximum Likelihood tree was constructed using the partial ACC deaminase DNA sequences from 750 bp region amplified with degenerate primers. In the tree, the actinobacterial *Rhodococcus* ACC deaminase gene separated from all Gram negative bacteria. Glick *et al*. (2007) took 154 available putative bacterial ACC deaminase gene (*acdS*) sequences to construct phylogenetic tree. From the tree, six *acdS* groups were defined. The first group consisted of Gammaproteobacteria and one

Betaproteobacterium. Group II and III contained sequences from the Betaproteobacteria, as well as two Gammaproteobacteria. Group IV includes Alphaproteobacteria and a small group of Betaproteobacteria, Group V consisted entirely of Actinobacteria, and the final, group VI consisted of Betaproteobacteria and Gammaproteobacteria.

However, a limited number of works have been done to elucidate the abilities of endophytic actinomycetes especially the ability to stimulate plant growth through ACC that has been detected in a wide range of soil and rhizosphere bacteria. In addition, the ACC deaminase genes of endophytic actinomycetes have not yet been characterized and identified so far.

3.4 As a source of novel bioactive compounds

The principal reason behind the actinomycetes having such important roles in soil and in plant relationships comes from the ability of the actinomycetes to produce a large number of bioactive compounds which are secondary metabolites with a wide range of activities such as antibiotics, anti-tumors and hydrolytic enzymes (Nolan and Cross, 1988; Berdy, 2005). Nowadays, actinomycetes produce approximately two-thirds of the known antibiotics produced by all microorganisms (Butler *et al*., 2003). The genus *Streptomyces* produces nearly 80% of the actinobacterial antibiotics, with the genus *Micromonospora* producing one-tenth as many as the *Streptomyces* (Baltz, 1998). However, the development of resistance to multiple drugs is a major problem in the treatment of infections by pathogenic microorganisms. Focus of research on new bioactive compounds is necessary to combat disease. Therefore, screening for new bioactive compounds are currently oriented towards the isolation of new strains from uncommon habitats and as the result, endophytic actinomycetes is the new entity (Igarashi, 2004; Mehdi, *et al*., 2006). Since, Castillo *et al*. (2002) isolated antibiotic Munumbicin from endophytic *Streptomyces munumbicin* NRRL 30562 and then several novel bioactive compounds from endophytic actinomycetes have been found. For example, Igarashi *et al*. (2007) have reported two novel anthroquinones, lupinacidins A and B. These compounds

produced by novel endophytic actinomycete, *Micromonospora lupini* sp. nov. Lupinacidins were found to act as anti-tumor and showed significant inhibitory effects on the invasion of murine colon carcinoma cell without inhibiting cell growth.

Streptomyces aureofaciens CMKUAc130 was isolated from root tissue of Thai medicinal plant, *Zingiber officinale* Rosc. (Taechowisan and Lumyong, 2003) and revealed the production of coumarin secondary metabolites, 5, 7-dimethoxy-4-ρ-methylphenylcoumarinand 5, 7-dimethoxy-4-phenylcoumarin with antifungal activities (Taechowisan *et al.,* 2005); and 4-arylcoumarins with anti-tumor activity (Taechowisan *et al.,* 2007). Liu *et al*. (2007) isolated 165 actinomycetes from medicinal plants and showed that over 42% of the isolates exhibited antagonism against pathogenic strains, and 54.5% displayed excellent inhibition against mouse melanoma cell line B16 or/and human alveolar epithelial cell line A549. These results are superior to those of soil actinomycetes indicating tremendous potential of endophytic actinomycetes for exploration. Coromycin is a complex of novel peptide antibiotics that produced by *Streptomyces* sp. MSU-2110 isolated from *Monstera* sp. Coromycin is active against malarial parasite *Plasmodium fasciparum* and human fungi pathogen *Crytococcus neoformans* (Ezra *et al*., 2004). The list of novel bioactive compounds isolated from endophytic actinomycetes was shown in Table 4.

Table 4 Novel bioactive compounds isolated from endophytic actinomycetes.

Endophytes	Host	Bioactive compounds	Type	Activity
Streptomyces sp. NRRL 30562	*Kennedia nigriscans*	Munumbicins A-D	Peptides	Antibiotic
Streptomyces sp. MaB-QuH-8	*Maytenus aquifolia* Mart.	Celastramycins A and B	Heterocyclic compounds	Antimicrobial
Streptomyces sp. NRRL 30566	*Grevillea pteridifolia*	Kakadumycins	Peptides	Antibiotic
Streptomyces sp. CS	*Maytenus hookeri*	24-demethyl-bafilomycin C1	Macrolides	Antimicrobial, antitumor
Streptomyces sp. CS	*M. hookeri*	24-demethyl-bafilomycin A2	Macrolides	Antimicrobial, antitumor
Streptomyces sp. MSU-2110	*Monstera* sp.	Coronamycins	Peptides	Antibiotic
Streptomyces sp. TP-A0595	*Allium tuberosum*	6-Prenylindole	Alkaloids	Antifungal
Streptomyces sp. TP-A0556	*Aucuba japonica* Thunb	Demethylnovobiocins	Coumarins	Antimicrobial
Streptomyces sp. TP-A0456	*Cryptomeria japonica*	Cedarmycins A and B	Butyrolactones	Antifungal
Streptomyces sp.ls9131	*M. hookeri*	Dimeric dinactin and Dimeric nonactin	Macrotetrolide	Antimicrobial, antitumor

Table 4 (Continued)

Endophytes	Host	Bioactive compounds	Type	Activity
Streptomyces griseus	*Kandelia candel*	7-(4-aminophenyl)-2,4-dimethyl-7-oxohept-5-enoic acid; 9-(4-aminophenyl)-7-hydroxy-2,4,6-trimethyl-9-oxo-non-2-enoic acid; 12-(4-aminophenyl)-10-hydroxy-6-(1-hydroxyethyl)-7,9-dimethyl-12-oxo-dodeca-2,4-dienoic acid	p-Aminoaceto phenonic acids	Antimicrobial
Streptomyces sp.NRRL 30562	*Kennedia nigriscans*	Munumbicins E-4 and E-5	Peptides	Antibiotic
Streptomyces laceyi	*Ricinus communis* L.	Salaceyins A and B	6-Alkylsalicylic acids	Antitumor
Streptomyces hygroscopicus TP-A0451	*Pteridium aquilinum*	Pterocidin	δ-lactone	Antitumor
Streptomyces sp. CS	*Maytenus hookeri*	Naphthomycin K	Ansamycins	Antitumor
Streptomyces aureofaciens CMUAc 130	*Zingiber officinale* Rosc.	5,7-dimethoxy-4-p-methoxylphenylcoumarin; 5,7-dimethoxy-4-phenylcoumarin	Arylcoumarins	Antifungal, antitumor, antiinflammatory

Table 4 (Continued)

Endophytes	Host	Bioactive compounds	Type	Activity
Micromonospora lupini	*Lupinus angustifolius*	Lupinacidins A and B	Anthraquinones	Antitumor
Streptomyces sp.SUC1	*Ficus benjamina*	Lansai B and C	Phenols	Weakly anticancer antiinflammatory
Streptomyces sp. Hedaya 48	*Aplysina fistularis* (sponge)	Saadamycin	Heterocyclic compound	Antifungal
Streptomyces sp.CS	*M. hookeri*	24-demethyl-bafilomycin A1; 21-O-methyl-24-demethyl-bafilomycinA1; 19,21-di-O-methyl-24-demethylbafilomycin A1; 17,18-dehydro-19,21-di-O-methyl-24-demethyl-bafilomycin A1; 24-demethyl-bafilomycin D	Macrolides	Antitumor
Streptomyces albidoflavus	*Bruguiera gymnorrhiza*	Antimycin A18	Macrolides	Antifungal

Source: Qin *et al*. (2011)

Based on all aforesaid reports, it is convinced that endophytic actinomycetes are a promising novel source for discovery new taxa and bioactive compounds apart from that they are high potential PGPB.

MATERIALS AND METHODS

Materials

1. Plant samples

Appendix Table A1 displays a total of plant samples belonging to 23 families, 31 species (32 varieties) including 29 medicinal and 3 agricultural plant samples. The plant samples were collected from: (1) Biology Department, Faculty of Science, Burapha University, Chonburi province in November 2006; (2) Khaohinsorn Royal Development Study Center, Chachoengsao province in November 2006; (3) Pathum Thani Rice Research Center, Pathum Thani province in August 2007; and (4) personal garden in Chumphon province in October 2007.

2. Actinomycetes

2.1 Endophytic actinomycetes

A total of 66 endophytic isolates (Appendix Table A2) recovered from surface sterilized tissue of plants were used in this study which were designated GMKU (Genetic-Microbiology-Kasetsart-University) culture collection.

2.2 Type strains

The list of actinobacterial type strains which used in this study was shown in Table 5.

3. Test microorganisms

Gram-positive bacteria and Gram-negative bacteria as well as fungi including phytopathogenic bacteria and fungi which used in this study were shown in Table 6.

Table 5 Type strains of actinomycetes

Strains	Species	Sources	Accession number of 16S rRNA gene
GMKU 931^{T}	*Actinoallomurus acaciae*	Root of wattle tree, Bangkok, Thailand= BCC 28622^{T}= NRRL B-24610^{T}	EU429322
TT04-09 ^{T}	*A. coprocola*	Cow dung, Chiba, Japan= KCTC 19542^{T} = NBRC 103688^{T}	AB364579
TT02-47 ^{T}	*A. iriomotensis*	Meadow soil, Okinawa, Japan=KCTC 19539^{T} = NBRC 103685^{T}	AB364586
KCC A-0146 ^{T}	*A. spadix*	Soil, Yamanashi, Japan= ATCC 27298^{T}= NBRC 14099^{T}	AF163120
KA-605 ^{T}	*Actinokineospora fastidiosa*	Soil, Egypt=ATCC 31181^{T}= NBRC 14105^{T}	AJ400710
K99-5278 ^{T}	*Streptomyces albus*	Straw=ATCC 25426^{T}= NRRL-ISP 5313^{T}	AB184257
K01-B0171^{T}	*Rhodococcus jostii*	Soil, Yunnan, China= NBRC 16295 ^{T} = CCM 4760 = JCM 11615 ^{T}	AB204817

Table 6 Test microorganisms

Oganisms	Comments
Bacillus cereus ATCC 11778	Gram-positive bacteria
Staphylococcus aureus subsp. *aureus* ATCC 25923	Gram-positive bacteria
Escherichia coli ATCC 8739	Gram-negative bacteria
Xanthomonas campestris pv. *glycine* ATCC 43912	Phytopathogenic bacteria
Erwinia carotovora pv. *Carotovora* ATCC 495	Phytopathogenic bacteria
Ralstonia solanacearum ATCC BAA-1114	Phytopathogenic bacteria
Aspergillus niger	Fungus
Candida albicans	Fungus
Fusarium proliferatum DOAC 0842	Phytopathogenic fungi
Colletotrichum sp.	Phytopathogenic fungi

Methods

1. Isolation and identification

1.1 Isolation

The whole plant samples were used to isolate endophytic actinobacteria by using the surface-sterilization method modified from those of Coombs and Franco (2003) and Cao *et al*. (2004). Before isolation, the plant samples were washed with tap water to remove soil particles and kept at 4 °C for a week to decrease surface bacteria. The roots, stems and leaves were cut into small pieces and then surface-sterilized using serial treatments of 95% EtOH for 10 minutes, 1% NaClO for 15 minutes, sterile DW for three times and then immersed in 10% (w/v) $NaHCO_3$ solution for 10 minutes. After air drying, the surface sterilized plant materials were ground in mortar containing Ringer's solution. Those of ground materials and their solution were placed and spread, respectively onto SCA (Küster and Williams, 1964) and WA media supplemented with 2.5 U/ml penicillin G and 50 mg/ml cyclohexamide. The solution of the third washed DW was also spread on the medium to ensure that the surface plant materials were actually sterilized. Colonies of endophytic actinobacteria appeared on the media after incubation at 30 °C for 1-8 weeks. The colony was isolated and purified on MS (Hobbs *et al*., 1989). After purification, the pure isolates were grown on several media, namely ISP 2, ISP 3, and ISP 4 to determine culture characteristics. The duplicate plates of each isolates were incubated at 27 °C for 3 weeks and the growth on the medium, color (reverse plate) of substrate, soluble pigment and sporulation/aerial mycelium were determine.

To maintain the pure cultures, the endophytic actinomycetes were grown either on MS, ISP3, PDA or Oatmeal-nitrate agar medium and incubated at 27 °C until sporulation. The spores were scraped from the plate and placed in a 1.5 ml sterilized tube containing 1 ml of 20% glycerol. The spore suspension was maintained at -20 ºC or -80 ºC. For long term preservation, lyophilization was carried out. The spores were taken from the plate and placed in a 50 ml tube containing 5 ml

of 10% skim milk. The spore suspension was then divided into 5 sterilized ampoule tubes and freeze-dried.

1.2 Identification of endophytic actinomycetes by 16S ribosomal RNA gene sequencing

1.2.1 Extraction of total DNA (Kieser *et al*., 2000)

After growing of endophytic actinomycetes on MS medium at 30 °C, two loopfuls of mycelia and spore were scraped from the plate and placed in a 1.5 ml tube containing 500 µl of lysozyme solution. The mixture was incubated at 37 °C for 1 h and inverted every 10 min during incubation. The lysate was cooleded to room temperature and 250 µl of 2% SDS was added and the tube was immediately inverted for several times till the lysate was clear. 250 µl of phenol-chloroform (25 phenol: 24 chloroform: 1 isoamyl alcohol) was then added and the tube was vigorously mixed by vortex for 30 sec and centrifuged at 12,000 rpm for 10 min at RT. The upper phase was transferred to a fresh sterilized tube and 0.1 V of 3 M sodium acetate pH 4.8 and 1 V of isopropanol were then added and the tube was inverted before keeping at -20 °C for at least 5 min and centrifuged at 12,000 rpm for 2 min at RT to precipitate DNA. The supernatant was discarded and the DNA pellet was collected and washed twice with 200 µl of 70% EtOH and dried at RT with the tube lid open. The pellet was then re-suspended in 20-50 µl of TE buffer. The total DNA was stored at 4 °C or -20 °C for a long period preservation.

1.2.2 Amplification of 16S rRNA gene

Approximately 50 ng of the chromosomal DNA was used to amplify almost complete the 16S rRNA gene with specific primer, STR1F and STR1530R (Table 7). The PCR was performed with the following reagents: 1X PCR buffer, 3 mM $MgCl_2$, 0.5 mM dNTP mixture, 0.125 ρM each primer, 1.25 U *Taq* DNA polymerase, 10% DMSO in a total volume of 20 µl with sterilized ultra pure water. The PCR reactions were carried out in a Biometra Thermocycler by following

temperature cycling profile: 1st at 94 °C for 4 min, followed by 35 cycles at 94 °C for 1 min, 55 °C for 1 min, 72 °C for 1 min and final cycle at 72 °C for 4 min. The presence of the 1.5 Kb PCR product coding 16S rRNA gene was confirmed by 0.8% agarose gel electrophoresis. The PCR products were purified using QIAquick PCR Purification Kit (QIAGEN, Germany), NucleoSpin Extract 2 in 1 (MACHEREY-NAGEL, Germany) or Gel/PCR DNA Fragments Extraction kit (Geneaid) following the manufacturer's protocol. After that the purified PCR products of all isolates were partially sequenced with STR1F primer to obtain the 5′-end sequences of 16S rRNA gene at BSU BIOTEC, Thailand or Macrogen, Korea.

1.2.3 Sequence similarity analysis

To analyze the similarity values, the 16S rRNA sequences were preliminarily compared with nucleotide sequences in the GenBank and the EzTaxon database at www.ncbi.nih.gov/BLAST and www.eztaxon-e.org, respectively.

1.2.4 Phylogenetic tree analysis

For phylogenetic tree construction, CLUSTAL_X version 2 (Larkin, 2007) was used to multiple align of 16S rRNA sequences. MEGA 4 (Tamura *et al*., 2007) and PHYLIP 3.68 (Felsenstein, 1981) were used for analysis of phylogenetic tree. Phylogenetic relationship was performed using three algorithms, namely neighbour-joining (Saitou & Nei, 1987), maximum-parsimony (Eck & Dayhoff, 1996) and maximum-likelihood (Felsenstein, 1981) in the case of novel species NJplot 2.3 and MEGA 4 were used for drawing a phylogenetic tree. The resultant neighbour-joining tree topology was evaluated for the confidence values of branches by 1,000 resamplings of bootstrap analysis (Felsenstein, 1985). The evolutionary distances were computed by using the Kimura 2-parameter method (Kimura, 1980).

2. Characterization of novel taxa

2.1 Phenotypic methods

2.1.1 Morphological characteristics

2.1.1.1 Cultural characteristics

To determine characteristics of culture, the putative novel genus/species were grown on several media of Difco, namely ISP 2, ISP 3, ISP 4, ISP 5, NA, PDA, oatmeal-nitrate agar (JCM medium 52) and 1/10 YS. The duplicate plates of each strain were incubated at 27 °C for 3 weeks and the color of mycelium and soluble pigment was determined by comparing its color with the color chips from Color Harmony Manual (Jacobson *et al*., 1958).

2.1.1.2 Spore characteristics

The putative novel genus/species were examined the spore characteristics by using SEM. The strains were grown on several media, namely ISP 2, ISP 3, ISP 4, ISP 5, oatmeal-nitrate agar, 1/10 YS, NA, PDA and HV for 4-6 weeks at 27 °C. After observation of sporulation of each strain under light microscopy, the selected spore areas were cut into smaller pieces (0.5×0.5 cm). The agar block was placed on the glass slide which was laid down on the coil in Petri dish and then fixation and dehydration for the SEM were carried out. Fixation of spores was carried out by adding 2-4 drops of 4% OsO_4 in the Petri dish for 16 h. To air dry of the spores, the Petri dish was opened in the fume hood for 16 h after that each piece of drying spores was introduced into the chamber of the coater JEOL JFC-1200 and coated with gold for 1 min. The morphology of spore chain was observed using the SEM model JSM5600; JEOL.

For spore motility, after sporulation of the putative strain, spore suspensions were obtained by gentle scraping of the agar surface and mixing with 10 ml sterile tap water. The spore suspension was incubated at 27 °C for about 1 h. The suspension was shaken allowed to settle down. About 0.1 ml supernatant was taken with a sterile pasteur pipette, spread onto a clean slide and covered with a coverslip. The motility of spores was observed under a light microscope at ×100 and ×200 magnifications.

2.1.2 Physiological characteristics

2.1.2.1 Acid production from carbohydrates

To analyze acid production from carbohydrates, each putative strain was cultured on the basal inorganic nitrogen medium added with bromocresol purple indicator and 1% of each sugar. 12 sugar types were used including, adonitol, L-arabinose, dulcitol, erythritol, D-fructose, D-glucose, D-galactose, *myo*-inositol, lactose, D-mannitol, raffinose and L-rhamnose. After inoculation of the washed cells in duplicate slants, the cultures of these carbohydrate agars were incubated at 27 °C for 4 weeks and observed for acid production by yellow color change of the indicator.

2.1.2.2 Catalase and oxidase activities

Catalase and oxidase activities were determined with a 3% (v/v) H_2O_2 solution and 1 % N,N,N',N'-tetramethyl-1,4-phenylenediamine dihydrochloride (TMPD) solution, respectively. For catalase test, a few drops of 3% H_2O_2 were added into culture broth and culture agar of each strain and a positive result, which is the rapid evolution of O_2 evidenced by bubbling, was observed. To test oxidase activity, a sterile swab was used to obtain a small amount of each strain from a culture plate. One drop of 1 % TMPD was placed onto the culture on the swab. After that the reaction turned violet to purple color within 10 - 30 sec indicated positive reaction.

2.1.2.3 Degradation of starch

ISP 4 medium was used to analyze degradation of starch of each strain. A loop of the washed cells was inoculated on the center of the plate. After incubation at 27 °C, the duplicate plates of the cultures were flooded with Lugol's solution at 1, 2, 3 and 4 weeks respectively. After standing for 15 - 30 min, the clear zone around the strains was observed for positive strain.

2.1.2.4 Coagulation and peptonization of milk

A loop of the washed cells of each strain was inoculated in 10 % skim milk of duplicate tubes. Coagulation and peptonization were observed after incubating at 37 °C for 3 weeks.

2.1.2.5 Liquefaction of gelatin

A loop of the washed cells of each strain was inoculated in 20 % gelatin of duplicate slants. The strains were grown at 20 °C and 27 °C for 3 weeks after that liquefaction of gelatin, which changing from the solid substance to the liquid state, was observed for positive reaction.

2.1.2.6 NaCl tolerance

To clarify the NaCl tolerance, each test novel strain was cultured on the ISP 2 or ISP 3 medium with 1%, 2%, 3%, 4%, 5%, 6% and 7% NaCl. The strain was streaked once across a plate and the duplicate plates of each culture were incubated at 27 °C for 3 weeks.

2.1.2.7 Production of melanin pigment

ISP6 and ISP7 media were used to determine production of melanin pigment of each strain. The strains were cultured on ISP6 and ISP7 media and observed the production of melanin after incubating for 3 weeks at 27 °C.

2.1.2.8 Range of temperature for growth

To determine range of temperature for growth, ISP 2 or ISP 3 was used. Each test strain was grown on slants in a temperature gradient incubator (5-50 °C) for 3 weeks and then the optimum temperature and range of temperature for growth were determined.

2.1.2.9 Range of pH for growth

To determine optimal pH and range of pH for growth, the strains were cultured on ISP 2 or NA medium which was various pH values which were pH 4, pH 5, pH 6, pH 7, pH 8, pH 9 and pH 10. The pH value of medium was adjusted after autoclaving. Each culture was done in duplicate plate and streaked once across a plate after that incubated at 27 °C for 3 weeks.

2.1.2.10 Reduction of nitrate

ISP 8 broth was used to study reduction of nitrate. A loop of washed cells was incubated in the ISP8 medium and duplicate of each strain were grown at 27 °C, 250 rpm for 1-4 weeks. The cultures were observed N_2 gas and were added 6-8 drops of nitrite reagent A and B, respectively and then a bit of zinc power was added into the tubes. The observation of pink color indicated positive strain.

2.1.2.11 Utilization of carbon sources

Utilization of carbohydrate as sole carbon sources was tested using ISP 9 or ISP 4 media (without soluble starch) that contained 1 % of a sugar. A loop of each washed cells was streaked once across a plate and the cultures of these carbohydrate agars were incubated at 27 °C for 4 weeks and observed the growth. Sixteen sugars, L-arabinose, dulcitol, D-fructose, D-glucose, D-galactose, *myo*-inositol, lactose, maltose, D-mannitol, D-mannose, raffinose, L-rhamnose, D-sorbitol, sucrose, D-trehalose and D-xylose, were used in this study.

For utilization of benzoate and citrate, modifications of Koser's citrate and benzoate agar were used. The strain was inoculated in the duplicate slant media and incubated at 27 °C for 4 weeks. The observation of alkaline color of phenol red indicated positive strain.

2.1.2.12 Utilization of nitrogen sources

Utilization of nitrogen sources was carried by using 5% casein medium, 5% xanthine-NA and 5% hypoxanthine-NA. Each strain was streaked once across a plate. The duplicate plates were incubated at 27 °C for 4 weeks. To observe utilization of casein, clearing of casein was appeared around the growth. For utilization of xanthine and hypoxanthine, disappearance of the crystals was observed around the growth.

2.1.3 Chemical characteristics

2.1.3.1 Cell wall peptidoglycan

Freeze-dried cells were prepared by growing cell-mass of the strain in appropriate media and the cell-mass were harvested by centrifugation at 3,000 g for 15 min. The resultant pellets were washed twice with sterile distilled water and then was freeze-dried. For dried cells, the washed pellets were immersed in 70% EtOH at 4 °C for 16 h and then dried at 50 °C for 16 h. The freeze-dried and dried cells were kept at -20 °C before using.

To determine acyl type of muramic acid, the method of Uchida & Aida (1984) was carried out. Freeze dried cells (10 mg) of the strain were hydrolyzed with 6N HCl in a small grass tube at 100 °C for 3 h. After cooleding to room temperature, 0.1 ml sterile DW and 2 ml saturated diethyl ether were added in the tube and the mixtures were strongly mixed by vortex for 1 min and centrifuged at 2,000 rpm at 4 °C for 5 min. Next, the lower phase of water layer was transfer to clean test tube and 2 ml saturated diethyl ether was added. The mixtures were strongly mixed by vortex for 1 min and centrifuged at 3,000 rpm at 4 °C for 10 min. The upper organic layer (1) was kept for color detection and the lower phase was continuously carried out by repeating the diethyl ether extraction for 2 times to colect the upper organic layer (2) and (3). Each tube of the upper organic layer (1), (2) and (3) was mixed with 10µl of 1N NaOH and then evaporated to dryness on a heating bath at 50 °C under a stream of nitrogen. To detect the color, each dried sample was dissolved in 2 ml of 0.02% 2,7-dihydroxynaphthalene-H_2SO_4 and kept at 100 °C for 10 min. As the standard, freeze dried cells of *Rhodococus jostii* K01-B0171 and *Streptomyces albus* K99-5278 were also prepared as aforesaid the method. The sample color was compared with the standard strain *R. jostii* K01-B0171 which show reddish purple indicating glycosyl type while *S. albus* K99-5278 show colorless indicating acetyl type.

To determine the isomer of DAP in the cell wall, the strains were analyzed according to the method of Hasegawa *et al.* (1983). Dried cells (10 mg) were hydrolyzed with 1 ml 6N HCl in a small grass tube at 100 °C for 18 h. After cooleding, the hydolysate is filtered using absorbent cotton to another clean small glass tube and 1 ml of sterile DW was added to the filter. The combined filtrate was concentrated to dryness by a rotary evaporator at 40 °C. The dried material was dissolved in 1 ml of water and dried again. After that, the residue was dissolved in 0.3 ml of sterile DW and 5 µl was applied on the base line of cellulose TLC plate (Merck No. 5716, 20 cm × 20 cm). A standard for 3-5 µl 0.01 M DL-DAP (sigma Chemical Co. St Louis,Mo.,USA) was used. TLC was developed with the solvent system methanol: water: 6N HCL: pyridine (80: 26: 4: 10, v/v) (freshly prepared). Development was taken about 16 h. The TLC plate was then dried for about 30 min-1 h in fume hood and then development was repeat again about 4-5 h in the same solvent system. The spots were visualized by spraying with 0.2% ninhydrin solution followed by heating at 100 °C about 5-15 min. in a dryer incubator.

To isolate and purify the composition of amino acids of peptidoglycan, the method of Schleifer & Kandler (1972) was carried out. Freeze dried cells (500 mg) were suspended in 6 ml of 0.05 M phosphate buffer (pH 7.2) and then homogenized with Sonicator Ultrasonic Processor for 45 min on ice. The homogenate was added with 6 ml of the phosphate buffer and centrifuged at 3,000 rpm for 15 min. The upper layer was transferred to clean test tube. Cell wall was then harvested by centrifugation at 9,000 rpm for 1 h. After centrifugation the resultant pellets consisted of a dark lower portion and a white top layer. The white layer was removed to clean test tube and suspended in 2 ml of 0.05 M phosphate buffer (pH 7.2) and the suspension were vigorously mixed by vortex for 30 sec. 450 µl of 25% SDS was added to the suspension and incubated at 100 °C for 40 min and followed by three times washing with 5 ml of warm 0.05 M phosphate buffer (pH 7.2) and centrifugation at 9,000 rpm at 30 °C for 1 h. After the third wash, 2 ml of 0.05 M phosphate buffer pH 7.2 and 100 µl of 1 mg/ml Pronease E were added to the pellet separated the soluble polysaccharide from the peptidoglycan. The mixture was incubated at 37 °C for 2 h. After that, the cell walls were washed and harvested by

centrifugation at 9,000 rpm for 1 h. After centrifugation the resultant pellets consisted of a dark lower portion and a white top layer. The white layer was removed to clean test tube and suspended in 2 ml of 0.05 M phosphate buffer (pH 7.2). Next, extraction of peptidoglycan with TCA was carried out. The resultant pellets were suspended in 5% (w/v) TCA and vigorously mixed by vortex for 30 sec. The mixture was incubated at 100 °C for 20 min. After that, the cell walls were washed and harvested by centrifugation at 9,000 rpm for 1 h. After centrifugation the resultant pellets consisted of a dark lower portion and a white top layer. The white layer was removed to clean test tube and suspended in 2 ml of 0.05 M phosphate buffer (pH 7.2) at least 3 times. To purify peptidoglycan, TCA was removed by extraction with absolute EtOH and diethyl ether (DEE). 3 ml absolute EtOH was added and mixed by vortex and then centrifuged at 9,000 rpm at 30 °C for 1 h and followed by adding 3 ml DEE and mixed by vortex. The mixture was centrifuged at 9,000 rpm at 30 °C for 1 h. The purified peptidoglycan was dried and kept at -20 °C until use.

The purified sample was hydrolysed (Becker *et al*., 1965) and determined by TLC (Hasegawa *et al.* 1983). The dried sample (1 mg) were hydrolyzed with 1 ml 6N HCl in a small grass tube at 100 °C for 16 h. After cooleding, the hydolysate is concentrated to dryness by a rotary evaporator at 40 °C. The dried material was dissolved in 300 µl of water and dried again. After that, the residue was dissolved in 300 µl of sterile DW and used as sample. 5 µl of the sample was applied on the base line of cellulose TLC plate (Merck No. 5716, 20 cm × 20 cm). 1 µl of amino acid standards (Sigma) was also applied. TLC was developed with the solvent system n-buthanol: acetic acid: water (4: 1: 2, v/v) (prepare fresh). Development was taken about 10 and 16 h. After development, the TLC plate was dried for about 30 min-1 h in fume hood and then development was replete again about 4-5 h in the the solvent system methanol: water: 10N HCL-pyridine (34: 12: 1: 4, v/v) (freshly prepared). The spots were visualized by spraying with 0.2% ninhydrin solution followed by heating at 100 °C about 5-15 min. in a dryer incubator.

Analysis of amino acid in peptidoglycan by using Pico Tag HPLC was carried out. The dried sample and standards were resuspended in 100 µl of PicoTag solution and then 1 µl of sample was injected into a PicoTag HPLC.

2.1.3.2 Cellular fatty acid composition

The cellular fatty acid composition was analyzed by TechnoSuruga Co., Ltd Japan according to the instructions of the MIDI System by using a gas chromatograph (model HP6890; Hewlett Packard) (Sasser, 1990).

2.1.3.3 Menaquinones

Menaquinones were extracted and purified by the method of Collins *et al*. (1977). Freeze dried biomass (300 mg) was placed in a flask added with 21 ml of chloroform-methanol (2: 1) and the flask was wrapped with aluminum foil. The contents were gently mixed on magnetic stirrer for overnight. The mixtures were filtered using Whatman filter paper No. 2 and the combined filtrate was concentrated to dryness by a rotary evaporator on a bath below 35 °C under a stream of nitrogen. The dried material was dissolved in 6 ml of acetone and dried again. After that, the extract containing the isoprenoid quinines was dissolved in 2 ml of acetone and used as sample. 2 ml of the sample were applied as 1-2 cm bands on the base line of silica gel plate (Merck, 20 PLC plates 20 × 20, silica gel 60 F254, 0.5 mm thickness). A standard 3-5 µl of vitamin K solution was co-migrated to help identify the position of extracted menaquinones. TLC was developed with the 100% benzene. Development was taken about 1 h. After development, the TLC plate was dried for 5 min in fume hood and then the single band containing the menaquinones was visualized and located under UV at 254 nm and mark the band with pencil. The band was scraped from the plate and deposited in a glass tube containing 2 ml of acetone to extract the menaquinones. The preparations were mixed thoroughly and centrifuged at 3,000 rpm for 15 min. The supernatants were transferred to a small vial, dried on a bath below 35 °C under a stream of nitrogen. The dried menaquinones were resuspended in 2 ml acetone and placed in a round-bottom flask filtered with

disposable syringe filter units - hydrophilic PTFE membrane filter (0.5 μm). The purified menaquinones were dried on a bath below 35 °C under a stream of nitrogen and kept at -20 °C until use.

Analysis of isoprenoid quinones by using HPLC was carried out. The purified menaquinones were resuspended in 100 μl of acetone and injected into HPLC using a JASCO 802-SC chromatograph equipped with a Shiseido CAPCELL PAK C18 column. Methanol-isopropanol (70:30, v/v) was used as a mobile phase and the samples were detected at 270 nm. Retention times and peak areas were determined using an integrator (Hitachi D2500 Chromato-integrator).

2.1.3.4 Polar phospholipid

Phospholipids were extracted and determined by using the method of Minnikin *et al*. (1984). Freeze dried cells (20 mg) were placed in a glass tube containing 2.5 ml DW. The lipids were extracted with a mixture of 2.5 ml chloroform and 1.25 ml methanol (2: 1, v/v) in a small grass tube and were roughly mixed by vortex and then centrifuged at 3,000 rpm for 5 min. The lower layer was transferred to a new glass tube and extracted again. The resultant lipids were evaporated to dryness on a heating bath at 50 °C under a stream of nitrogen. Phospholipids were separated on TLC of 10 × 10 cm silica gel plates (Merck, 20 PLC plates 20 × 20, silica gel 60 F254, 0.5 mm thickness) with a solvent system of chloroform: methanol: DW (10: 5: 1, v/v). 5 μg/ml of PE, PC, PG, PI and DPG solutions were used as standards. For visualization of amines, phosphorylated molecules, glycolipids and sugar molecules each TLC plate was sprayed with ninhydrin, phosphorus, 50% H_2SO_4 and anisaldehydrate, respectively.

2.1.3.5 Whole-cell sugars

Whole-cell sugars were analyzed according to the method of Becker *et al.* (1965). 50 mg of dried cells were hydrolyzed with 1 ml of 1N H_2SO_4 in a screw grass tube which covered with teffon and cap, incubated at 100 ˚C for 2 h. The hydrolysate were allow to cooled at room temperature and saturated $Ba(OH)_2$ was added to get pH to 5-7 (detected by pH paper). The supernatant was harvested by centrifugation at 3,000 rpm for 15-20 min. Methanol: chloroform (4:2 ml) was added and then tube was strongly vortexed. Centrifugation at 3,000 rpm for 15-20 min was conducted and the upper supernatant was keet at 4˚C over night. The centrifugation 3,000 rpm for 15-20 minutes was repeated and the supernatant was harvested and dried by a rotary vacuum evaporator. The remainder was resuspended with 200 µl sterile DW and use as the sample. 40 µl of sample was applied on base line of paper chromatography size 10×40 cm. 20 µl of 2 mg/ml standard sugars were used as standards. Paper chromatography was developed with the solvent system n-butanol : toluene: pyridine: water (20:16:12:12, v/v and use the upper layer of the mixture about 25 ml) (prepare with in 24 h before use) for 51 h. After development, the paper was dried for about 30 min in fume hood. The spots are visualized by spraying with aniline-butanol-phthelic acid solution followed by heating at 100 ˚C in a dryer incubator a few minutes.

2.1.3.6 Mycolic acid

Mycolic acids were detected by TLC using the method of Tomiyasu (1982). The lipids were extracted three times with a mixture of chloroform and methanol (2:1, v/v). The fatty acids were obtained by hydrolysis with 10% KOH in methanol of the extractable lipids and the residual cell wall-bound lipids. After acidification with 6 N HCI, fatty acids were extracted with n-hexane and transmethylated with benzene: methanol: H_2SO_4 (10:20:1, v/v) for 1.5 h under reflux. The resultant methyl esters were extracted with n-hexane and separated on a thin-layer plate of Silica Gel G (Merck) with a solvent system of n-hexane: diethyl ether (4:1, v/v). After the mycolic acid esters were eluted from the thin-layer plate with

chloroform, they were further separated on 10% silver nitrate-impregnated silica gel according to the number of double bonds. The saturated monoenoic, dienoic, trienoic, and tetraenoic mycolic acid esters were well separated with a solvent system of chloroform: methanol (80:1.5, v/v).

2.2 Genotypic methods

2.2.1 DNA base composition

2.2.1.1 Extraction and purification of total genomic DNA

Genomic DNA was isolated and purified by using the method modified from Saito and Miura (1963). 0.1 g of freeze-dried cells were ground in the mortar and placed in a 50 ml tube containing 5 ml saline-EDTA. A small spatula of lysozyme, a microspatula of achromopeptidase and 30 µl of N-acetylmuramidase (1mg/100 µl) were added in the mixture and vigorously mixed by vortex. The contents of the tube were left in the water-bath shaker at 37 °C for overnight. After that 500 µl of 20% SDS were added and further incubated at 60 °C for 2 h and allowed to cool down at room temperature. Equal volume of Tris-phenol was added and the lysate was strongly mixed by vortex for 30 second and centrifuged at 10,000 rpm at 4 °C for 15 min. The supernatant was transferred to a 50 ml of fresh sterilized tube followed by addition of 0.025 volumn of RNase A and RNase T solution. The mixture was incubated in the water-bath shaker at 37 °C for overnight and was extracted by the equal volume of Tris-phenol. The supernatant was removed after centrifuge to a 50 ml of new sterilized tube followed by addition of 0.1 volumn of 3 M sodium acetate pH 5.2 and the content in the tube was thoroughly mixed. Equal volume of absolute EtOH was added and incubated a few minute on ice. Gently swirled the tube and DNA was pool by sterilized glass rod. DNA was washed with 70% EtOH 3 times, dried in the air for 1 h and dissolved in 15 ml tube containing 4 ml of 0.1X SSC at 4 °C for 16 h.

Purity of DNA was measured by OD_{260}/OD_{280} ratio. The concentration of DNA was calculated by following equation:

50 x OD_{260} of the sample =concentration of DNA (µg/ml)

2.2.1.2 G+C content analysis

G+C content was determined by HPLC of deoxyribonucleosides as described by Tamaoka and Komagata (1984). 300 µg of purified DNA was incubated at 100 °C for 10 min and allowed to cool down on ice. 10 µl the denature DNA was transfered to the new tube and 10 µl nuclease P1 was added and incubated at 50 °C for 1 h. After that 10 µl bacterial alkaline phosphatase was added. As a quantitative standard, the mixture of 5 µl dNMP was also digested with bacterial alkaline phosphatase. Both sample and standard were incubated at 37 °C for 1 h. 5 µl of sample and standard were injected into a HPLC using a JASCO 802-SC chromatograph equipped with a GC column. Ammonium phosphate: acetonitrile (40: 1, v/v) was used as the mobile phase and the samples were detected at 270 nm. Retention times and peak areas were determined using an integrator (Hitachi D2500 Chromato-integrator).

2.2.2 Analysis of almost complete sequencing of 16S rRNA gene

2.2.2.1 Extraction and purification of genomic DNA

Genomic DNA was extracted and purified by method modified from Saito and Miura's (1963) as described in 2.2.1.1

2.2.2.2 Amplification

Approximately 50 ng of the genomic DNA was used to amplify the 16S rRNA gene with 11F sense primer and 1510R anti-sense primer (Table 7). The PCR was performed with the following reagents: 1X PCR buffer, 2.5 µM dNTP mixture, 5µM each primers, 1.25 U *Taq* DNA polymerase in a total volume of 50 µl with ddH_2O. The reaction mixtures were carried out in a TaKaRa PCR Thermal Cycler by following temperature cycling profile: the first cycle at 94 °C for 1 min, followed by 30 cycles at 94 °C for 1 min, 50 °C for 1 min, 72 °C for 1 min and the final cycle at 72 °C for 1 min. The presence of the 1.5 Kb PCR products coding for 16S rRNA gene was detected using 0.8% agarose gel electrophoresis.

2.2.2.3 16S rRNA gene sequencing

PCR products of 16S rRNA gene were purified using QIAquick PCR Purification Kit (QIAGEN, Germany) before sequencing. The sequencing was performed by using a BigDye terminator cycle sequencing kit (Applied Biosystems) with eight sequencing primers (11F, 520F, 685F, 926F, 704R, 925R, 1115R, 1510R; Table 7) on ABI model 3130 automatic DNA sequencer (Applied Biosystems). The first step was to remove excess dNTPs and primers, 5 µl of purified PCR products were added in the mixture of 0.65 µl ddH_2O, 0.2 µl of 10X *Exo I* buffer, 1 µl of SAP (1U/ µl) and 0.25 µl of *Exo I* (20U/ µl) and then incubated at 37 °C for 15 min followed by 80°C for 15 min. The second step, the PCR products were purified using a QIAquick Gel Extraction kit (QIAGEN, Germany). In the third step, 10 µl reaction mixtures (0.5 µl of the purified PCR product, 3.5 µl of BigDye Terminator Ready Reaction Kit (ABI), 0.5 µl of single primer (10 µM) and 5.5 µl of ddH_2O) were placed in TaKaRa thermal cycler using temperature cycling profile: the first cycle at 96 °C for 10 sec, followed by 25 cycles at 50 °C for 5 sec, 60 °C for 41 sec.

The PCR products were purified using a QIAquick Gel Extraction kit (QIAGEN, Germany). The PCR products were then dried for 2 h. The pellets were dissolved in 15 µl of Hi-Di™ formamide and transfered to 96 well plates and heated plate at 95 °C for 2 min and then immediately tranfered on ice. The PCR products were directly sequenced by an ABI model 3130 automatic DNA sequencer (Applied Biosystems).

Table 7 Oligonucleotide primers used for PCR amplification and sequencing of 16S rRNA gene.

Primers	Nucleotide sequences (5′ to 3′)	Size (bp)	References
STR1F	TCACGGAGAGTTTGATCCTG	20	Kataoka *et al.* (1997)
STR1530R	AAGGAGATCCAGCCGCA	18	Kataoka *et al.* (1997)
ATT025F	GATTAGATACCCTGGTAGTCCA	22	Bunyoo (2008)
ATT026R	TGGACTACCAGGGTATCTAATC	22	Bunyoo (2008)
11F	AGTTTGATCATGGCTCAG	18	Tajima *et al.* (2001)
520F	CAGCAGCCGCGGTAATAC	18	Tajima *et al.* (2001)
685F	GTAGCGGTGAAATGCGTA	18	Tajima *et al.* (2001)
926F	AAACTCAAAGGAATTGACGG	20	Tajima *et al.* (2001)
704R	TCTACGCATTTCACCGCTAC	20	Tajima *et al.* (2001)
925R	CGTCAATTCATTTGAGTT	18	Tajima *et al.* (2001)
1115R	AGGGTTGCGCTCGTTG	16	Tajima *et al.* (2001)
1510R	GGTTACCTTGTTACGACT	18	Tajima *et al.* (2001)

2.2.2.4 Analysis of sequence similarity

The DNA fragments were assembed by using CAP3 Sequence Assembly Program at http://pbil.univ-lyon1.fr/cap3.php. The similarity values were compared to the nucleotide sequences in the EzTaxon database.

2.2.2.5 Phylogenetic tree analysis

Phylogenetic trees were constructed by using the method that described in 1.2.4.

2.2.3 DNA - DNA hybridization

DNA was isolated and purified according to the method of Saito and Miura (1963) as described in 2.2.1.1. DDH experiment was carried out by fluorometric hybridization method (Ezaki *et al.*, 1989). The purified DNA solution (100 µg/ml DNA in 0.1xSSC buffer) was incubated at 100 °C for 10 min and immediately cooled on ice. Calf thymus DNA was also paralelly prepared control DNA. Next, 100 µl of the denatured DNA (10 ng/ml DNA in PBS/$MgCl_2$) were loaded on each well of the 96 well black polystyrene microplates and sealed with self-adhesive vinyl tape before incubation at 37 °C for overnight. The plates were then washed once with 250 µl PBS per well with the aid of a multichannel pipette. A pre-hybridization step was performed by adding 200 µl pre-hybridization solution (2x SSC, 05x Denhardt's solution, 50% formamide, 0.1 mg/ml denatured salmon sperm DNA) per well, sealing the microplate with vinyl tape and incubated at 37 °C for 30 min in the hybridization oven.

For the DNA probe, the DNA sample was fragmented with sonicator for 2 min. DNA probe was then labelled by mixing 10 µg/ml DNA in 0.1 SSC with 3 volumn of photobiotin solution (Photoprobe Biotin Kit, Vector Laboratories, Canada) in a 1.5 ml tube and illuminated the mixture for 30 min under a 300 W mercury vapor lamp positioned 10 cm above the tube while the open tube was kept upright on ice. After coupling, brough the volume of labeling reaction to 144 µl with distilled water and 16 µl of 0.1 M Tris/HCl (pH 9.0) was added. The remaining free photobiotin was removed by adding 160 µl 1-butanol to the nucleic acid solution, vigorously vortexed and centrifuged 1,000 g for 1 min to separate the phases. Discarded the upper butanol phase and repeat the butanol extraction. The water layer phase was then boiled for 10 min and immediately cooleded on ice.

For the actual hybridization, the pre-hybridization solution was removed and 100 µl hybridization solution (pre-hybridization solution plus 2.5 % dextran sulfate and 10 µg/ml DNA probe) was added per well. The microplates were sealed again with vinyl tape and incubated at 54 °C for 3 h. The microplate was then washed three times with 200 µl 1x SSC per well. For the enzymic development, 100 µl streptavidin-β-D-galactosidase solution was added per well (0.5 U/ml in PBS plus 0.5 % BSA and 0.01 % Triton) and the microplate was covered with vinyl tape and incubated at 37 °C for 30 min. Subsequently, the plate was washed three times with 250 µl 1x SSC per well. Finally, the substrate for β-D-galactosidase, 100 µl of 4-methylumbelliferyl-β-D-galactopyranoside (Sigma) 0 .1 mg/ml was added per well. Percentage of hybridization will be detected by using Cytofluor multi-well plate reader series 4000 and by manual calculation.

3. Determination of plant growth promoting agents

3.1 Auxin production

Auxin production was analyzed by growing each isolate in glucose-beef extract supplemented with 0.2 mg/ml tryptophan. The flasks were incubated at 28 ºC, 250 rpm in the dark for 1 week. After incubation, colorimetric assay using method modified from Ahmad *et al*. (2006) was carried. 4 ml of Salkowski's reagent was added into 1 ml of the supernatant and vigorously mixed. The mixture was incubated in the dark at room temperature for 20 min and then measure OD 530 nm.

3.2 Phosphate solubilization

The isolates were streaked on the Pikovskaya's agar (Pikovskaya, 1948) and incubated at 30 ºC. After good growth, the lawn of colony was plugged that had already plugged out (6 mm diameter) and transferred into the hole (6 mm diameter) of the Pikovskaya's agar. The solubilization of phosphate was determined by the formation of transparent "halo" zone around each bacterial plug after incubation at 30 ºC for 3 weeks.

3.3 Siderophore production

Siderophores were detected using method modified from Schwyn *et al*. (1987). The isolates were streaked onto the MS medium and incubated at 30 ºC. After good growth the lawn of colony was plugged (6 mm diameter) and transferred onto CAS agar plate and incubated at 30 ºC for a week. The orange halo zone around bacterial plug indicated siderophores production.

3.4 Ammonia production

Ammonia production was studied by growing the isolates in peptone water broth and incubation at 30 ºC, 250 rpm for 1-3 weeks. After incubation, 200 µl of the supernatant were loaded per well on the 96 well microplates and then 20 µl of Nesler's reagent was added. The production of ammonia was observed by development of yellow to brown color.

3.5 ACC deaminase production

All isolates were screened for their potential to produce ACC deaminase. Isolates were grown in seed media and incubation at 28 ºC, 250 rpm for 4-7 days. After good growth, the cells were inoculated in the 5 ml of minimum media (Hopwood, 1967) supplemented with either 3.0 mM ACC or 2 g $(NH_4)_2SO_4$ per liter as a sole nitrogen source (Tarabily, 2008) and incubation at 28 ºC, 250 rpm for 4 weeks. The cultures were harvested and the pellets washed twice with sterilized DW. 50 µg of the washed cells were then re-inoculated and incubated in the same experimental condition. New colonies formed in the media supplemented with ACC were obseved as positive for ACC deaminase activity.

4. Identification of *acdS* (ACC deaminase) gene

4.1 Extraction of genomic DNA

Genomic DNA was extracted and purified by using the method of Kieser *et al*. (2000) that described in 1.2.1

4.2 Degenerate primer design

To identify *acdS* gene of endophytic actinomycetes, amino acid sequences of putative ACC deaminases i.e. *Actinosynnema mirum* DSM 43827^T (accession number YP003099210), *Brevibacterium linens* BL2 (accession number ZP05915229), *Kribbella flavida* DSM 17836^T (accession number YP003380387), *Mycobacterium abscessus* ATCC 19977^T (accession number YP001702443), *M. smegmatisstr* MC 2155 (accession number YP006571279), *Nakamurella multipartita* DSM 44233^T (accession number YP_003202162), *Streptomyces sviceus* ATCC 29083^T (accession number ZP_06916233), *S. ghanaensis* ATCC 14672^T (accession number ZP06579762), *S. griseus* NBRC 13350^T (accession number YP001824466), *S. albus* J1074 (accession number ZP06590640) and *Saccharopolyspora erythraea* NRRL 2338^T (accession number YP001104984) were retrived from the GenBank database. Multiple sequence alignment was conducted with ClustalW2 (http://www. ebi.ac.uk/ Tools/msa/clustalw2/). The forward and reverse primers based on conserved amino acid regions with minimal degeneracy were designed for PCR amplification of *acdS* gene.

4.3 PCR amplification

50 ng of genomic DNA of each isolate was used to amplify *acdS* gene with ATT082F sense primer and ATT082R anti-sense primer. The PCR was performed with the following reagents: 1X PCR buffer, 3 mM $MgCl_2$, 0.5 mM dNTP mixture, 0.125 ρM each primer, 1.25 U *Taq* DNA polymerase, 10% DMSO to a total volume of 20 µl with sterilized ultra pure water. The reaction mixtures were carried

out in a Biometra Thermocycler by following temperature cycling profile: the first cycle at 94 °C for 3 min, followed by 30 cycles at 94 °C for 30 sec, 63 °C for 30 sec, 72 °C for 1.5 min and the last cycle at 72 °C for 4 min. The presence of the 600 bp long DNA fragment coding for *acdS* gene confirmed by 0.8% agarose gel electrophoresis. PCR products were purified using QIAquick PCR Purification Kit (QIAGEN, Germany), NucleoSpin Extract 2 in 1 (MACHEREY-NAGEL, Germany) or Gel/PCR DNA Fragments Extraction kit (Geneaid) following the manufacturer's protocol. After that the purified PCR products of the isolates were directly sequenced using ATT082F primer (Macrogen, Korea).

4.4 Sequence analysis

The analysis of similarity values was compared nucleotide sequences in the GenBank database.

4.5 Phylogenetic tree analysis

Phylogenetic trees were constructed by using the method that described in 1.2.4.

5. Screening of anti-microbial activity

5.1 Antibacterial activities

Antibacterial activities were determined using agar plug assay method (Jeffry, 1994). The isolates were grown on MS agar for a week and then were plugged using 6 mm cork borer and placed on NA medium that was flooded with 10^8 cfu/ml of test bacterial suspension. 1 mg of ampicillin on a paper disk was used as a positive control. All plates were incubated at 30 °C overnight. Antibacterial activities of the isolates were observed by clear halo zone (inhibition zone) around the agar plugs. The inhibition zone was measured after 24 h.

5.2 Antifungal activities

To test ability of individual isolate to inhibit the growth of fungi, the modified protocol of Crawford *et al*. (1993) was carried out. The isolate was cultured in ISP2 media and incubation at 28 ºC, 250 rpm for 4-7 days. After good growth, the cells were inoculated on NA and PDA plates and incubated at 30 °C for a week or until the culture had sporulated. A PDA block (0.5 cm^2) containing actively growing test and fungi was then aseptically placed in the center of the plate. Incubation was continued at 30 °C. Antifungal activities were indicated when mycelia growth in the direction of the isolates were retarded or prevented. For determination of antifungal activity against *C. albicans*, the protocol that descried in 5.1 was used.

6. Identification of a novel compound

6.1 Fermentation

The selected strain of endophytic actinomycetes was cultured on Bn-2 slant agar medium and then was inoculated into 500-mL flasks each containing 100 ml of the V-22 seed medium (pH 7.0). The cultures were cultivated on a rotary shaker (200 rpm) at 30 °C for 4 days. The seed culture (3 ml) was transferred into 500 ml flasks each containing 100 ml of the A-11M production medium (pH 7.0) The flasks were inoculated on a rotary shaker (200 rpm) at 30 °C for 6 days.

6.2 Extraction and isolation

At the end of the fermentation period, 100 ml of 1-butanol were added to each flask, and they were allowed to shake for 1 additional hour. The mixture was centrifuged at 6,000 rpm for 10 min and the organic layer was separated from the aqueous layer containing the mycelium. Evaporation of the organic layer gave crude extract. The crude extract was subjected to silica gel column chromatography with a step gradient of $CHCl_3$-MeOH (1: 0, 20: 1, 10: 1, 4: 1, 2: 1, 1: 1 and 0: 1 v/v). Fraction were collected and evaporated and further purified by preparative C-18

HPLC using a gradient of MeCN/0.1% HCO_2H (MeCN concentration: 15 to 40% over 60 min). Finally, fraction that contained the putative peak was evaporated and concentrated.

6.3 Identification of the putative novel compound

The pure fraction was subjected to the spectroscopic analyses. Optical rotations were measured using a JASCO DIP-3000 polarimeter. UV spectra were recorded on a Hitachi U-3210 spectrophotometer. IR spectra were measured on a Perkin-Elmer Spectrum 100. NMR spectra were obtained on a Bruker AVANCE 400 or a Bruker AVANCE 500 spectrometer and referenced to residual solvent signals. J-resolved HMBC experiments were performed on a Varian INOVA-500 spectrometer. HRESITOFMS were recorded on a Bruker microTOF focus.

RESULTS AND DISCUSSION

1. Isolation and identification of endophytic actinomycetes

1.1 Isolation

A total of 66 endophytic actinobacterial isolates were obtained. Thirty one species (32 varieties) of healthy Thai medicinal and agricultural plants belonging to 24 families were used. The majority of plants (22 species, 69%) are woody plants and the rests are herbaceous plants. The plant materials including roots, stems and leaves were surface-sterilized by using the method modified from Coombs and Franco (2003) and Cao *et al*. (2004). The pieces of those and crushed solution were distributed and spread onto starch casein agar (SCA) and water agar (WA) media.

Surface sterilization is an important step to eliminate all epiphytic microbes and to gain true endophytic microbes. In this study, the surface sterilization was carried out by using three-step procedure. The plants were washed in 95% ethanol for 10 min, sterilized with 1% NaClO for 15 min and immersed in 10% $NaHCO_3$ solution for 10 min (to inhibit the growth of endophytic fungi) (Tan *et al*., 2006; Nimnoi *et al*., 2010). In addition, this alkaline environment favors the growth of actinomycetes especially *Streptomyces* (Verma *et al*., 2009). Ethanol (70-99%) and NaClO (0.87-10%) are commonly used in the sterilization protocol which made it possible to isolate the actinomycetes associated with plant tissues (Coombs and Franco, 2003; Cao *et al*., 2004; Tian *et al*., 2004; Tan *et al*., 2006; Qin *et al*., 2011). Moreover, to ensure that the surface microbes were completely eliminated the final wash was spread on the WA and SCA in duplicate plates. None of the representative colonies appeared on any of the plates indicating that the protocol was effective in removing the epiphytic microbes and that the isolates obtained can be considered to be true endophytes (Coombs and Franco, 2003).

After 1-8 weeks of incubation at 30 °C on the SCA and WA plates, the actinobacterial colonies were morphologically observed on the SCA plates spread with ground root solution while mycelia of actinomycetes which took at least 3 weeks to grow out from tissues were detected on pieces of roots and leaves on the both media (Figure 9). No actinomycete was obtained from stems of any plant species. Growth of microbes in the laboratory is dependent on the composition of the media and the cultivation conditions (Qin *et al*. 2011). In this experiment, SCA (Küster and Williams, 1964) and WA were selected as isolation media following the report of Sardi *et al*. (1992). Both media were supplemented with 2.5 U penicillin G ml^{-1} and 50 mg cyclohexamide ml^{-1} to inhibit the growth of endophytic bacteria and fungi, respectively (Bunyoo *et al*., 2008; Thamchaipenet *et al*., 2008). However, the long time incubation of the plant materials onto the media at 30 °C made the antibiotics less effective and commonly allowed the growth of endophytic bacteria and fungi. In this study, contamination of endophytic bacteria and fungi was found lower on WA than on SCA, while the colonies of actinomycetes were higher obtained from the pieces of plant materials laid on WA than SCA. The results corresponded to the report of Sardi *et al*. (1992) that nearly 100% of plant materials incubated on WA were colonized whereas less colonies were observed on SCA. Coombs and Franco (2003), Qin *et al*. (2009) and Li *et al*. (2009) agree that low nutrient medium is effective for isolation of endophytic actinomycetes including WA.

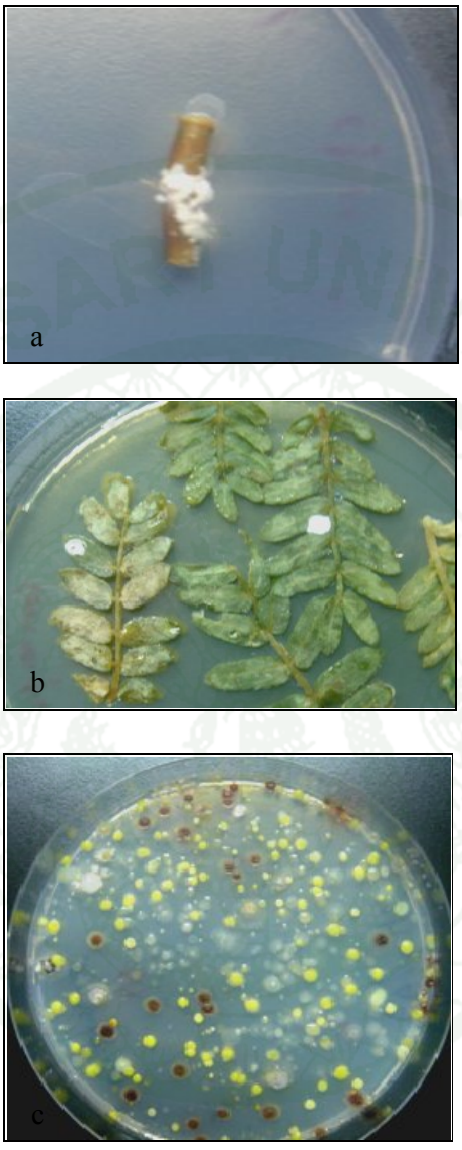


Figure 9 Example of endophytic actinomycetes isolation. (a) Colonization on the root of Pak-bong-ta-lay (*Ipomoea pescaprae* Sweet) plated onto WA medium. (b) Colonization on leaves of Kok-ka-soon (*Tribulus cistoides* Linn.) plated on SCA medium. (c) Colonization from the solution of ground roots of Ka-jae (*Hesperethusa crenulata* Roem.) spread on SCA medium. Incubation time was 4 weeks at 30 °C.

Different morphology of actinobacterial colonies were picked and purified on MS medium. A total of 66 isolates were obtained from 22 plant species belonging to 17 families including Acanthaceae, Araliaceae, Compositae, Convolvulaceae, Ebenaceae, Euphorbiaceae, Lauraceae, Leguminosae, Moraceae, Myristicaceae, Myrsinaceae, Palmae, Plumbaginaceae, Poaceae, Rutaceae, Verbenaceae and Zygophyllaceae. The isolates designated GMKU (Genetics-Microbiology-Kasetsart-University) culture collection, the numbers were shown in Appendix Table A2. In this experiment, most of endophytic actinomycetes (n=46; 70%) obtained from woody plants (15 species) and the remainder (n=20; 30%) gained from herbaceous plants (7 species). In contrast, Taechowisan *et al*. (2003) using 36 plant species collected in Chiang Mai, Thailand gained a few actinomycetes from woody plants (2 species) while 99% of actinomycetes were recovered from herbaceous plants (34 species). However, both experiments indicated that actinomycetes are widely distributed in the internal tissues of Thai tropical plants ranging from herbaceous to woody plants. It is evident that plants in nature harbor a community of endophytic bacteria (Long *et al*., 2008) and colonization inside the plant tissue of actinomycetes is ubiquitous and common in the nature (Qin *et al*., 2011). In addition, the highest number of the actinobacterial isolates (n = 55, 83 %) was obtained from roots and their crushed solutions, and the rest were gained from leaves (Table 8). These results were in agreement with previous report that roots represent a good habitat for endophytes and this may relate to the fact that actinomycetes reside abundantly in rhizosphere and could easily move into the plant roots (Lamb *et al*., 1996). No isolate was recovered from 10 plant species namely, *Adhatoda vasica* Nees, *Polyscias fruticosa* Harás, *Terminalia chebula* Retz., *Garcinia mangostana* Linn., *Cinnamomum camphora* Th. Fries, *Tinospora cordifolia* Mier, *Parkia speciosa* Hassk, *Rivina humilis* Linn, *Lepisanthes senegalensis* Leenh and *Boesenbergia pandurata* Holtt (Table 8). This may be because of the long time incubation that made the antibiotics less effective and allowed the growth of endophytic bacteria or fungi or these trees have mycorrhizal fungi associated with their roots which may form a barrier to the infection of other endophytic microoganisms (Taechowisan *et al*., 2003).

Table 8 Actinobacterial endophytes and host plants.

Host plants		Isolates				A total number of isolates
Family	Scientific name	Leaf	Stem	Root	Solution of ground roots	
Acanthaceae	*Adhatoda vasica* Nees [b]	–	–	–	–	–
	Barleria strigosa Willd.[b]	354	–	–	355, 356, 357, 358	5
	Climacanthus nutans Lindau [a]	346	–	–	–	1
	Climacanthus siamensis Brem.[a]	362, 363	–	360, 361	–	4
Araliaceae	*Polyscias fruticosa* Harás [b]	–	–	–	–	–
	Schefflera venulosa Harms [b]	–	–	–	330, 331	2
Combretaceae	*Terminalia chebula* Retz. [b]	–	–	–	–	–
Compositae	*Eupatorium stoechadosmum* Hance [a]	–	–	350	–	1
	Ageratum conyzoides Linn. [a]	348	–	–	–	1
Convolvulaceae	*Ipomoea pescaprae* Sweet [a]	312, 313	–	311	309,314	5
Ebenaceae	*Diospyros mollis* Griff. [b]	–	–	315	316, 317, 318	4
Euphorbiaceae	*Croton caudatus* Geisel [b]	352	–	341, 342	353	4
	Phyllanthus pulcher Wall.[b]	324	–	–	–	1
Guttiferae	*Garcinia mangostana* Linn.[b]	–	–	–	–	–
Lauraceae	*Cinnamomum camphora* Th. Fries [b]	–	–	308	347	–
	Cinnamomum bejolghota Sweet[b]	–	–	322, 323	320	3
Leguminosae	*Abrus pulchellus* Wall.[b]	–	–	–	325, 326, 327, 328, 329, 351	6

Table 8 (continued)

Host plants		Isolates				A total number of isolates
Family	Scientific name	Leaf	Stem	Root	Solution of ground roots	
Menispermaceae	*Tinospora cordifolia* Miers[b]	–	–	–	–	–
Mimosaceae	*Parkia speciosa* Hassk.[b]	–	–	–	–	–
Moraceae	*Streblus asper* Lour. [b]	–	–	–	364	1
Myristicaceae	*Myristica fragrans* Linn. [b]	–	–	–	377	1
Myrsinaceae	*Ardisia polycephala* Wall.[a]	–	–	344	343, 345	3
Palmae	*Elaeis guineensis* Jacq. [b]	–	–	–	378	1
Phytolaccaceae	*Rivina humilis* Linn. [b]	–	–	–	–	–
Plumbaginaceae	*Plumbago zeylanica* Linn.[b]	–	–	–	359	1
Poaceae	*Oryza sativa* L. cv KDML 105 [a]	–	–	–	368, 369, 370, 372	4
	Oryza sativa L. cv RD 6 [a]	–	–	–	366, 367	2
Rutaceae	*Hesperethusa crenulata* Roem. [b]			302	301, 303	3
Sapindaceae	*Lepisanthes senegalensis* Leenh. [b]	–	–	–	–	–
Verbenaceae	*Clerodendrum serratum* Moon [b]	340 (1)	–	334, 335	319, 333, 336, 337, 365	8
Zingiberaceae	*Boesenbergia pandurata* Holtt. [a]	–	–	–	–	–
Zygophyllaceae	*Tribulus cistoides* Linn. [a]	305 (1)	–	306, 307 (2)	–	3
	Total of isolates	11	–	16	39	66
	Total (%)	11 (17%)	–	55 (83%)		

[a] herbaceous plant; [b] woody plant

All isolates were cultured on MS until good growth to preliminarily identify their morphology as actinomycetes (Figure 10). After that, all isolates were preliminarily identified on the distinguished basis of characteristic colonial morphology notably the ability to form substrate and aerial mycelia as well as spore. The pure isolates were examined by growing on the media of Difco, namely ISP 2, ISP 3, and ISP 4 incubating at 27 °C for 3 weeks. The growth on each media, color of substrate mycelium (reverse plate), soluble pigment and sporulation were determined. The results of preliminary identification indicate that there were two groups including *Streptomyces* and rare actinomycetes (Table 9).

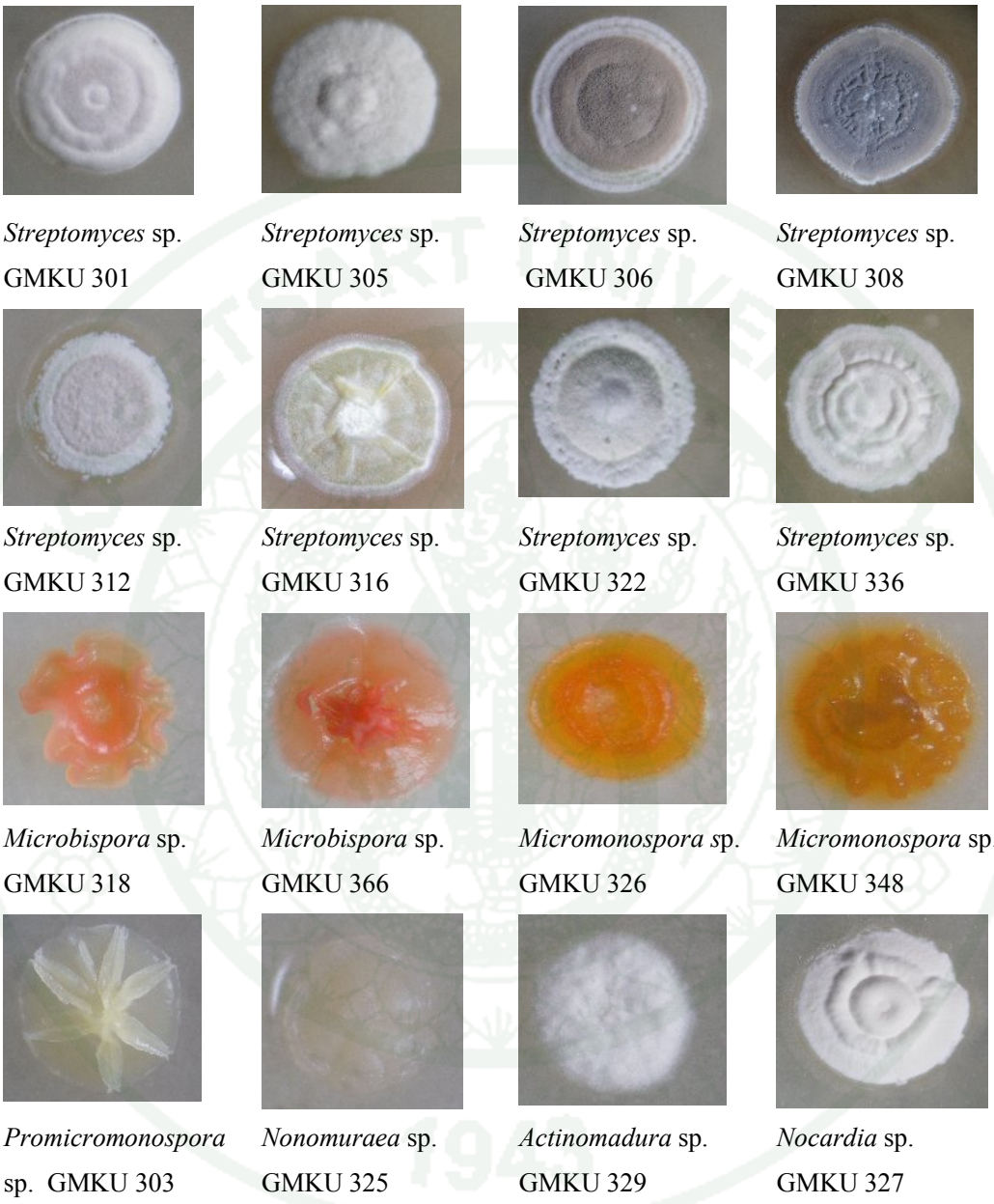


Figure 10 Morphology of some endophytic actinomycetes cultured on MS media.

Table 9 Characteristics of actinobacterial endophytes on ISP2, ISP3, and ISP4 media incubated at 27 °C for 2 weeks. 1, growth on the medium; 2, color of substrate mycelium; 3, soluble pigment; 4, sporulation. VG, very good; G, good; M, moderate; P, poor; VP very poor; − absent.

GM	ISP No.2 medium				ISP No. 3 medium				ISP No. 4 medium			
KU	1	2	3	4	1	2	3	4	1	2	3	4
301	G	Golden yellow	−	−	G	Golden yellow	−	M	G	Golden yellow	−	VG
303	G	Yellow	−	−	M	Light yellow	−	−	M	White	−	−
305	G	Golden yellow	−	G	G	White	−	M	G	Light yellow	−	G
306	G	Light yellow	−	G	VG	Greenish gray	−	VG	G	Light yellow	−	G
307	G	Light yellow	−	G	G	Golden brown	−	VG	G	Light yellow	−	G
308	VG	Light yellow	−	G	VG	Dark olive green	Pale brown	G	VG	Light yellow	−	G
309	G	Gold	Gold	G	G	Golden	−	VG	G	Light yellow	−	G
311	G	Light yellow	−	P	G	Light yellow	−	−	G	Light yellow	−	G
312	G	Light yellow	−	G	M	Light yellow	−	VP	G	Light yellow	−	VG
313	M	Yellow	−	P	M	Yellow	−	M	G	Light yellow	−	G
314	G	Yellow	−	G	VG	Yellow	Yellow	VG	G	Yellow	−	G
315	VG	Light yellow	−	VP	M	Yellow	−	P		Light yellow	−	G
316	VG	Yellow	Gold	−	VG	Light yellow	Pale brown	P	G	Yellow	−	M
317	−	−	−	−	G	Orange		G	VP	White	−	−

Table 9 (continued)

GM	ISP No.2 medium				ISP No. 3 medium				ISP No. 4 medium			
KU	1	2	3	4	1	2	3	4	1	2	3	4
318	P	Orange	–	–	G	Orange	–	G	P	White	–	P
319	G	Yellow	–	P	G	Yellow	–	M	G	Light Yellow	–	G
320	G	Light Yellow	–	G	G	Gray	–	VG	G	Light Yellow	–	G
322	G	Light Yellow	Light Yellow	G	G	Brown	–	M	G	Light Yellow	–	G
323	G	Light Yellow	–	G	G	Yellow	–	M	G	Light Yellow	–	M
324	VP	Orange	–	–	G	Orange	–	G	P	Light yellow	–	P
326	P	Orange	–	–	M	Orange	–	–	–	–	–	–
328	G	Orange	–	–	G	Orange	–	–	G	Orange	–	–
330	G	Orange	–	–	M	Orange	–	–	VP	Orange	–	–
333	G	Light Yellow	–	M	VG	Yellow	–	VG	G	Light yellow	–	G
334	G	Light Yellow	–	P	G	Light Yellow	–	M	M	Light Yellow	–	M
335	VG	Dark brown	–	VG	VG	Dark brown	Pale brown	G	VG	Light Yellow	–	VG
336	P	Yellow	Gold	–	M	Gold	–	M	G	Golden yellow	Light yellow	G
337	G	Gold	Gold	G	G	Golden brown	–	G	G	Light yellow	–	G
340	P	Light Yellow	–	–	G	Orange	–	P	VP	White	–	–
341	G	Dark brown	Pale brown	G	G	Dark brown	Pale brown	G	G	Dark brown	–	G
342	G	Gray	–	G	M	Reddish brown	–	P	G	Light yellow	–	G

Table 9 (continued)

GM	ISP No.2 medium				ISP No. 3 medium				ISP No. 4 medium			
KU	1	2	3	4	1	2	3	4	1	2	3	4
344	G	Light yellow	Purple	G	G	Pink	−	G	G	Gold	−	VG
345	G	Light yellow	Purple	G	G	Pink	−	G	G	Gold	−	VG
346	M	Reddish brown	−	G	P	Light yellow	−	M	G	Yellow	−	G
348	G	Orange Black	−	−	G	Orange	−	−	−	−	−	−
352	G	Light yellow	yellow	G	P	Light yellow	yellow	P	M	Light yellow	yellow	G
350	P	Dark brown	−	−	M	Dark brown		P	VP	Dark brown	−	−
353	G	Orange	−	−	M	Orange	−	−	−	−	−	−
354	G	Yellow	−	G	G	Brown	−	VG	G	Light yellow	−	VG
355	G	Yellow	−	G	G	Brown	Gold	G	G	Light yellow	−	VG
357	P	Light Yellow	−	−	G	Reddish brown	−	−	P	Light yellow	−	−
358	G	Orange	−	−	G	Orange	−	−	G	Orange	−	−
359	G	Light yellow	−	−	M	White	−	−	P	Light yellow	−	P
360	G	Yellow	Gold	G	G	Gold	Gold	G	G	Light yellow	−	G
361	G	Light yellow	−	G	G	Dark brown	Brown	G	G	Light Yellow	−	G
362	G	Orange	−	−	G	Orange	−	−	G	Orange	−	−
367	M	yellow	−	−	G	yellow	−	M	P	yellow	−	P
368	P	Dark brown	Gold	−	G	Orange	−	−	VP	yellow	−	−

Table 9 (continued)

GM	ISP No.2 medium				ISP No. 3 medium				ISP No. 4 medium			
KU	1	2	3	4	1	2	3	4	1	2	3	4
369	P	Dark brown	−	−	G	Orange	−	M	P	White	−	−
370	P	Light yellow	−	−	M	White	−	P	−	−	−	−
372	P	Light yellow	−	−	M	Light yellow	−	G	VP	White	−	−
377	G	yellow	Gold	−	P	Pale brown	−	−	G	Tan	−	G

1.2 Identification based on partial sequences of 16S ribosomal RNA gene

Partial 16S rRNA gene sequencing was initially used to identify the 66 actinobacterial endophytes to the genus level and compared with EzTaxon web base tool and then the phylogenetic tree using the neighbour-joining method based on partial 16S rRNA gene sequence was constructed. The results of partial 16S rRNA sequencing analysis revealed that the majority (50%) of the isolates matched the EzTaxon database entries to genus *Streptomyces* and the remainder (50%) matched the entries to members of rare actinomycetes.

1.2.1 Genus *Streptomyces*

The majority of isolates that matched the EzTaxon database entries belong to genus *Streptomyces* which shared 98-100% identity to those of type strains in this genus. Results of partial 16S rRNA sequencing displayed in Appendix B and their similarity values with the closest species show in Table 10. To display the taxonomic position of 33 *Streptomyces* isolates with closely related type strains of *Streptomyces*, the phylogenetic tree using the neighbour-joining method based on partial 16S rRNA gene sequences was constructed (Figure 11). The sequence data for all closely related *Streptomyces* type strains were recovered from EzTaxon database and used to perform the phylogenetic tree, *Streptosporangium purpuratum* ATCC

12428^{T} (AF191735) was used as an out group. The results indicated that 33 isolates formed distinct clade within the members of the genus *Streptomyces*. From the tree, five groups were defined. The first group consists of 22 isolates including GMKU 301, GMKU 302, GMKU 306, GMKU 307, GMKU 308, GMKU 309, GMKU 311, GMKU 312, GMKU 313, GMKU 314, GMKU 316, GMKU 319, GMKU 320, GMKU 322, GMKU 323, GMKU 335, GMKU 336, GMKU 342, GMKU 344, GMKU 345, GMKU 354 and GMKU 355. Group II consists of only GMKU 315. Group III contains isolates GMKU 341 and GMKU 361. Group IV contains sequences from 4 isolates GMKU 305, GMKU 333, GMKU 334 and GMKU 337. And the final group V consists of GMKU 343, GMKU 365, GMKU 352 and GMKU 360. From the analysis, it is likely that 8 isolates of *Streptomyces* including, GMKU 301, GMKU 312, GMKU 315, GMKU 316, GMKU 320, GMKU 336, GMKU 344 and GMKU 345 have potential to be novel species (Figure 11). However, full length sequencing of 16S rRNA gene should be performed to verify the confidential potential of novel species.

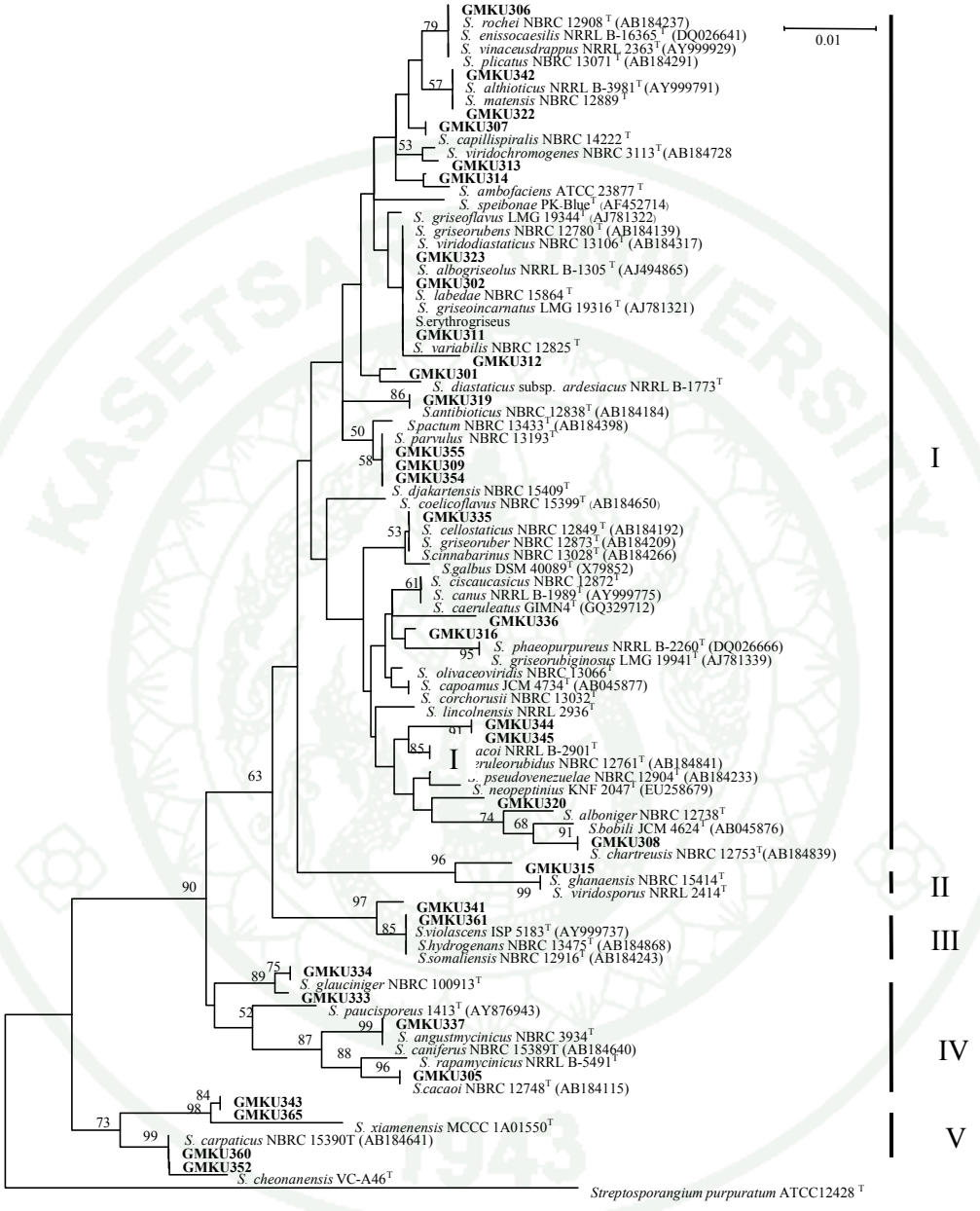


Figure 11 Phylogenetic tree based on partial 16S rRNA gene sequence using the neighbour-joining method of 33 *Streptomyces* strains. The sequence data for all closely related *Streptomyces* type strains were recovered from EzTaxon database and *Streptosporangium purpuratum* ATCC12428^{T} (AF191735) was used as an out group. Numbers at nodes indicate levels of bootstrap support (%) based on neighbour-joining analysis of 1000 resampled datasets and only values above 50 % are shown. Bar, 0.005 substitutions per site.

Table 10 Identification of endophytic actinomycetes based on partial sequencing of 16S rRNA gene.

GMKU	Closest actinomycete species	Sequence similarity (%)	Diff/ Total nucleotides	GenBank Accession no.
301	*Streptomyces coelicoflavus* NBRC 15399 ^{T}	99.50	3/608	AB184650
302	*S. viridodiastaticus* NBRC 13106^{T}	99.85	1/700	AB184317
	S. albogriseolus NRRL B-1305 ^{T}			AJ494865
	S. erythrogriseus LMG 19406 ^{T}			AJ781328
	S. griseoincarnatus LMG 19316 ^{T}			AJ781321
	S. griseorubens NBRC 12780 ^{T}			AB184139
	S. labedae NBRC 15864 ^{T}			AB184704
	S. variabilis NBRC 12825 ^{T}			AB184884
305	*S. cacaoi* subsp. *cacaoi* NBRC 12748^{T}	99.50	3/609	AB184115
306	*S. vinaceusdrappus* NRRL 2363^{T}	100	0/817	AY999929
	S. rochei NBRC 12908 ^{T}			AB184237
	S. plicatus NBRC 13071 ^{T}			AB184291
	S. enissocaesilis NRRL B-16365 ^{T}			DQ026641
307	*S. althioticus* NRRL B-3981 ^{T}	99.86	1/748	AY999791
	S. matensis NBRC 12889 ^{T}			AB184221
	S. capillispiralis NBRC 14222 ^{T}	99.47	4/755	AB184577
308	*S. chartreusis* NBRC 12753^{T}	100	0/825	AB184839
309	*S. djakartensis* NBRC 15409^{T}	99.88	1/809	AB184657
311	*S. labedae* NBRC 15864 ^{T}	100	0/825	AB184704
	S. griseoincarnatus LMG 19316 ^{T}			AJ781321
	S. variabilis NBRC 12825 ^{T}			AB184884
312	*S. griseorubens* NBRC 12780^{T}	99.22	5/647	AB184139
	S. viridodiastaticus NBRC 13106^{T}			AB184317
	S. albogriseolus NRRL B-1305 ^{T}			AJ494865

Table 10 (continued)

GMKU	Closest actinomycete species	Sequence similarity (%)	Diff/ Total nucleotides	GenBank Accession no.
313	*S. speibonae* PK-Blue^{T}	99.64	3/828	AF452714
	S. viridochromogenes NBRC 3113^{T}	99.15	7/826	AB184728
314	*S. viridochromogenes* NBRC 3113^{T}	99.76	2/815	AB184728
	S. ambofaciens ATCC 23877^{T}	99.27	6/816	M27245
315	*S. ghanaensis* NBRC 15414^{T}	98.50	12/786	AB184662
316	*S. capoamus* JCM 4734^{T}	99.75	2/795	AB045877
319	*S. antibioticus* NBRC 12838^{T}	100	0/809	AB184184
320	*S. galbus* DSM 40089^{T}	98.77	10/812	X79852
	S. curacoi NRRL B-2901^{T}			EF626595
	S. corchorusii NBRC 13032^{T}			AB184267
	S. coeruleorubidus NBRC 12761^{T}			AB184841
322	*S. althioticus* NRRL B-3981^{T}	100	0/802	AY999791
	S. matensis NBRC 12889^{T}			AB184221
323	*S. viridodiastaticus* NBRC 13106^{T}	100	0/810	AB184317
	S. griseorubens NBRC 12780^{T}			AB184139
	S. albogriseolus NRRL B-1305^{T}			AJ494865
333	*S. glauciniger* NBRC 100913^{T}	99.63	3/809	AB249964
334	*S. glauciniger* NBRC 100913^{T}	99.74	1/378	AB249964
335	*S. griseoruber* NBRC 12873^{T}	99.88	1/822	AB184209
	S. cellostaticus NBRC 12849^{T}			AB184192
336	*S. phaeopurpureus* NRRL B-2260^{T}	98.73	11/866	DQ026666
	S. griseorubiginosus LMG 19941^{T}			AJ781339
	S. ciscaucasicus NBRC 12872^{T}			AB184208
	S. canus NRRL B-1989^{T}			AY999775
	S. caeruleatus GIMN4^{T}			GQ329712
337	*S. caniferus* NBRC 15389^{T}	99.877	1/815	AB184640
341	*S. violascens* ISP 5183^{T}	99.76	2/835	AY999737
342	*S. althioticus* NRRL B-3981^{T}	100	0/698	AY999791
	S. matensis NBRC 12889^{T}			AB184221
343	*S. xiamenensis* MCCC 1A01550^{T}	98.87	10/886	EF012099

Table 10 (continued)

GMKU	Closest actinomycete species	Sequence similarity (%)	Diff/ Total nucleotides	GenBank Accession no.
344	*S. neopeptinius* KNF 2047^{T}	98.85	9/780	EU258679
345	*S. neopeptinius* KNF 2047^{T}	98.91	9/824	EU258679
	S. pseudovenezuelae NBRC12904 ^{T}			AB184233
352	*S. carpaticus* NBRC 15390^{T}	99.88	1/808	AB184641
354	*S. parvulus* NBRC 13193^{T}	100	0/822	AB184326
355	*S. parvulus* NBRC 13193^{T}	100	0/771	AB184326
360	*S. carpaticus* NBRC 15390^{T}	99.88	1/826	AB184641
361	*S. somaliensis* NBRC 12916^{T}	100	0/814	AB184243
365	*S. xiamenensis* MCCC 1A01550 ^{T}	98.68	12/910	EF012099

1.2.2 Rare actinomycetes

Analysis of the remainder of the isolates (n = 33), results of partial 16S rRNA sequencing indicated that they matched EzTaxon database entries to 9 genera as follows: *Actinomadura* (n = 6), *Kibdelosporangium* (n = 1), *Microbispora* (n = 14), *Micromonospora* (n = 7), *Nocardia* (n = 1), *Nocardiopsis* (n = 1), *Nonomuraea* (n = 1), *Promicromonospora* (n = 1) and *Saccharopolyspora* (n = 1). And their similarity values with the closest species show in Table 11.

Table 11 Identification of endophytic actinomycetes based on partial sequencing of 16S rRNA gene.

GMKU	Closest actinomycete species	Sequence similarity (%)	Diff/ Total nucleotides	GenBank Accession no.
303	*Promicromonospora citrea* DSM 43110^T	100	0/810	X83808
317	*Microbispora rosea* subsp. *rosea* IFO 14044^T	99.50	3/595	D86936
318	*M. rosea* subsp. *rosea* IFO 14044^T	98.88	9/805	D86936
324	*M. rosea* subsp. *rosea* IFO 14044^T	99.24	6/785	D86936
325	*Nonomuraea fastidiosa* IFO 14680^T	98.90	9/815	U48844
326	*Micromonospora auratinigra* TT1-11^T	99.03	7/724	AB159779
327	*Nocardia jiangxiensis* 43401^T	98.65	11/815	AY639902
328	*Micromonospora chalcea* DSM 43026^T	99.76	2/825	X92594
329	*Actinomadura nitritigenes* DSM 44137^T	99.64	3/827	AY035999
330	*Micromonospora aurantiaca* ATCC 27029 ^{T}	99.82	1/555	CP002162
331	*A. nitritigenes* DSM 44137 ^{T}	99.31	6/874	AY035999
340	*M. amethystogenes* JCM 3021 ^{T}	99.34	5/762	U48988
346	*Nocardiopsis dassonvillei* subsp. *dassonvillei* DSM 43111 ^{T}	99.68	3/946	ABUI01000017
347	*M. corallina* DF-32^T	98.80	11/918	AB018046
348	*Micromonospora tulbaghiae* TVU1^T	100	0/807	EU196562
351	*A. nitritigenes* DSM 44137^T	98.60	11/783	AY035999
	A. bangladeshensis 3-46-b(3)^T			AB331652
353	*Micromonospora echinospora* ATCC 15837^T	99.63	3/810	U58532

Table 11 (continued)

GMKU	Closest actinomycete species	Sequence similarity (%)	Diff/ Total nucleotides	GenBank Accession no.
356	*A. bangladeshensis* 3-46-b(3)^T	99.14	7/810	AB331652
357	*M. mesophila* JCM 3151^T	98.94	6/566	AF002266
358	*Micromonospora chalcea* DSM 43026^T	99.12	6/685	X92594
359	*A. spadix* JCM 3146^T	96.79	25/779	AF163120
362	*Micromonospora chalcea* DSM 43026^T	99.36	6/936	X92594
363	*M. mesophila* JCM 3151^T	99.57	4/938	AF002266
364	*M. mesophila* JCM 3151^T	99.88	1/802	AF002266
366	*M. rosea subsp. rosea* IFO 14044^T	99.80	2/824	D86936
367	*Kibdelosporangium aridum* subsp. *largum* DSM 44150^T	94.19	46/791	AJ512463
368	*M. rosea* subsp. *rosea* IFO 14044^T	99.78	2/927	D86936
369	*M. amethystogenes* JCM 3021^T	99.23	6/774	U48988
370	*A. spadix* JCM 3146^T	97.26	23/839	AF163120
372	*M. amethystogenes* JCM 3021^T	99.10	7/774	U48988
377	*Saccharopolyspora shandongensis* 88^T	99.88	1/797	EF104116
378	*M. corallina* DF-32^T	98.08	19/988	AB018046

1.2.2.1 Genus *Actinomadura*

There were six isolates of which four isolates (GMKU 329, GMKU 331, GMKU 351 and GMKU 356) shared 98.60-99.65% identity to those of genus *Actinomadura* type strains, while 2 isolates, GMKU 359 and GMKU 370, shared 96.79% and 97.26% identity with *A. spadix* JCM 3146^T (AF163120), respectively. For GMKU 359 and GMKU 370, the results indicated that they had highly potential to be novel species in the genus *Actinomadura* because the isolate that shows ≤97% 16S rRNA sequence similarity to all known taxa are considered to

belong to a new species (Amann *et al*., 1992; Stackebrandt and Goebel, 1994; Vandamme *et al*., 1996; Stackebrandt *et al.,* 2002; Gevers *et al*., 2005 and Janda and Abbott, 2007). To display the taxonomic position of six isolates with the corresponding 16S rRNA gene sequence of all the type strains validly described as *Actinomadura*, the phylogenetic tree using the neighbour-joining method based on 729 nucleotides of 16S rRNA gene sequence was constructed (Figure 12). In the List of Prokaryotic names with Standing in Nomenclature (LPSN: http:// www.bacterio. cict.fr/) at the time of identification, this genus was known to compose of 38 species with validly published names. The sequence data of 38 species in this genus were recovered from EzTaxon database. *Nocardiopsis compostus* KS8^{T} (AF360733) was used as an out group. The results of phylogenetic tree analysis indicated that six isolates formed clade within the members of the genus *Actinomadura*. GMKU 329 and GMKU 331 formed distinct clade with *A. nitritigenes* DSM 44137^{T} (AY035999) and this was supported by 1000 bootstrap value. GMKU 351 formed clade with *A. macra* IFO 14102^{T} (U49009) and this was supported by moderately bootstrap value and formed polyphyletic group with a type species *A. madurae* DSM4 367^{T} (X97889). For GMKU 356, the isolate formed a clade with *A. latina* DSM 43382^{T} (AY035998) but it was supported by low bootstrap value. From the tree, it is likely that GMKU 351, GMKU 356, GMKU 359 and GMKU 370 have highly potential to be novel species. As the result of partial sequence and phylogenetic tree analysis, the almost complete 16S rRNA gene sequences of GMKU 359 and GMKU 370 were determined by direct sequencing to further identification by using polyphasic taxonomy.

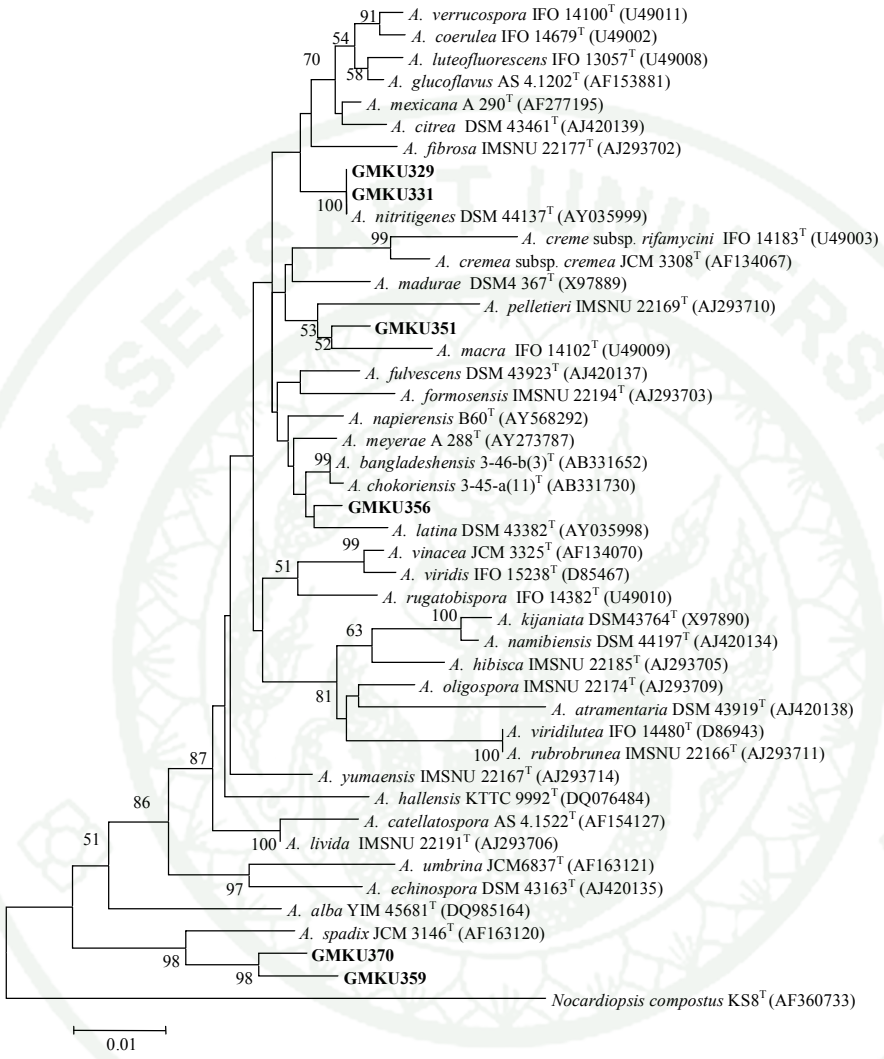


Figure 12 Phylogenetic tree based on 729 nucleotides of 16S rRNA gene sequences of six isolates and all the type strains of the genus *Actinomadura* using the neighbour-joining method. *Nocardiopsis compostus* KS8^T (AF360733) was used as an out group. Numbers at nodes indicate levels of bootstrap support (%) based on neighbour-joining analysis of 1000 resampled datasets and only values above 50 % are shown. Bar, 0.01 substitutions per site.

1.2.2.2 Genus *Kibdelosporangium*

For the result of an isolate matched with genus *Kibdelosporangium*, GMKU 367 showed highest sequence similarity to *K.aridum* subsp. *largum* DSM 44150 ^{T} (94.185%). To display the taxonomic position of GMKU 367 with all the type strains of the genus *Kibdelosporangium*, phylogenetic tree was constructed. In the LPSN at the time of identification, this genus was known to compose of 2 species with validly published names. The sequence data of 2 species in this genus were recovered from EzTaxon database. *Amycolatopsis nigrescens* CSC17-Ta-90^{T} (DQ486888) was used as the root organism and the topology of tree was shown in Figure 13. The results of phylogenetic tree analysis indicated that the isolates separated from the members of genus *Kibdelosporangium*. From the results of the partial 16S rRNA sequence and the phylogenetic tree analysis, it was indicated that GMKU 367 did not belong to the genus *Kibdelosporangium* but might represent a novel genus of the family *Pseudonocardiaceae.* Thus the almost complete 16S rRNA gene sequence of the isolate was further determined to validate the isolate by using polyphasic taxonomy.

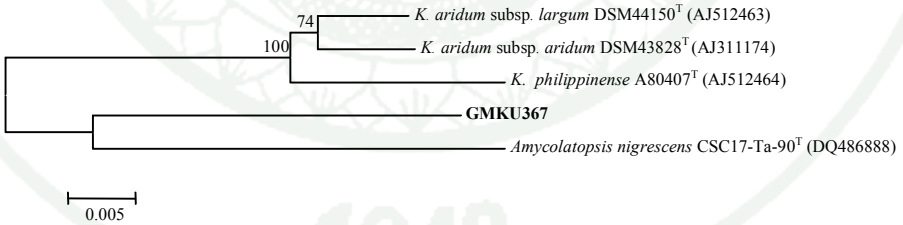


Figure 13 Phylogenetic tree based on 791 nucleotides of 16S rRNA gene sequences of isolate GMKU367 with all the type strains of the genus *Kibdelosporangium* using the neighbour-joining method. *Amycolatopsis nigrescens* CSC17-Ta-90^{T} (DQ486888) was used as an out group. Numbers at nodes indicate levels of bootstrap support (%) based on neighbour-joining analysis of 1000 resampled datasets and only values above 50 % are shown. Bar, 0.005 substitutions per site.

1.2.2.3 Genus *Microbispora*

On the basic of partial 16S rRNA gene sequence analysis, there are 14 isolates showed the sequence similarity to the genus *Microbispora* of which 13 isolates share 98.08-99.88% identity to those of type strains. But one isolate, GMKU 350, revealed poor sequencing data and was not included in the phylogenetic analysis. To display the taxonomic position of 13 isolates with the corresponding 16S rRNA gene sequences of all type strains validly described as *Microbispora*, the phylogenetic tree using the neighbour-joining method was constructed. The results of phylogenetic tree analysis indicated that the isolates formed distinct clade within the members of the genus *Microbispora* (Figure 14).

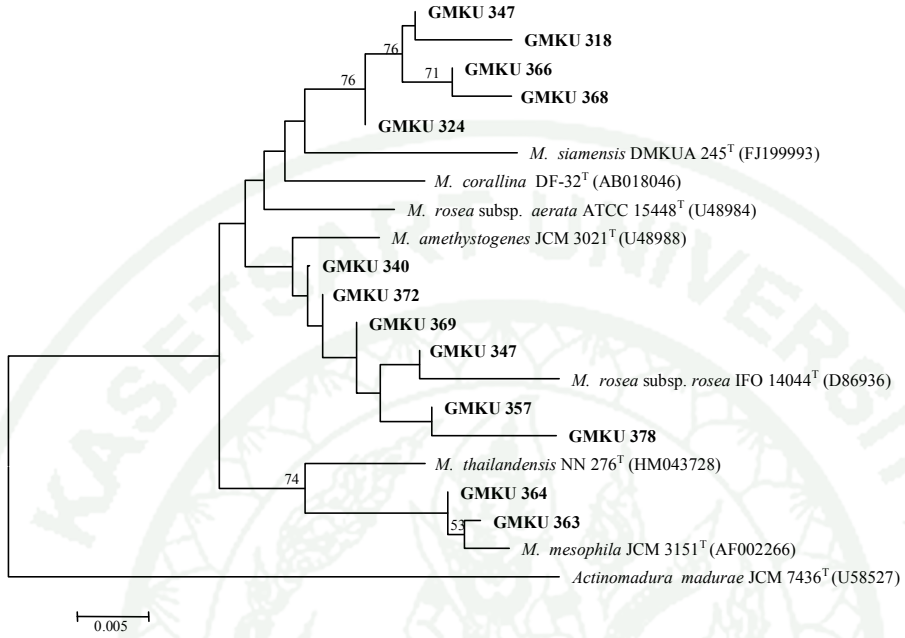


Figure 14 Phylogenetic tree based on 540 nucleotides of 16S rRNA gene sequences of 13 isolates and all the type strains of genus *Microbispora* using the neighbour-joining method. Numbers at nodes indicate levels of bootstrap support (%) based on neighbour-joining analysis of 1000 resampled datasets and only values above 50 % are shown. Bar, 0.005 substitutions per site.

1.2.2.4 Genus *Micromonospora*

There are seven isolates belonged to the genus *Micromonospora* based on partial sequencing of 16S rRNA gene which shared 99.03-100 % identity to those of type strains. To display the taxonomic position of 7 isolates with the corresponding 16S rRNA gene sequences of all type strains validly described as *Micromonospora*, the phylogenetic tree using the neighbour-joining method based on 493 nucleotides of 16S rRNA gene sequence was constructed. In the LPSN at the time of identification, this genus was known to compose of 40 species with validly published names. The sequence data of 40 species in this genus were recovered from EzTaxon database. Apart from that, *Verrucosispora sediminis* MS426^{T} (EU870859), *Salinispora arenicola* CNH-643^{T} (AY040619), *Salinispora tropica* CNB-440^{T} (CP000667) and *Microbispora mesophila* JCM3151^{T} (AF002266) were used as the root organisms and the topology of tree was shown in Figure 15. The results of phylogenetic tree analysis indicated that seven isolates formed distinct clade within the members of the genus *Micromonospora*.

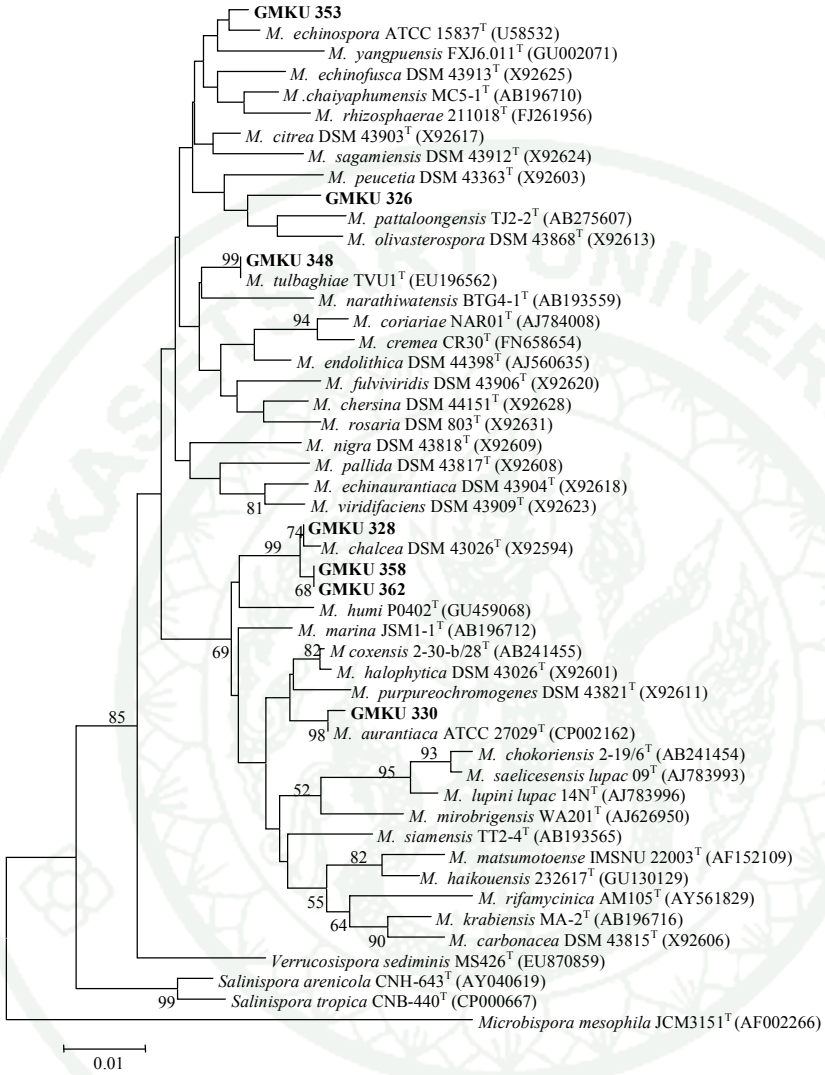


Figure 15 Phylogenetic tree based on 493 nucleotides of 16S rRNA gene sequences of seven isolates and all the type strains of the genus *Micromonospora* using the neighbour-joining method. Numbers at nodes indicate levels of bootstrap support (%) based on neighbour-joining analysis of 1000 resampled datasets and only values above 50 % are shown. Bar, 0.01 substitutions per site.

1.2.2.5 Genus *Nocardia*

For the result of an isolate matched with genus *Nocardia*, GMKU 327 showed sequence similarity to *N. jiangxiensis* 43401^T (98.65%). To display the taxonomic position of GMKU 327 with closely related type strains of the genus *Nocardia*, phylogenetic tree based on 792 nucleotides of 16S rRNA gene sequences with type strains was constructed. *Saccharopolyspora rosea* IMMIB RIV-085^T (AJ854055) was used as an out group and the topology of tree was shown in Figure 16. The results of phylogenetic tree analysis indicated that the isolate GMKU 327 formed distinct clade with the number of the genus *Nocardia* and showed monophyletic group with *N. jiangxiensis* 43401^T, *N. nova* JCM 6044^T (Z36930) and *N. miyunensis* 117^T (AY639901) but this was supported by low bootstrap value. It is likely that the isolate has potential to be novel species in this genus.

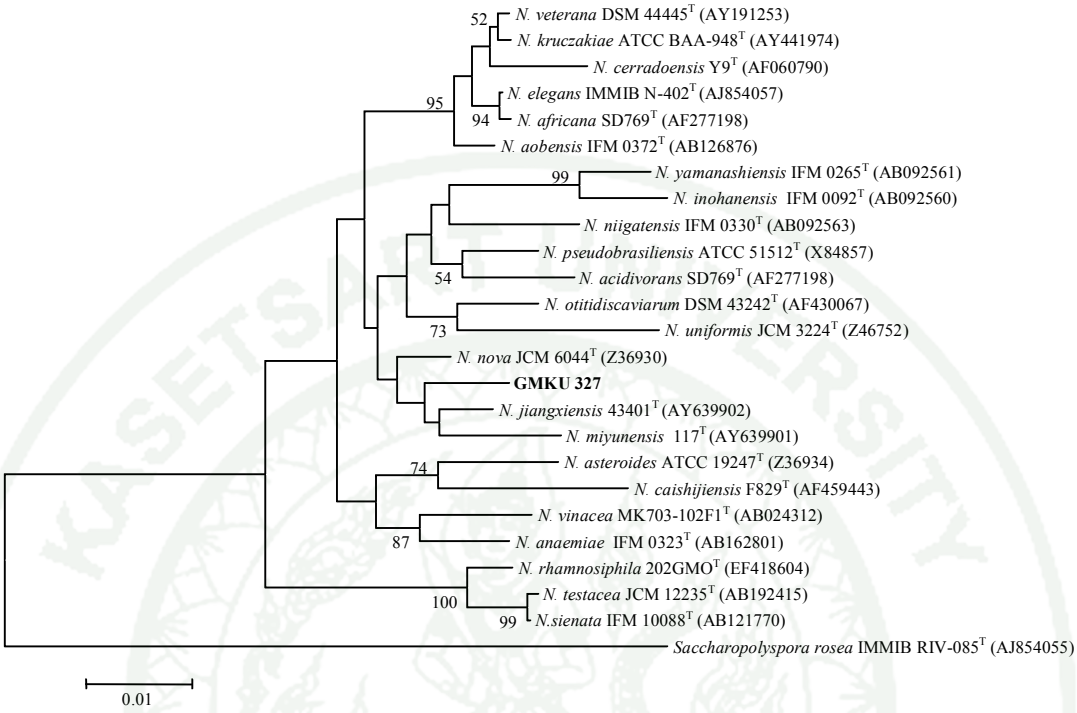


Figure 16 Phylogenetic tree based on 791 nucleotides of 16S rRNA gene sequences of isolate GMKU327 and all the type strains of the genus *Nocardia* using the neighbour-joining method. *Saccharopolyspora rosea* IMMIB RIV-085^T (AJ854055) was used as an out group. Numbers at nodes indicate levels of bootstrap support (%) based on neighbour-joining analysis of 1000 resampled datasets and only values above 50 % are shown. Bar, 0.01 substitutions per site.

1.2.2.6 Genus *Nocardiopsis*

On the basic of partial 16S rRNA gene sequence analysis, there is an isolate GMKU 346 showed the sequence similarity to the genus *Nocardiopsis* which shared 99.68 % identity to *N. dassonvillei* subsp. *dassonvillei* DSM 43111 ^{T}. To display the taxonomic position of the isolate with the corresponding 16S rRNA gene sequences of closely related type strains, the phylogenetic tree using the neighbour-joining method was constructed. The results of phylogenetic tree analysis indicated that the isolate formed distinct clade with

N. dassonvillei subsp. *dassonvillei* DSM 43111 ^{T} and this was supported by 99 bootstrap values as shown in Figure 17.

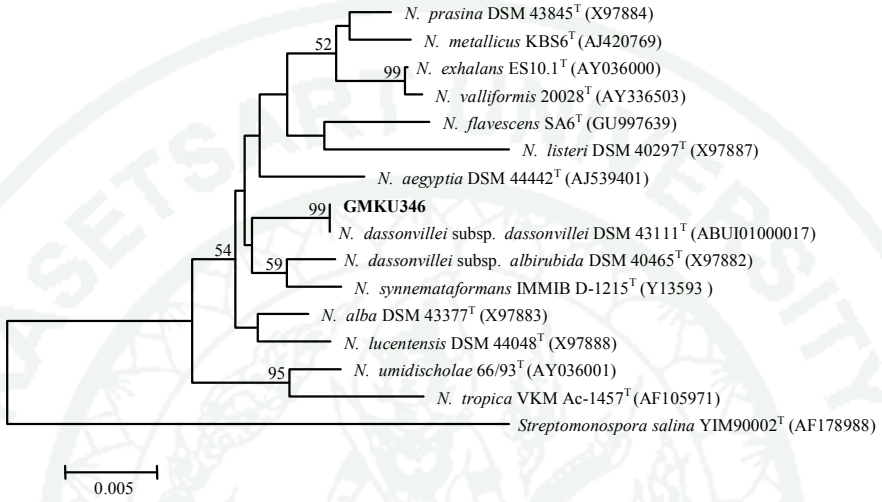


Figure 17 Phylogenetic tree based on 901 nucleotides of 16S rRNA gene sequence of isolate GMKU346 and all the type strains of the genus *Nocardiopsis* using the neighbour-joining method. *Streptomonospora salina* YIM90002^{T} (AF178988) was used as an out group. Numbers at nodes indicate levels of bootstrap support (%) based on neighbour-joining analysis of 1000 resampled datasets and only values above 50 % are shown. Bar, 0.005 substitutions per site.

1.2.2.7 Genus *Nonomuraea*

For the result of an isolate matched with genus *Nonomuraea*, GMKU 325 showed sequence similarity to *N. fastidiosa* IFO 14680^{T} (98.90%). To display the taxonomic position of the isolate with the corresponding 16S rRNA gene sequence of all type strains validly described as *Nonomuraea*, the phylogenetic tree using the neighbour-joining method based on 768 nucleotides of 16S rRNA gene sequence was constructed. In the LPSN at the time of identification, this genus was known to compose of 30 species with validly published names. The sequence data of 30 species in this genus were recovered from EzTaxon database.

Sphaerisporangium album YIM 48782^{T} (EU499344) was used as an root organism and the topology of tree was shown in Figure 18. The results of phylogenetic tree analysis indicated that the isolates formed distinct clade with *N. maritima* FXJ7.203^{T} that supported by highly bootstrap value (98%).

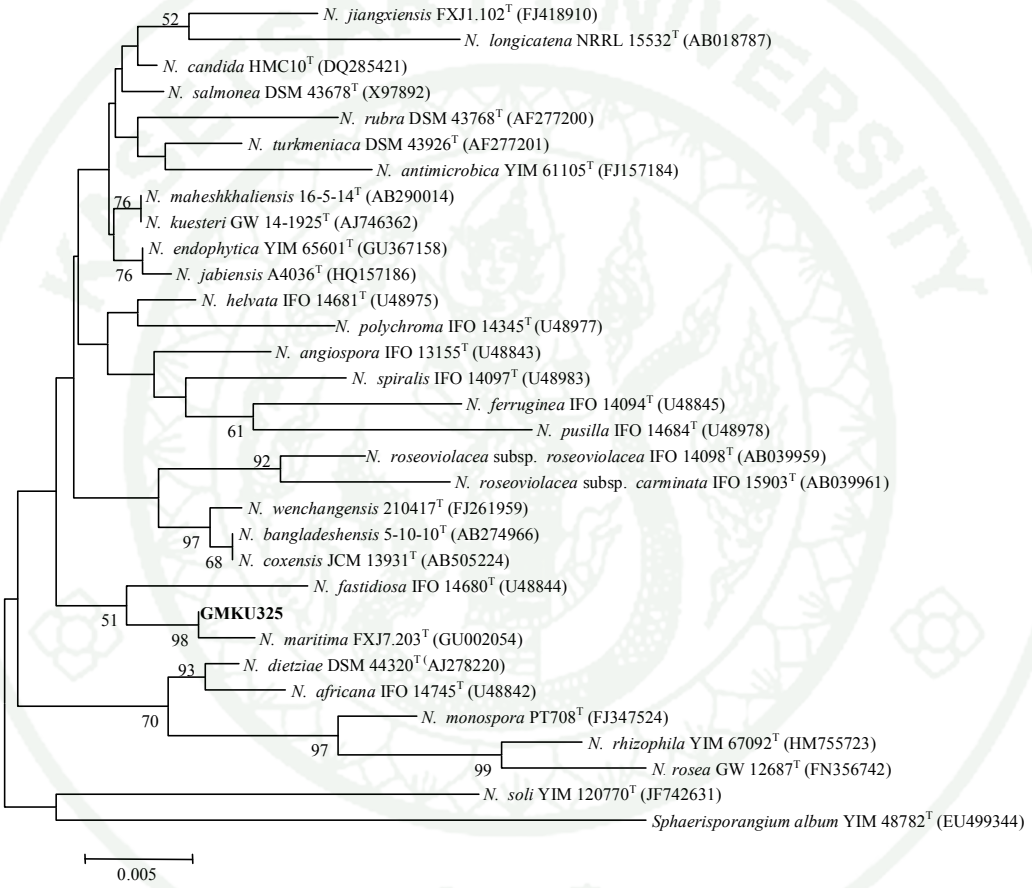


Figure 18 Phylogenetic tree based on 768 nucleotides of 16S rRNA gene sequences of an isolate GMKU325 and all the type strains of the genus *Nonomuraea* using the neighbour-joining. *Sphaerisporangium album* YIM 48782^{T} (EU499344) was used as the out group. Numbers at nodes indicate levels of bootstrap support (%) based on neighbour-joining analysis of 1000 resampled datasets and only values above 50 % are shown. Bar, 0.005 substitutions per site.

1.2.2.8 Genus *Promicromonospora*

An isolate GMKU 303 matched with genus *Promicromonospora* showed sequence similarity to *P. citrea* DSM 43110^T (100%). To display the taxonomic position of the isolates with the corresponding 16S rRNA gene sequence of all the type strains validly described as *Promicromonospora*, the phylogenetic tree using the neighbour-joining method based on 757 nucleotides of 16S rRNA gene sequence was constructed. In the LPSN at the time of identification, this genus was known to compose of 10 species with validly published names. The sequence data of 10 species in this genus were recovered from EzTaxon database. *Cellulosimicrobium terreum* DS-61^T (EF076760) was used as an out group and the topology of tree was shown in Figure 19. The results of phylogenetic tree analysis indicated that the isolates formed distinct clade with *P. citrea* DSM 43110^T that supported by highest bootstrap values (100%).

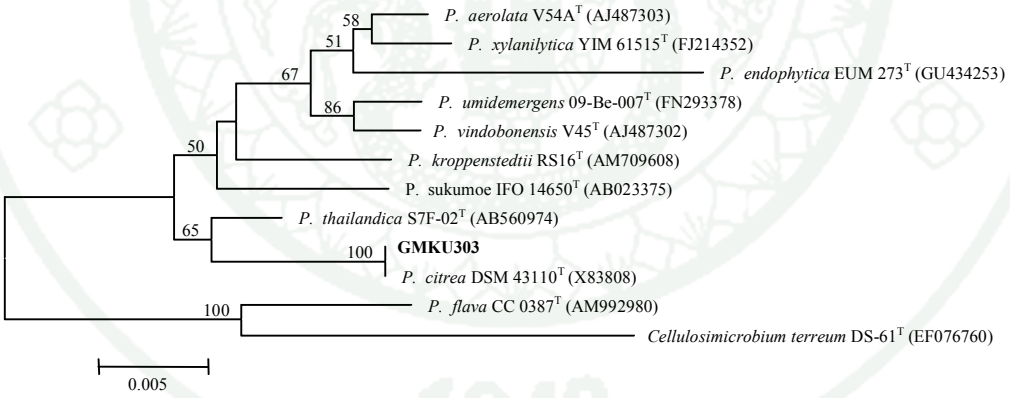


Figure 19 Phylogenetic tree based on 757 nucleotides of 16S rRNA gene sequences of an isolate GMKU303 and all the type strains of the genus *Promicromonospora* using the neighbour-joining method. *Cellulosimicrobium terreum* DS-61^T (EF076760) was used as an out group. Numbers at nodes indicate levels of bootstrap support (%) based on neighbour-joining analysis of 1000 resampled datasets and only values above 50 % are shown. Bar, 0.005 substitutions per site.

1.2.2.9 Genus *Saccharopolyspora*

For the result of an isolate matched with genus *Saccharopolyspora*, GMKU 377 showed sequence similarity to *S. shandongensis* 88^T (99.88%). To display the taxonomic position of the isolates with the corresponding 16S rRNA gene sequences of all type strains validly described as *Saccharopolyspora*, the phylogenetic tree using the neighbour-joining method based on 744 nucleotides of 16S rRNA gene sequence was constructed. In the LPSN at the time of identification, this genus was known to compose of 20 species with validly published names. The sequence data of 20 species in this genus were recovered from EzTaxon database. Apart from that, *Actinokineospora terrae* IFO 15668^T (AB058394) was used as an out group and the topology of tree was shown in Figure 20. The results of phylogenetic tree analysis indicated that the isolates formed distinct clade with *S. shandongensis* 88^T that supported by highest bootstrap values (100%).

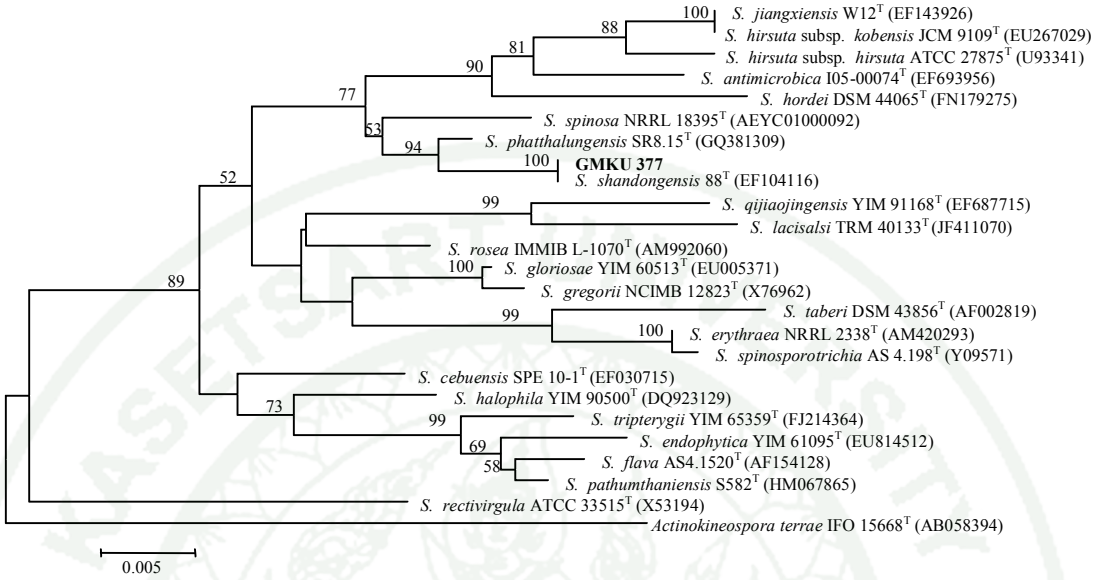


Figure 20 Phylogenetic tree based on 744 nucleotides of 16S rRNA gene sequences of an isolate GMKU377 and all the type strains of the genus *Saccharopolyspora* using the neighbour-joining method. *Actinokineospora terrae* IFO 15668^T (AB058394) was used as an out group datasets and only values above 50 % are shown. Numbers at nodes indicate levels of bootstrap support (%) based on neighbour-joining analysis of 1000 resampled datasets and only values above 50 % are shown. Bar, 0.005 substitutions per site.

2. Characterization of novel taxa

2.1 Phenotypic methods

The results of partial 16S rRNA sequences and the phylogenetic tree analysis indicated that isolates GMKU 367 is likely to represent novel genus of the family *Pseudonocardiaceae* while GMKU 359 and GMKU 370 might be a novel species of genus *Actinomadura*. Thus, polyphasic taxonomy was carried out to validate the taxonomic position of three isolates.

2.1.1 Morphological characteristics

2.1.1.1 Cultural characteristics

GMKU 367 grew well on ISP 3 and oatmeal-nitrate agar after incubation at 27 °C for 3 weeks. However, moderate growth was observed on ISP 2 and YS, poor growth was found on ISP 4 and ISP 5. The trace of rust tan soluble pigment was produced only on oatmeal-nitrate agar. GMKU 367 produced pale peach aerial mycelium and melon yellow substrate mycelium on ISP 3. No aerial mycelium was generated on ISP 2 and YS agar.

A growth of GMKU 359, good growth was observed on ISP 2 and ISP 5, while moderate growth was observed on ISP 3 and oatmeal-nitrate agar after incubation at 27 °C for 2 weeks. Poor growth was detected on ISP 4 and YS agar. However, GMKU 359 grew well on PDA and NA but did not produce spore. No soluble pigment was produced on any of the media tested. The whitish aerial mycelia were produced on ISP 4 and oatmeal-nitrate agar after growing for 3 weeks.

Moderate growth of GMKU 370, was observed on ISP 3, oatmeal-nitrate agar and YS agar. Poor growth was detected on ISP 2 and ISP 5 after incubation at 27 °C for 2 weeks. No growth was found on ISP 4. However, GMKU

370 grew well on PDA and NA but did not produce spore. No soluble pigment was produced on any of the media tested. The whitish aerial mycelia were produced on ISP 3 and oatmeal-nitrate agar after growing for 3 weeks.

2.1.1.2 Spore characteristics

In order to examine spore characteristics, GMKU 367 was grown on humic acid vitamin agar (HV medium; Hayakawa & Nonomura, 1987) tap-water agar and sucrose-nitrate agar at 27 °C for 8 weeks and then spore morphology was observed using light and scanning electron microscopy (JSM5600; JEOL). For GMKU 359 and GMKU 370, the isolates grown on oatmeal-nitrate agar at 27 ºC for 5 weeks before spore characteristics were observed. Spore morphology of the isolate GMKU 367 shows cylindrical spore on aerial mycelium, no sporangium-like structure nor fragmentation of substrate mycelium (Figure 21). Spore morphology of GMKU 359 and GMKU 370 showed short spiral chains of smooth-surfaced spores (Figure 22-23).

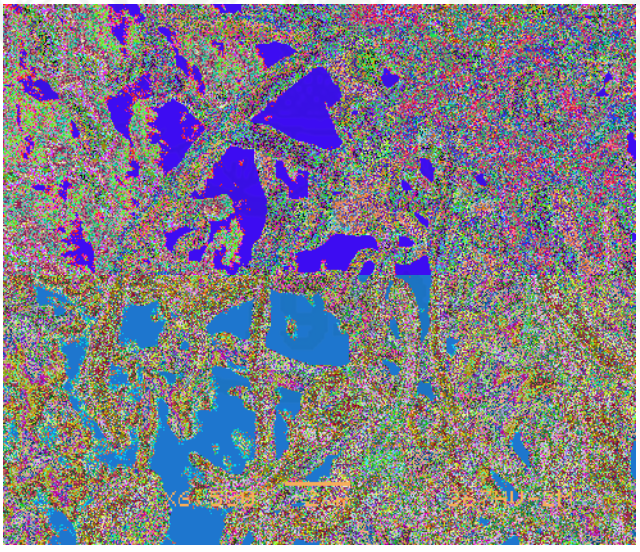


Figure 21 Scanning electron micrograph of spores of GMKU 367 grown on HV medium after incubation at 27 °C for 8 weeks. Bar, 2 µm.

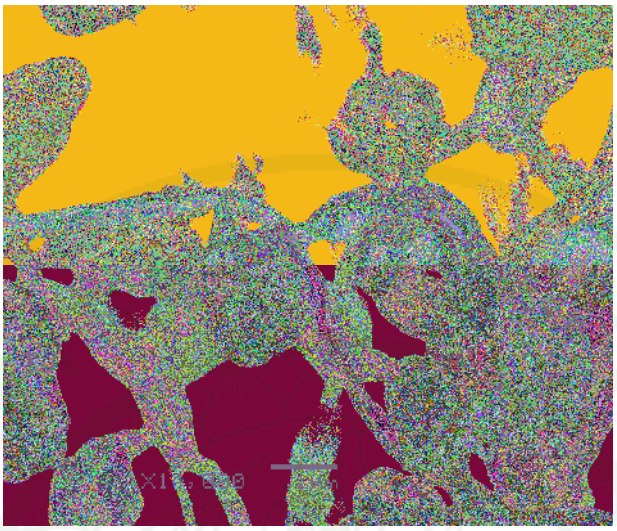


Figure 22 Scanning electron micrograph of spores of GMKU 359 grown on oatmeal nitrate agar at 27 ºC for 5 weeks. Bar, 1 µm.

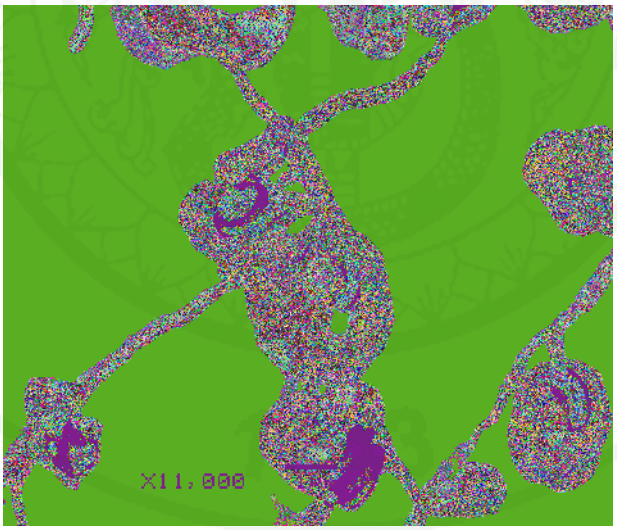


Figure 23 Scanning electron micrograph of spores of GMKU 370 grown on oatmeal nitrate agar at 27 ºC for 5 weeks. Bar, 1 µm.

2.1.2 Physiological characteristics

2.1.2.1 Acid production from carbohydrates

To analyze acid production from carbohydrates, the washed cells of each isolate was cultured on the basal inorganic nitrogen medium added with bromocresol purple as an indicator and 1% of each differ type of sugar. Twelve sugar types were used namely, adonitol, L-arabinose, dulcitol, erythritol, D-fructose, D-glucose, D-galactose, *myo*-inositol, lactose, D-mannitol, raffinose and L-rhamnose. GMKU 367 showed yellow color from L-arabinose, D-fructose and D-glucose. GMKU 370 showed yellow color from D-galactose, D-glucose and raffinose. GMKU 359 wasn't analyzed for acid product from carbohydrate.

2.1.2.2 Catalase and oxidase activities

GMKU 367 and GMKU 370 showed catalase positive by producing bubbles intensively. For oxidase test, GMKU 370 changed violet to purple color, while GMKU 367 showed colorless. These results indicated that oxidase of GMKU 370 was positive while GMKU 367 was negative. GMKU 359 wasn't analyzed for catalase and oxidase activities.

2.1.2.3 Degradation of starch

Degradation of starch on ISP 4 of each isolate was observed when the cultures were flooded with Lugol's solution. The clear zone around the strain GMKU 367 and GMKU 359 were observed indicated that both degraded starch, while GMKU 370 did not utilize starch.

2.1.2.4 Coagulation and peptonization of milk

Coagulation and peptonization of milk were observed in wash cells grew in 10% skim milk. The results showed that three isolates, GMKU367, GMKU 359 and GMKU 370, were negative.

2.1.2.5 Liquefaction of gelatin

Liquefaction of gelatin, which changing from the solid substance to the liquid state was observed in GMKU 359 while GMKU 367 and GMKU 370 could poorly degrade gelatin.

2.1.2.6 NaCl tolerance

To clarify the NaCl tolerance, each test novel strain was cultured on the ISP 2 or ISP 3 supplements with 1%, 2%, 3%, 4%, 5%, 6% and 7% NaCl. The results showed that GMKU 367 and GMKU 359 tolerates up to 2% NaCl while GMKU 370 was not able to grow in 2% NaCl.

2.1.2.7 Production of melanin pigment

ISP6 and ISP7 were used to determine production of melanin pigment of each strain. The strains were cultured on ISP6 and ISP7 to observe production of melanin. The results showed that neither diffusible pigment nor melanin of three isolates was produced on ISP6 and ISP7.

2.1.2.8 Range of temperature for growth

To determine range of temperature for growth, ISP 2 or ISP 3 was used. Each test strain was grown on slants in a temperature gradient incubator (5-50 °C). GMKU 367 showed temperature range for growth 12-30 °C, with the optimal temperature for good growth at 18-28 ºC. GMKU 359 was able to

grow at temperature range 20-40 °C and the optimal temperature for growth was 26-38 °C. GMKU 370 showed temperature range for growth at 20-44 °C and the optimal temperature for growth was 30-32 °C.

2.1.2.9 Range of pH for growth

To determine optimal pH and range of pH for growth, the strains were cultured on ISP 2 or NA which have various pH values of pH 4, pH 5, pH 6, pH 7, pH 8, pH 9 and pH 10 respectively. The results showed that GMKU 367 was able to grow on ISP 2 in arrange of pH 5.0-10.0, with the optimal pH at 6.0-7.0. GMKU 359 and GMKU 370 was able to grow on NA at the pH range 5.0-9.0, with the optimal pH at 7.0-8.0.

2.1.2.10 Reduction of nitrate

ISP 8 broth was used to study reduction of nitrate. The cultures were observed N_2 gas and pink color indicated positive strain. The results showed that GMKU 367, GMKU 359 and GMKU 370 not seen N_2 gas and pink color in the tubes indicated that all isolates did not reduce nitrate.

2.1.2.11 Utilization of carbon sources

Utilization of carbohydrate as sole carbon sources was tested using ISP 9 for GMKU 359 and GMKU 370 and ISP 4 medium (without soluble starch) for GMKU 367 (as it could not grow on ISP9). Sixteen sugars, L-arabinose, dulcitol, D-fructose, D-glucose, D-galactose, *myo*-inositol, lactose, maltose, D-mannitol, D-mannose, raffinose, L-rhamnose, D-sorbitol, sucrose, D-trehalose and D-xylose, were used in this study.

The results showed that GMKU 367 utilized D-fructose, D-glucose and L-rhamnose as sole carbon sources, but not L-arabinose, dulcitol, D-

galactose, *myo*-inosital, lactose, maltose, D-mannitol, D-mannose, raffinose, D-sorbitol, sucrose, D-trehalose and D-xylose.

GMKU 370 was able to use dulcitol, D-galactose, *myo*-inositol, lactose, maltose, D-mannose, raffinose, L-rhamnose, D-sorbitol, sucrose, D-trehalose and D-xylose as sole carbon sources but not L-arabinose, D-fructose, D-glucose and D- mannitol.

GMKU 359 was able to use dulcitol, *myo*-inositol, D-mannose, L-rhamnose, D-xylose and D-sorbitol as sole carbon sources but not arabinose, D-fructose, D-glucose, D-galactose, maltose, lactose, raffinose, sucrose, D-trehaloseand and D- mannitol.

For utilization of benzoate and citrate, modifications of Koser's citrate and benzoate agar were used. The results showed that GMKU 367 could degrade citrate by changing color of phenol red to yellow while GMKU 359 and GMKU 370 were negative. Decomposition of benzoate was negative in all three strains.

2.1.2.12 Utilization of nitrogen sources

Utilization of nitrogen sources was carried out by using 5% casein medium, 5% xanthine-NA and 5% hypoxanthine-NA. To observe utilization of casein, clearing of casein was appeared around the growth. For utilization of xanthine and hypoxanthine, disappearance of the crystals was observed around the growth. The results showed that GMKU 367 and GMKU 370 did not utilized casein, hypoxantine and xanthine, while GMKU 359 was able to utilize casein.

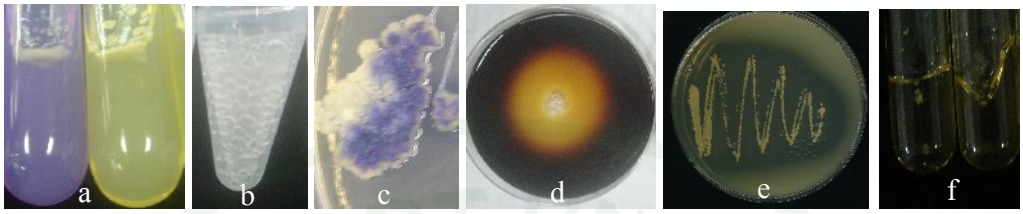


Figure 24 Physiological characteristics of GMKU 367, GMKU 359 and GMKU 370. (a) GMKU 367 did not produce acid in 1% L-arabinose (blue) and produce acid in D-fructose (yellow). (b) GMKU 370 was catalase positive (bubble formation). (c) GMKU 370 was oxidase positive (purple color). (d) GMKU 367 degraded starch (clear zone). (e) GMKU 359 utilized casein (clear zone). (f) GMKU 359 liquefied gelatin.

2.1.3 Chemical characteristics

2.1.3.1 Cell wall peptidoglycan

To determine acyl type of muramic acid in cell wall peptidoglycan, the method of Uchida and Aida (1984) was carried out and the color was detected by comparing with the standard strains. *Rhodococus jostii* K01-B0171 shows reddish purple (glycosyl type) while *Streptomyces albus* K99-5278 shows colorless (acetyl type). The results showed that GMKU 367, GMKU 359 and GMKU 370 showed colorless. Therefore, the peptidoglycan of the three isolates was acetyl type.

The isomer of diaminopimelic acid (DAP) in the cell wall was analyzed according to the method of Hasegawa *et al.* (1983). GMKU 367, GMKU 359 and GMKU 370 contained the *meso*-DAP in the cell wall peptidoglycan (Figure 25).

For the cell-wall amino acids, the results of TLC plate with Pico Tag HPLC showed that GMKU 370 contained alanine, glutamic acid and lysine

(Figure 26) while GMKU 367 contained alanine and glutamic acid (Figure 26) at a molar ratio of *meso*-DAP: alanine: glutamic acid = 1.0: 1.2: 1.0 (Figure 27).

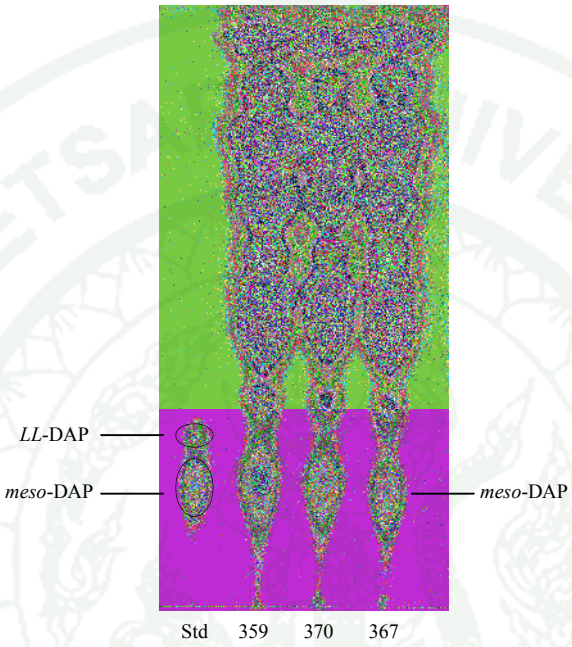


Figure 25 TLC analysis of the *meso*-DAP in the cell wall peptidoglycan of GMKU 367, GMKU 359 and GMKU 370.

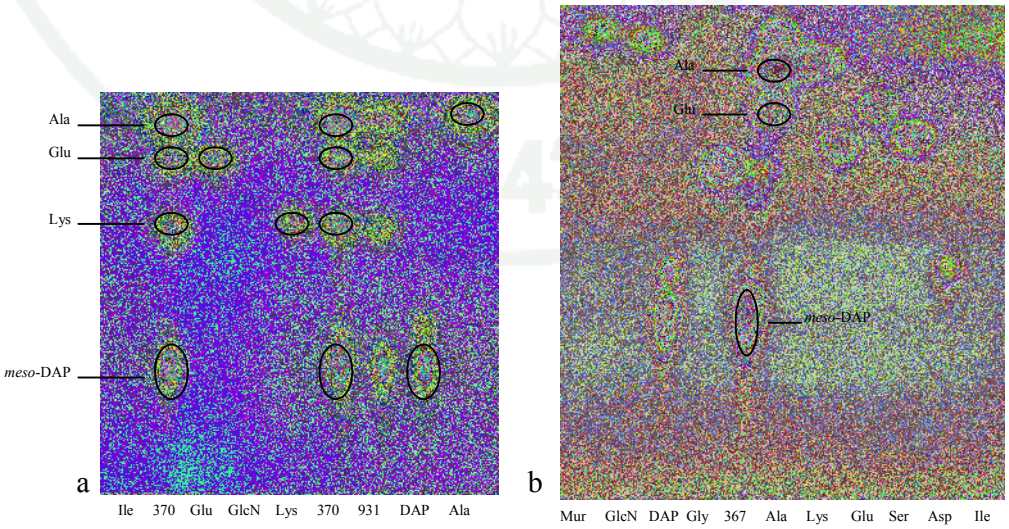


Figure 26 The TLC analysis of (a) GMKU 370 (b) GMKU 367.

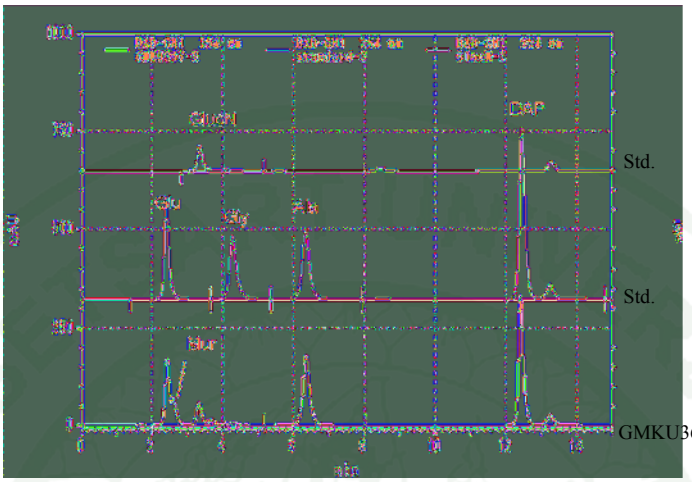


Figure 27 Pico Tag HPLC chromatogram of GMKU 367 contains glutamic acid, alanine and *meso*-DAP.

2.1.3.2 Cellular fatty acid composition

Composition of cellular fatty acid was analyzed by TechnoSuruga Co., Ltd (Japan) according to the instructions of the MIDI System by using a gas chromatograph (model HP6890; Hewlett Packard) (Sasser, 1990). The results of GMKU 367 showed the major fatty acids (above 10%) as iso-$C_{16:0}$ (40.24%), iso-$C_{15:0}$ (12.17%) and $C_{16:0}$ (10.39%) and minor fatty acids (3-10%) as iso-$C_{16:0}$ -2-OH (7.67%), $C_{15:0}$ (3.39%), iso-$C_{16:1}$ (3.27%) and iso-$C_{17:0}$ (3.15%). For GMKU 370, the major fatty acids were iso-$C_{16:0}$ (37.3%), iso-$C_{16:1}$ iso G (15.0%) and anteiso -$C_{17:0}$(13.7%) and minor fatty acids were $C_{17:1}$ ω9c (9.3%), 10-Methyl $C_{17:0}$ (6.9%) and anteiso-$C_{17:1}$ C (3.0%). For GMKU 359, the composition of cellular fatty acid was not analyzed.

2.1.3.3 Menaquinones

Isoprenoid quinones were determined by the method of Collins *et al*. (1977) using HPLC analysis. The results showed that diagnostic menaquinone of GMKU 367 was MK-9(H_4) (100%) while GMKU 370 were MK-9(H_6) (30%) and MK-9(H_8) (30%) with the minor was MK-9(H_2) (11%) and MK-9(H_4) (23%). GMKU 359 was MK-9(H_6) (16%) and MK-9(H_8) (84%).

2.1.3.4 Polar phospholipid

Phospholipids were extracted and determined by using the method of Minnikin *et al*. (1984) using TLC analysis. The results showed that the phospholipids of GMKU 367 included phosphatidylethanolamine (PE) and hydroxyphosphatidylethanolamine (OH-PE) representing type PII (Lechevalier *et al*., 1977). Phospholipids of GMKU 359 and GMKU 370 included phosphatidylglycerol (PG) and phosphatidylinositol (PI), representing type PI (Lechevalier *et al*., 1977).

2.1.3.5 Whole-cell sugars

Whole-cell sugars were analyzed according to the method of Becker *et al.* (1965) using paper chromatography. The results showed that the whole-cell sugars of GMKU 367 were arabinose, galactose, mannose, rhamnose and ribose as shown in the Figure 28. The sugars presented in whole-cell hydrolysates of GMKU 370 were galactose, glucose, madurose, mannose and ribose. Madurose was the characteristic sugar, indicating type B whole-cell sugars (Lechevalier & Lechevalier, 1970). The sugars presented in whole-cell hydrolysates of GMKU 359 were galactose, mannose and ribose.

Figure 28 Paper chromatography of GMKU 367 were arabinose, galactose, mannose, rhamnose and ribose and KA 605, standard sugar; galactose, glucose, manose, arabinose, madurose, xylose, ribose and rhamnose.

2.1.3.6 Mycolic acid

Mycolic acids were detected by TLC using the method of Tomiyasu (1982). No mycolic acid was detected in GMKU 367, GMKU 359 and GMKU 370.

All chemical characteristics of GMKU 367, GMKU 359 and GMKU 370 were shown in Table 12.

Table 12 Chemotaxonomic characterizations of GMKU 367, GMKU 359 and GMKU370.

Characteristics	**GMKU367**	**GMKU359**	**GMKU370**
Cell wall diamino acid	meso-DAP	meso-DAP	meso-DAP
Characteristic sugar(s)			
Galactose	+	+	+
Glucose	-	-	+
Mannose	+	+	+
Arabinose	+	-	-
Madurose	-	-	+
Ribose	+	+	+
Rhamnose	+	-	-
Polar lipids			
PE	+	-	-
OH-PE	+	-	-
PC	-	-	-
PG	-	+	+
PI	-	+	+
DPG	-	-	-
Menaquinone			
MK-9(H_0)	-	-	5%
MK-9(H_2)	-	-	11%
MK-9(H_4)	100%	-	23%
MK-9(H_6)	-	16%	30%
MK-9(H_8)	-	83%	30%
Mycolic acid	None	None	None
N-acyl type of muramic acid	Acetyl	Acetyl	Acetyl

Table 12 (continued)

Characteristics	GMKU367	GMKU359	GMKU370
Major fatty acid	isoC16:0 (40.24%) iso-$C_{15:0}$ (12.17%) $C_{16:0}$ (10.39%)	ND	iso-$C_{16:0}$ (37.3%) iso-$C_{16:1}$ iso G (15.0%) anteiso-$C_{17:0}$(13.7%)

ND = not determine

2.2 Genotypic methods

2.2.1 DNA base composition

G+C content was determined by HPLC as described by Tamaoka and Komagata (1984). The G+C content of GMKU 367, GMKU 359 and GMKU 370 was 71.1 mol%, 72.57 mol % and 65.37 mol %, respectively.

2.2.2 Analysis of almost complete sequence of 16S rRNA genes

2.2.2.1 GMKU 367

An almost-complete 16S rRNA gene sequence of GMKU 367 was determined. The sequence (1444 bp) was preliminarily compared with those of 16S rRNA genes available in the GenBank and EzTaxon database and revealed that strain GMKU 367 belonged to the member of suborder *Pseudonocardineae* of which comprises 2 family, *Actinosynnemataceae* and *Pseudonocardiaceae*. GMKU 367 showed high sequence identity values to *Kibdelosporangium aridum* DSM 43828^T (95.5%), *Streptoalloteichus hindustanus* IFO 15115^T (95.4%), *Actinokineospora enzanensis* IFO 16517^T (95.4%) and *Amycolatopsis nigrescens* CSC 17Ta-90^T

(95.3%) (Table 13). No other type strains in the suborder *Pseudonocardineae* showed higher sequence similarity than 95.5 % to GMKU 367.

Table 13 Sequence identity of GMKU 367 to member in suborder *Pseudonocardineae* based on an almost complete sequence of 16S rRNA genes.

Closest actinomycetes species	GenBank Accession no.	Sequence identity (%)	References
Kibdelosporangium aridum DSM 43828^T	AJ311174	95.5	Sheaere *et al*., 1986
Streptoalloteichus hindustanus IFO 15115^T	D85497	95.4	Tomita *et al*., 1987
Actinokineospora enzanensis IFO 16517^T	AB058395	95.4	Otoguro *et al*., 2003
Amycolatopsis nigrescens CSC 17Ta-90^T	DQ486888	95.3	Groth *et al*., 2007

The nearly complete 16S rRNA sequence (1444 bp) of GMKU 367 was aligned with the representative type strains of the other 23 genera of the suborder *Pseudonocardineae* using CLUSTAL_X 2 (Larkin *et al*., 2007).

The phylogenetic trees were deduced using methods of neighbour-joining (Saitou & Nei, 1987), maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Eck & Dayhoff, 1966). The resultant neighbour-joining tree topology was evaluated the confidence values of branches by 1000 resamplings of bootstrap analysis (Felsenstein, 1985). The evolutionary distances were computed by using the Kimura 2-parameter method (Kimura, 1980). Strain GMKU 367 was phylogenetically separated from *Kibdelosporangium aridum* DSM 43828^T and *Amycolatopsis nigrescens* CSC 17Ta-90 ^{T} (Figure 29). It is evident from the phylogenetic tree that GMKU 367 formed a distinct subclade within the members of the family *Pseudonocardiaceae.* For the family *Pseudonocardiaceae*, it was firstly described by Embley *et al*. (1988), and was emended by Stackebrandt *et al*. (1997) on the basis of 16S rRNA gene sequence analysis. The family currently comprises 17 genera with validly published names, including *Actinoalloteichus* (Tamura *et al*.,

2000), *Actinomycetospora* (Jiang *et al*., 2008), *Actinopolyspora* (Gochnauer *et al*., 1975), *Allokutzneria* (Labeda & Kroppenstedt, 2008), *Amycolatopsis* (Lechevalier *et al*., 1986), *Crossiella* (Labeda, 2001), *Goodfellowiella* (Labeda *et al*., 2008), *Kibdelosporangium* (Shearer *et al*., 1986), *Kutzneria* (Stackebrandt *et al*., 1994), *Prauserella* (Kim & Goodfellow, 1999), *Pseudonocardia* (Henssen, 1957), *Saccharomonospora* (Nonomura & Ohara, 1971), *Saccharopolyspora* (Lacey & Goodfellow, 1975), *Sciscionella* (Tian *et al*., 2009), *Streptoalloteichus* (Tomita *et al*., 1987), *Thermobispora* (Wang *et al*., 1996) and *Thermocrispum* (Korn-Wendisch *et al*., 1995).

Strain GMKU 367 was recovered as a sister group of the genus *Kibdelosporangium*. Although the bootstrap value of neighbour-joining tree at the corresponding node is moderate (43%), the close relationship was solidly supported by maximum-likelihood and maximum-parsimony trees (Figure 29).

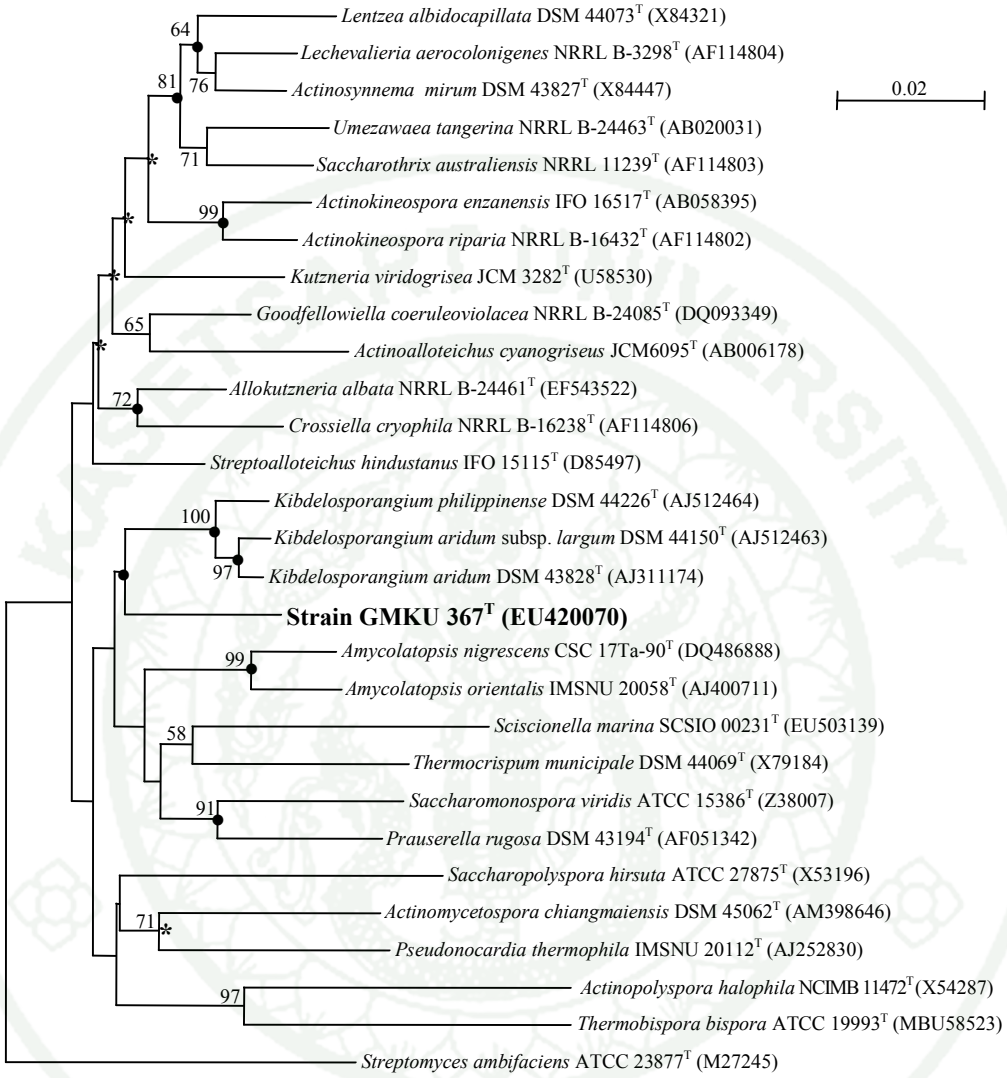


Figure 29 Phylogenetic tree for taxa of suborder *Pseudonocardineae* was constructed using the neighbour-joining method based on almost complete 16S rRNA sequences to display the taxonomic position of GMKU 367. *Streptomyces ambofaciens* ATCC 23877^T was used as the out group. Numbers at nodes indicate levels of bootstrap support (%) based on neighbour-joining analysis of 1000 resampled datasets; only values above 50 % are shown. Solid circles indicate corresponding nodes that were also recovered from maximum-likelihood and maximum-parsimony trees. Stars indicate corresponding nodes that were recovered from maximum-parsimony trees. Bar, 0.02 substitutions per site.

From the results of almost complete sequence of 16S rRNA gene and phylogenetic analysis, *Amycolatopsis orientalis* IMSNU 20058^T (data from Lechevalier *et al*., 1986; Lee and Hah, 2001), *Kibdelosporangium aridum* DSM 43828^T (Shearer *et al*.1986; Labeda & Kroppenstedt, 2008), *Pseudonocardia thermophila* ATCC 19285^T (Warwick *et al*., 1994; Reichert *et al*., 1998; Huang *et al*., 2002; Park *et al*., 2008), *Saccharopolyspora hirsuta* NRRL B-5792^T (Korn-Wendisch *et al*., 1989) and *Thermocrispum municipale* DSM 44069^T (Korn-Wendisch *et al*., 1995) were selected to compare morphological and chemotaxonomic characteristics with GMKU 367. The results revealed that morphological and chemotaxonomic characteristics of GMKU 367 distinguished from closely related members in the family *Pseudonocardiaceae*. Scanning electron microscopic observations of GMKU 367 showed cylindrical spore on aerial mycelium (Figure 33) but did not reveal sporangium-like structure or fragmentation of substrate mycelium which is absolutely distinct from the nearest neighbouring genus *Kibdelosporangium* and phylogenetically closely related genera. Phospholipid profile of strain GMKU 367 is clearly distinct from other closely related genera as it contains only phosphatidylethanolamine and hydroxyphosphatidylethanolamine without the present of phosphatidylcholine, phosphatidylglycerol, diphosphatidylglycerol or phosphatidylinositol. The fatty acid profile of GMKU 367 is also dissimilar from those of phylogenetically nearest genera as shown in Table 14.

Table 14 Comparison of the morphological and chemotaxonomic profiles of GMKU 367 with those of phylogenetically nearest genera.

Characters	1	2	3	4	5	6
Morphology:						
Aerial hyphae	+	v	+	v	+	+
Fragmented mycelium	−	+	+	v	+	−
Sporangium-like	−	−	+	−	−	+
Whole-cell sugar pattern	Ara, Gal, Man, Rha, Rib	Ara, Gal	Ara, Gal, Glc, Mad^{t}, Man, Rha, Rib	Ara, Gal	Ara, Gal	Ara, Gal^{t}, Glc, Man
Phospholipids	PE, OH-PE	PE, DPG, PI, PIM	PE, PME, PG, PI, PIM, DPG	PC, PE, PME	PC, PE, *lyso*-PE, PME, DPG, PI, PG	PE, OH-PE, PI
Major menaquinone(s)	MK-9(H_4)	MK-9(H_2), MK-9(H_4)	MK-9(H_4)	MK-8(H_4) or MK-9(H_0)	MK-9(H_4)	MK-9(H_4)
Major fatty acids	i-$C_{15:0}$, i-$C_{16:0}$ $C_{16:0}$	i-$C_{15:0,}$ i-$C_{16:0,}$ ai-$C_{17:0}$, $C_{17:0}$	ai-$C_{15:0,}$ i-$C_{16:0}$, ai-$C_{17:0}$	i-$C_{16:0}$	i-$C_{15:0,}$ i-$C_{16:0}$, i-$C_{17:0,}$ ai-$C_{17:0}$	i-$C_{16:0}$
G+C content (mol%)	71.1	66−69	66	68−79	70.4−71.5	69−73

1, GMKU 367; 2, *Amycolatopsis orientalis* IMSNU 20058^{T} (data from Lechevalier *et al*., 1986; Lee, S.D. & Hah, Y.C., 2001); 3, *Kibdelosporangium aridum* DSM 43828^{T} (Shearer *et al*.1986; Labeda & Kroppenstedt, 2008); 4, *Pseudonocardia thermophila* ATCC 19285^{T} (Warwick *et al*., 1994; Reichert *et al*., 1998; Huang *et al*., 2002; Park *et al*., 2008); 5, *Saccharopolyspora hirsuta* NRRL B-5792^{T} (Korn-Wendisch *et al*., 1989); 6, *Thermocrispum municipale* DSM 44069^{T} (Korn-Wendisch *et al*., 1995).–, absent; v, variable; ND, no data, ^{t}, trace.

On the basis of combination of the significant difference data of phylogenetic, chemotaxonomic and morphological analyzes, strain GMKU 367 accommodated in the family *Pseudonocardiaceae* and should be assigned as representing a novel genus and species, for which the name *Actinophytocola oryzae* gen.nov., sp.nov. is proposed. For nomenclature, the type strain GMKU 367^T (= BCC 31372^T, = NBRC 105245^T) was *Actinophytocola* [Ac.ti.no.phy´to.co.la.Gr.n. *actis*, *actinos* a ray, beam; Gr.n. *phyto* (from Gr.n.*phuton*) plant; L.masc.suff.-*cola* (from L.n.*incola*) a dweller, inhabitant; N.L.masc.n. *Actinophytocola* actinobacterial dweller inside plant], *Actinophytocola oryzae* [o.ry'zae.Gr.n.*oryza*, rice; M.L.gen.n.*oryzae*; denoting the isolation of the strain from root of Thai glutinous rice plant (*Oryza sativa* L.cv. RD6)].

2.2.2.1.1 Description of *Actinophytocola* gen.nov.

Aerobic, Gram-positive, non-acid-fast, non-motile actinomycetes. Non-fragmented substrate mycelium and, on some media, aerial mycelia are produced. Cylindrical spores are produced on aerial mycelia but not sporangium-like. Good growth occurs at 18-28 °C. The organism shows good growth on oatmeal agar and oatmeal-nitrate agar. Cell wall contains *meso*-diaminopimelic acid, alanine, glutamic acid and acetylated muramic acid. The whole-cell sugars are arabinose, galactose, mannose, rhamnose and ribose. Mycolic acids are absent. The major fatty acids are iso-$C_{15:0}$, iso-$C_{16:0}$ and $C_{16:0}$. The diagnostic menaquinone is MK-9(H_4). The polar phospholipids consist of phosphatidylethanolamine and hydroxyphosphatidylethanolamine. The type species is *Actinophytocola oryzae*.

2.2.2.1.2 Description of *Actinophytocola oryzae* sp.nov.

In addition to the characteristics given in the genus description, the type species has the following properties. Strain GMKU 367^T grows well on ISP 3 and oatmeal-nitrate agar, moderate growth on ISP 2 and 1/10 yeast extract starch agar, and poor growth on ISP 4 and ISP 5. The pale peach (5ca)

aerial mycelium produces on ISP 3, substrate mycelium is light melon yellow (3ea) and no soluble pigment. The trace of rust tan (5le) soluble pigment produces on oatmeal-nitrate agar. Absence of aerial mycelium on ISP 2 and 1/10 yeast extract starch agar. No growth on ISP 9 and ISP 6. Temperature range for growth is 12−30 °C, with the optimal temperature for good growth at 18–28 ºC. The strain is able to grow between pH 5.0−10.0, with the optimal pH at 6.0−7.0 and tolerates NaCl up to 2%. Reduction of nitrate and production of melanin pigments and H_2S are negative. Catalase is positive but oxidase is negative. Degradation of starch is positive while decomposition of benzoate, hypoxantine, xanthine and milk are negative. Liquefaction of gelatin is weakly positive. Degradation of citrate is positive. D-fructose, D-glucose and L-rhamnose can be utilized as sole carbon sources, but L-arabinose, dulcitol, D-galactose, *myo*-inosital, lactose, maltose, D-mannitol, D-mannose, raffinose, D-sorbitol, sucrose, D-trehalose and D-xylose are not utilized. Production of acid is observed from L-arabinose, D-fructose and D-glucose, while no acid produces from adonitol, dulcitol, erythritol, D-galactose, *myo*-inositol, lactose, D-mannitol, raffinose and L-rhamnose.

2.2.2.2 GMKU 370

The almost complete 16S rRNA gene sequence (1468 bp) of GMKU 370 was preliminary compared with 16S rRNA genes in the GenBank and EzTaxon database. The result indicated the close relationship with genus *Actinomadura*, particularity *A. spadix* that GMKU 370 shared 97.8% identity.

However, *A. spadix* is a distinct species possessing low 16S rRNA gene similarity with the other species in the genus *Actinomadura* (Nonomura & Ohara, 1971). Polyphasic investigation revealed that *A. spadix* is clearly distinguished at the genus level from other members of *Actinomadura* species and therefore, was assigned the status as new genus, *Actinoallomurus spadix* comb. nov. (Tamura *et al*., 2009). The genus *Actinoallomurus* is the newest genus belonging to the family *Thermomonosporaceae* (Tamura *et al*., 2009). This family includes *Actinocorallia*, *Actinomadura, Excellospora, Spirillospora* and

Thermomonospora (Kroppenstedt & Goodfellow, 1991; Zhang *et al*., 1998; 2001). Within this genus, other 8 species isolated from the soil and dung samples collected from various places in Japan were also chemotaxonomically characterized and were proposed as novel species belonging to the new genus *Actinoallomurus* with *Actinoallomurus spadix* as the type species (Tamura *et al*., 2009). Thus, an almost-complete 16S rRNA gene sequence of GMKU 370 was recompared. The result revealed that the sequence close relationship with members of the genus *Actinoallomurus* (Tamura *et al*., 2009).

For phylogenetic tree analysis of GMKU 370, multiple alignments of the sequences obtained from 10 validly described *Actinoallomurus* species (Tamura *et al*., 2009; Thamchaipenet *et al*., 2011) and the GMKU 370 (using *Actinomadura madurae* NBRC 14623^T as an outgroup) was performed using CLUSTAL X, version 2 (Larkin *et al*., 2007). The phylogenetic trees were deduced using methods of neighbour-joining (Saitou & Nei, 1987), maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Eck & Dayhoff, 1966) and constructed by NJplot (Perrière & Gouy, 1996), phylip 3.68 and MEGA 4 (Tamura *et al*., 2007), respectively. The resultant neighbour-joining tree topology was evaluated the confidence values of branches by 1000 resamplings of bootstrap analysis (Felsenstein, 1985). The evolutionary distances were computed by using the Kimura 2-parameter method (Kimura, 1980). The result of the phylogenetic analysis indicated that GMKU 370 formed a distinct clade within the members of the genus *Actinoallomurus* (Figure 30). The closest phylogenetic neighbour were *A.coprocola* NBRC 103688^T and *A.iriomotensis* NBRC 103685^T with the level of 16S rRNA gene sequence similarity at 99.20 % corresponding to 11 nucleotides substitutions with both of the type strains.

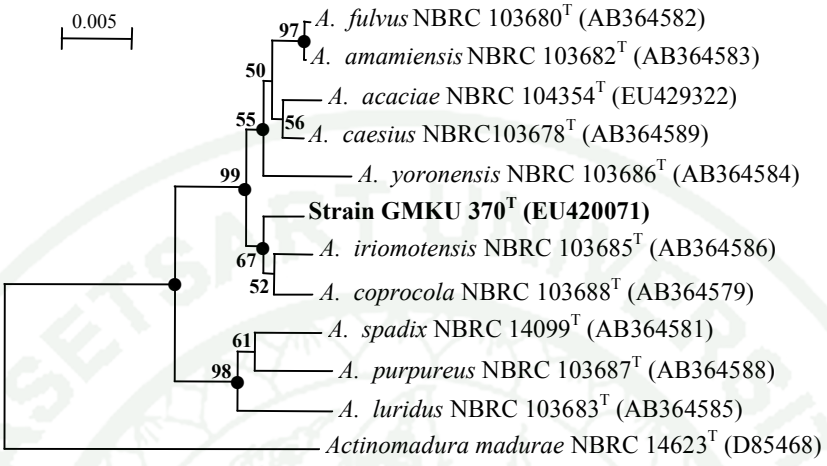


Figure 30 Phylogenetic tree was constructed by using the neighbour-joining method based on almost complete of the 16S rRNA sequences to display relationship between strain GMKU 370^T and all members of the genus *Actinoallomurus*. *Actinomudura madureae* was used as an outgroup. Numbers at nodes indicate levels of bootstrap support (%) based on neighbour-joining analysis of 1000 resampled datasets. Solid circles indicate corresponding nodes that were also recovered from maximum-likelihood and maximum-parsimony trees. Bar, 0.005 substitutions per site.

2.2.2.2.1 DNA - DNA hybridization

The analysis of 16S rRNA sequence produces numerical values of 16S rRNA similarities that can be used as circumscription of a species. Strains that show ≤ 97% 16S rRNA sequence similarity to all known taxa are considered to belong to a new species and isolates that have ≥ 97% identity is not as clear. This latter value can represent a new species or, alternatively, indicate clustering within a previously defined taxon (Amann *et al*., 1992; Stackebrandt and Goebel, 1994; Vandamme *et al*., 1996; Stackebrandt *et al.,* 2002; Gevers *et al*., 2005 and Janda and Abbott, 2007). For this reasons, the percent DDH is unequivocally the gold standard for proposed new species (Wayne *et al*., 1987). Thus, DDH of GMKU

370 was employed using the method of Ezaki *et al*. (1989) to determine the distinct taxonomic relationship among closely related species at species level.

Genomic DNA of GMKU 370 type strains, *A.iriomotensis* NBRC 103685^T and *A.coprocola* NBRC 103688^T were extracted and purified according to the method of Saito & Miura (1963) and DDH was carried out by fluorometric hybridization method (Ezaki *et al.*, 1989). Percentage of hybridization was detected by using Cytofluor multi-well plate reader series 4000. DNA-DNA relatedness value among type strains *A.coprocola* NBRC 103688^T and *A.iriomotensis* NBRC 103685^T with GMKU 370 were 17 % and 51 % (mean of duplication), respectively. Thus, the results clearly indicated that GMKU 370 does not belong to either *A. coprocola* NBRC 103688^T or *A. iriomotensis* NBRC 103685^T as this value were well below the threshold value of 70 % for the definition of bacterial species according to Wayne *et al*. (1987).

For the description of the genus *Actinoallomurus*, it contains *meso*-diaminopimelic acid in the cell wall and madurose as a characteristic sugar in the whole-cell hydrolysates the same as closely related genus *Actinomadura* (Lechevalier & Lechevalier, 1970; Goodfellow, 1989). Additionally, the cell wall peptidoglycan encloses D- and L-lysine that is an important differentiated characteristic to distinct *Actinoallomurus* from members in the genus *Actinomadura* (Tamura *et al*., 2009). The acyl type of the muramic acid is *N*-acetyl. The fatty acid profiles include iso-hexadecanoic (iso-$C_{16:0}$) as the major component and the phospholipid pattern comprises phosphatidylinositolmannoside. Main menaquinones are MK-9(H_6) and MK-9(H_8). Thus, comparison of the morphological and chemotaxonomic profiles of GMKU 370 with the closest phylogenetic related species, *A.coprocola* NBRC 103688^T and *A.iriomotensis* NBRC 103685^T was carried out (Table 15).

Table 15 Phenotypic characteristics that differentiate strain GMKU 370 from the type strains of the most phylogenetically related *Actinoallomurus* species.

Characteristics	1	2	3
Growth on ISP2			
Growth	Poor	Absent	Good
Color of substrate mycelium	Pale yellow brown		Moderate orange yellow
Soluble pigment	None		None
Growth on ISP3			
Growth	Moderate	Good	Moderate
Color of substrate mycelium	White	Gray purplish red	Light yellow to moderate orange
Soluble pigment	None	Moderate to strong yellow	None
Growth on ISP4			
Growth	Absent	Moderate	Poor
Color of substrate mycelium		Pale yellow	Light yellow
Soluble pigment		None	None
Growth on YS agar			
Growth	Moderate	Moderate	Poor
Color of substrate mycelium	White	Pale yellow	White
Soluble pigment	None	Pale greenish green	None
Range of temperature for growth (°C)	20–44	20–37	20–37
Growth at 2% NaCl	-	+	+
Growth at pH 8–9	+	-	-
Utilization of:			
Arabinose	-	+	+
D-Fructose	-	+	+

Table 15 (continued)

Characteristics	1	2	3
Glucose	-	+	+
D-Mannitol	-	+	+
Hydrolysis of:			
Starch	-	+	+
Gelatin	w	+	+

Strains: 1, GMKU 370^T; 2, *A. coprocola* NBRC 103688^T; 3, *A. iriomotensis* NBRC 103685^T (strains: 2 and 3, data from Tamura *et al*., 2009) +, Positive; –, negative; v, variable; w, weakly positive.

The comparison revealed that GMKU 370 exhibits phenotypic characters dissimilar to both type strains. GMKU 370 generated whitish aerial mycelia on ISP 3 and did not produce soluble pigment on any media tested. The strain was able to grow at temperature range 20-44 °C and moderately alkaline condition (pH 8-9) but not tolerate NaCl at 2 %. GMKU 370 was not able to utilize arabinose, fructose, glucose and mannitol, did not degrade starch but poorly hydrolyzed gelatin and not reduced nitrate.

Based on the chemotaxonomic and phylogenetic analysis, it is confirmed that strain GMKU370^T belongs to the genus *Actinoallomurus*. Phenotypic and genotypic data demonstrated in this study supported that strain GMKU 370^T represents a species belonging to the genus *Actinoallomurus*. It is also evident from physiological characteristics and the levels of DNA-DNA relatedness that strain GMKU 370^T is distinct from the type strains of closely phylogenetic related species. It is, therefore, proposed that strain GMKU 370^T represents a novel species of the genus *Actinoallomurus*, for which the name *Actinoallomurus oryzae* sp. nov. is proposed. For nomenclature, the type strain *Actinoallomurus oryzae* GMKU 370^T (BCC 31373^T = NBRC 105246^T), [o.ry'zae. Gr.n.*oryza*, rice; M.L.gen.n. *oryzae*; denoting the isolation of the strain from root of Thai jasmine rice plant

(*Oryza sativa* L.cv. KDML 105)].

2.2.2.2.2 Description of *Actinoallomurus oryzae* sp.nov.

Aerobic, Gram-positive. Cells grow well on PDA and NA and show moderate growth on ISP 3, oatmeal-nitrate agar and 1/10 yeast-starch agar, forming a well-developed white aerial mycelium that differentiated into short spiral spore chain with smooth surfaced. Neither diffusible pigment nor melanin is produced. The optimal temperature and pH for growth are 30–32 °C and pH 5.0-9.0. The strain be able to grow in 1% NaCl. Catalase and oxidase positive. Nitrate reduction is negative. Hydrolysis of casein is positive and gelatine is weakly positive. Degradation of hypoxantine, starch and xanthine are negative. No peptonization of milk. Dulcitol, D-galactose, *myo*-inosital, β-lactose, D-maltose, D-mannose, raffinose, L-rhamnose, D-sorbitol, sucrose, D-trehalose and D-xylose are utilized as sole carbon sources but L-arabinose, D-fructose, D-glucose, D-mannitol are not. Acid production appears from D-galactose, D-glucose and raffinose but not from L-arabinose, dulcitol, D-fructose, *myo*-inositol, β-lactose, D-mannitol and L-rhamnose. Strain GMKU 370^T contained *meso*-diaminopimelic acid and lysine in the cell wall peptidoglycan. Whole cell sugars contain galactose, glucose, madurose, mannose and ribose. The glycan moiety of the murein is acetylated. The predominant menaquinones are MK-9(H_6) and MK-9(H_8). The minor menaquinones are MK-9(H_4) and MK-9(H_2) and MK-9(H_0) is also detected. No mycolic acids. The major fatty acid is iso-$C_{16:0}$. Main phospholipid pattern comprises phosphatidylglycerol and phosphatidylinositol. The G+C content of the DNA is 65.4 mol %.

2.2.2.3 GMKU 359

Almost complete 16S rRNA gene sequence of GMKU 359 was preliminary compared with 16S rRNA genes in the EzTaxon database and indicated the close relationship with genus *Actinomadura*, *A.spadix* that GMKU 359 shared 97.7% identity. However, *A.spadix* was later assigned the status as new genus,

Actinoallomurus spadix comb.nov. (Tamura *et al*., 2009). Therefore, GMKU 359 was re-compared with the members of *Actinoallomurus.* The phylogenetic trees were deduced and the result indicated that GMKU 359 formed a distinct clade within the members of the genus *Actinoallomurus* (Figure 31). The closest phylogenetic neighbour were *A. iriomotensis* NBRC 103685^{T} with the 100% bootstrap support (Figure 31).

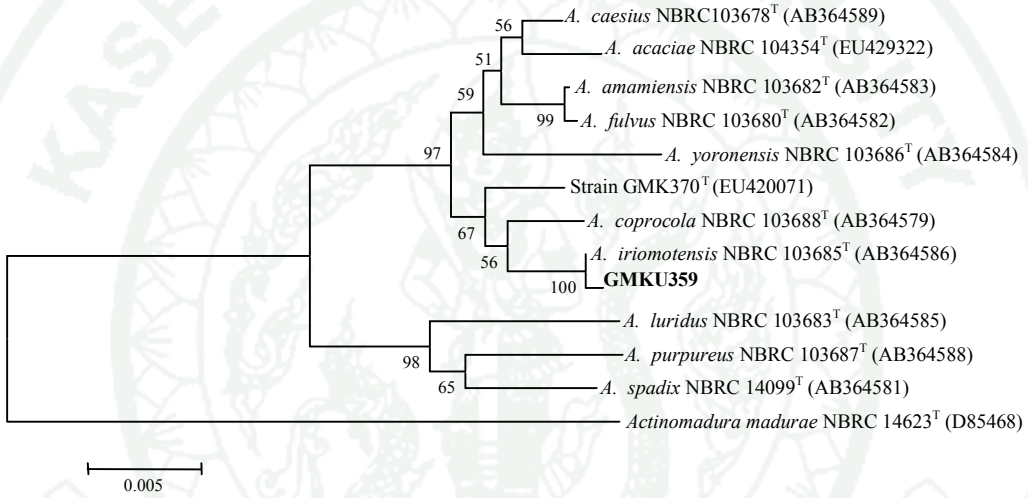


Figure 31 Phylogenetic tree was constructed by using the neighbour-joining method based on almost complete of the 16S rRNA sequences to display relationship between strain GMKU 359 and all members of the genus *Actinoallomurus*. *Actinomudura madureae* was used as an outgroup. Numbers at nodes indicate levels of bootstrap support (%) based on neighbour-joining analysis of 1000 resampled datasets. Bar, 0.005 substitutions per site.

2.2.2.3.1 DNA-DNA hybridization

DNA-DNA relatedness value among type strains *A. iriomotensis* NBRC 103685^{T} with GMKU 359 were 73%. Thus, the results clearly indicated that GMKU 359 belong to *A. iriomotensis* because the value was higher than the threshold value of 70% for the definition of bacterial species according to Wayne *et al*. (1987).

2.3 Diversity

The results of endophytic actinomycetes identification in this study showed that the majority (n=33, 50%) of endophytic actinomycetes belonged to genus *Streptomyces* and the remainder belonged to 10 genera including *Microbispora* (n=14), *Micromonospora* (n=7), *Actinomadura* (n=4), *Actinoallomurus* (n=2; GMKU 370 is a newly propose species), *Nocardia* (n=1), *Nocardiopsis* (n=1), *Nonomuraea* (n=1), *Promicromonospora* (n=1), and *Saccharopolyspora* (n=1) and a newly propose genus *Actinophytocola* (Figure 32).

This study is different from several reports that *Streptosporangium* spp. were commonly isolated and are the common endophytic microflora of a variety of plant species ranging from crop plants to woody tree species (Tan *et al*., 2006; Qin *et al*., 2011). There were 11 genera discovered in this study demonstrated that endophytic actinomycetes were diversed in endemic Thai tropical plants. This results supported several reports that the greatest diversity of endophytic actinomycetes is likely to occur in the tropical plants (Strobel and Dai, 2003; Qin *et al*., 2009). Likewise, Taechowisan *et al*. (2003) isolated 330 actinomycetes from 26 different Thai medicinal plant species and they belonged to four different genera (*Streptomyces*, *Microbispora*, *Nocardia* and *Micromonospora*). Moreover, there are a novel genus and a novel species verified in this study which demonstrated that endemic plants of Thailand are a novel valuable source of novel strains.

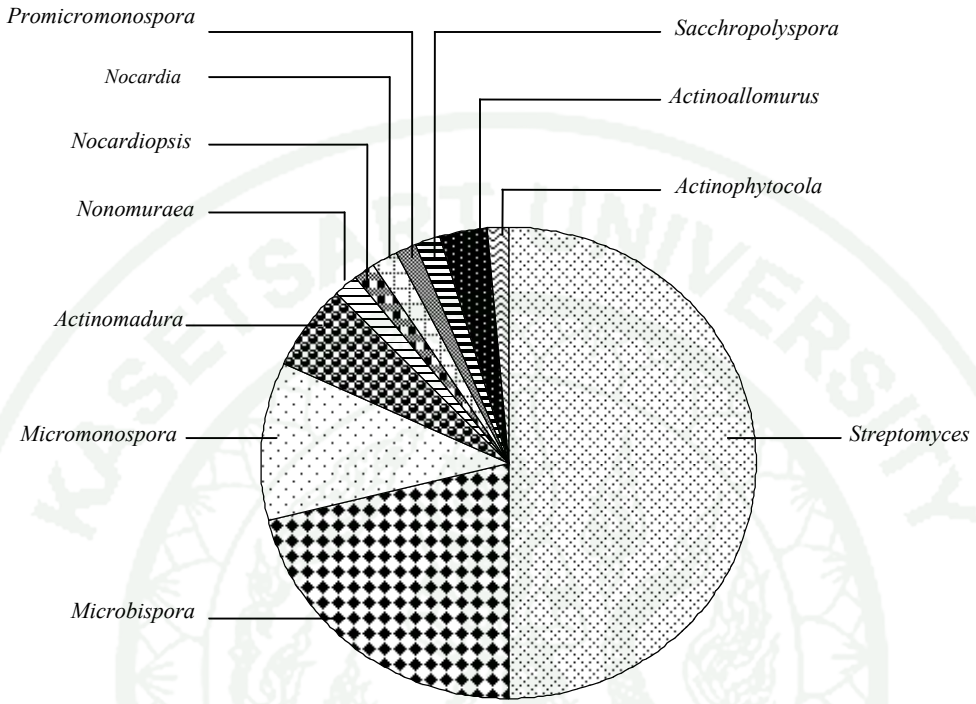


Figure 32 The diagram displays percentage of all actinobacterial endophytes (n=66) isolated from plant materials. The majority (n=33,) belonged to genus *Streptomyces* and the remainder belonged to 10 genera including *Microbispora* (n=14), *Micromonospora* (n=7), *Actinomadura* (n=4), *Actinoallomurus* (n=2), *Nocardia* (n=1), *Nocardiopsis* (n=1), *Nonomuraea* (n=1), *Promicromonospora* (n=1), *Saccharopolyspora* (n=1) and *Actinophytocola* (n=1).

3. Determination of plant growth promoting agents

3.1 Auxin production

Auxin production was analyzed by colorimetric assay using method modified from Ahmad *et al*. (2006). The results revealed that 13 isolates (20%) could produce auxin of which 12 isolates belonged to genus *Streptomyces* namely GMKU 306, GMKU 307, GMKU 308, GMKU 311, GMKU 334, GMKU 335, GMKU 342, GMKU 343, GMKU 352, GMKU 354, GMKU 361, GMKU 365 and an isolate GMKU 377 belonged to genus *Saccharopolyspora* (Table 16). Auxin is a key plant hormone which plays important role in cell division but bacteria can also synthesis auxin (Sarwar and Kremer, 1995 and Tao *et al*., 2002). Soil or rhizosphere actinomycetes revealed ability to produce IAA but a little work has shown that endophytic actinomycetes were also able to produce auxin (El-Tarabily *et al*., 1997; El-Tarabily and Sivasithamparam, 2006; El-Tarabily *et al*., 2008). In 2002, Igarashi *et al*. isolated *Streptomyces hygroscopicus* from *Pteridium aquilinum* and reported that the strain was able to produce auxin-like compound. Endophytic actinomycetes isolated from cucumber, tomato and eagle wood showed ability to produce IAA (El-Tarabily *et al*., 2009; Fialho *et al*., 2010 and Nimnoi *et al*., 2010) and they belonged to genus *Actinomadura*, *Nocardia*, *Nonomuraea*, *Pseudonocardia* and*Streptomyces* . In this study, 13 isolates of endophytic actinomycetes belonged to genus *Streptomyces* and *Saccharopolyspora* could produce IAA. Thus, it is firstly reported that an endophytic *Saccharopolyspora* isolated form Thai medicinal plant *Myristica fragrans* Linn. showed ability to produce IAA.

3.2 Phosphate solubilization

To determine the solubilization of phosphate, the isolates were cultured onto Pikovskaya's medium (Pikovskaya, 1948) and the formation of transparent "halo" zone around each bacterial plug was observed (Figure 33). The results revealed that 20 isolates (30%) of endophytic actinomycetes namely, *Streptomyces* sp. GMKU 301, GMKU 306, GMKU 307, GMKU 308, GMKU 309, GMKU 312, GMKU 314, GMKU 316, GMKU 319, GMKU 336, GMKU 337, GMKU 344,

GMKU 345, GMKU 354, and GMKU 355 as well as *Actinoallomurus* sp. GMKU 359, *Microbispora* sp. GMKU 318 and GMKU 324, *Nocardiopsis* sp. GMKU 346 and *Saccharopolyspora* sp. GMKU 377 could solubilize phosphate (Table 16). Recently, endophytic actinomycetes isolated from tomato, wheat and rice have been reported to solubilize phosphate (Fialho *et al*., 2010; Misk and Franco, 2011; Gangwar *et al*., 2012a; 2012b) and they belonged to genus *Streptomyces*. In this study, the ability of phosphate solubilization not only found in the members of the genus *Streptomyces* but also found in several genera of rare actinobacteria including *Actinoallomurus*, *Microbispora*, *Nocardiopsis* and *Saccharopolyspora*. Phosphate solubilizing bacteria (PSB) is a group of beneficial bacteria capable of hydrolysing organic and inorganic phosphorus from insoluble compounds (Young *et al*., 1998; Goldstein *et al*., 1999; Chen *et al*., 2006). This study firstly demonstrated that *Streptomyces*, *Actinoallomurus*, *Microbispora*, *Nocardiopsis* and *Saccharopolyspora* could be grouped as PSB.

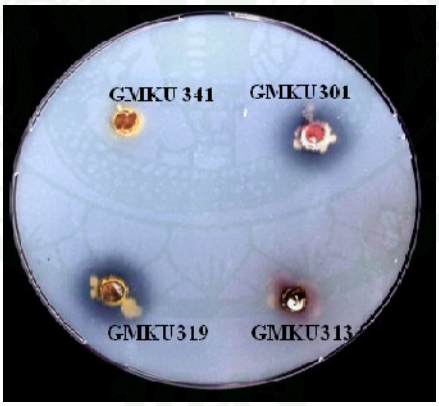


Figure 33 The formation of halo zone around plug of endophytic *Streptomyce* sp. GMKU 301 and *Streptomyces* sp. GMKU 319 on PVK medium after incubation at 30 ºC for 3 weeks.

3.3 Siderophore production

Siderophores was detected using modified method of Schwyn *et al*. (1987) by observation of orange halo zone formation around each bacterial plug. The results revealed that 33 isolates (50 %) showed orange halo zone indicated they could produce siderophores (Figure 34). Most of siderophore producing strains belonged to genus *Streptomyces* (18 isolates; GMKU 306, GMKU 307, GMKU 308, GMKU 313, GMKU 314, GMKU 315, GMKU 316, GMKU 333, GMKU 334, GMKU 335, GMKU 336, GMKU 344, GMKU 345, GMKU 352, GMKU 354, GMKU 355, GMKU 360 and GMKU 361) and the remainder belonged to 7 genera including *Actinoallomurus* (GMKU359 and GMKU 370), *Actinomadura* (GMKU 329, GMKU331 and GMKU 351), *Microbispora* (GMKU 363, GMKU 364 and GMKU 366), *Micromonospora* (GMKU 328, GMKU 330, GMKU 358 and GMKU 362), *Nocardiopsis* (GMKU 346), *Saccharopolyspora* (GMKU 377) and a new genus *Actinophytocola oryzae* GMKU 367 (Table 16). In previous reports, siderophores production was observed in several genera of endophytic actinomycetes including *Actinomadura*, *Kibdelosporangium*, *Nocardia*, *Nonomuraea*, *Streptomyces* and *Pseudonocardia* (Nimnoi *et al*., 2010; Misk and Franco, 2011; Xing *et al.*, 2011). In this study, the results showed that there were diversity of genera including *Actinoallomurus*, *Actinomadura*, *Actinophytocola Microbispora*, *Micromonospora*, *Nocardiopsis*, *Saccharopolyspora* and *Streptomyces* to produce siderophores.

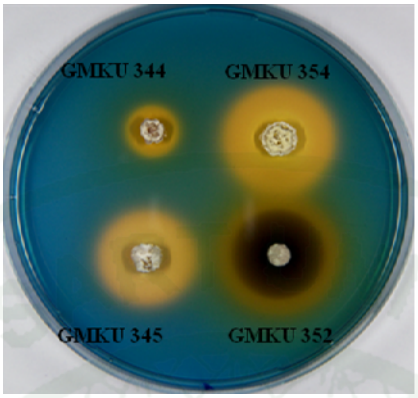


Figure 34 The orange halo zone of siderophore formation on Chrome Azurol S (CAS) medium around plugs of *Streptomyces* sp. GMKU 344, GMKU 345, GMKU 354 and GMKU 352.

3.4 Ammonia production

Ammonia production was studied by growing the cultures in peptone water broth and observed development of yellow to brown using Nesler's reagent. The results showed that 34 isolates (52%) produced ammonia (Table 16). *Streptomyces* are ammonifying bacteria (Savant and DeDatta, 1982; Kadlec and Knight, 1996; Rebollido *et al*., 2008). In this study, there were 34 isolates that showed ability of ammonifying bacteria of which 27 isolates (74%) belonged to genus *Streptomyces* including GMKU 301, GMKU 302, GMKU 305, GMKU 306, GMKU 309, GMKU 312, GMKU 314, GMKU 315, GMKU 316, GMKU 319, GMKU 320, GMKU 322, GMKU 323, GMKU 333, GMKU 335, GMKU 336, GMKU 337, GMKU 341, GMKU 342, GMKU 343, GMKU 344, GMKU 345, GMKU 352, GMKU 354, GMKU 355, GMKU 360 and GMKU 361 and the remainder were rare actinomycetes belonged to 3 genera including *Microbispora* (GMKU 363), *Micromonospora* (GMKU 326, GMKU 330, GMKU 348, GMKU 358 and GMKU 362) and *Promicromonospora* (GMKU 303).

3.5 ACC deaminase

All isolates were screened for their potential to produce ACC deaminase using minimum media (Hopwood, 1967) supplemented with either 3.0 mM ACC or 2g $(NH_4)_2SO_4$ (El-Tarabily, 2008). Growth on ACC as a sole source of nitrogen indicated bacteria were able to produce ACC deaminase. For example, Figure 35 showed *Streptomyces* sp. GMKU 336 was able to grow in minimum media supplemented with 3.0 mM ACC while no growth was observed in minimum media. In this study, the results showed that 16 isolates (24%) of endophytic actinomycetes were able to grow on ACC as a sole source of nitrogen including *Streptomyces* (11 isolates; GMKU 308, GMKU 313, GMKU 314, GMKU 315, GMKU 316, GMKU 319, GMKU 320, GMKU 335, GMKU 336, GMKU 344 and GMKU 345), *Microbispora* (GMKU 363 and GMKU 364), *Nonomuraea* (GMKU 325) and *Micromonospora* (GMKU 362) and a new genus *Actinophytocola oryzae* GMKU 367 (Table 16). However, the ACC deaminase production has been found to be associated with a large number of different soil microorganisms and the high frequency has been found in many rhizospheric bacteris (Glick *et al*., 2007; El-Tarabily *et al*., 2009).

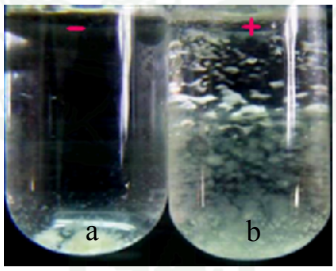


Figure 35 ACC deaminase production of *Streptomyces* sp. GMKU 336 (a) no growth in minimum media (b) growth in minimum media supplemented with 3.0 mM ACC.

Table 16 Production of plant growth promoting agents of all isolates

Isolates	ACCD production	Ammonia production	IAA production	Siderophore production	Phosphate solubiliza-tion
Streptomyces sp. GMKU 301	-	+	-	-	+
Streptomyces sp. GMKU 302	-	+	-	-	-
Streptomyces sp. GMKU 305	-	+	-	-	-
Streptomyces sp. GMKU 306	-	+	+	+	+
Streptomyces sp. GMKU 307	-	-	+	+	+
Streptomyces sp. GMKU 308	+	-	+	+	+
Streptomyces sp. GMKU 309	-	+	-	-	+
Streptomyces sp. GMKU 311	-	-	+	-	-
Streptomyces sp. GMKU 312	-	+	-	-	+
Streptomyces sp. GMKU 313	+	-	-	+	-
Streptomyces sp. GMKU 314	+	+	-	+	+
Streptomyces sp. GMKU 315	+	+	-	+	-
Streptomyces sp. GMKU 316	+	+	-	+	+
Streptomyces sp. GMKU 319	+	+	-	-	+
Streptomyces sp. GMKU 320	-	+	-	-	-
Streptomyces sp. GMKU 322	-	+	-	-	-
Streptomyces sp. GMKU 323	-	+	-	-	-
Streptomyces sp. GMKU 333	-	+	-	+	-
Streptomyces sp. GMKU 334	-	-	+	+	-
Streptomyces sp. GMKU 335	+	+	+	+	-
Streptomyces sp. GMKU 336	+	+	-	+	+
Streptomyces sp. GMKU 337	-	+	-	-	+
Streptomyces sp. GMKU 341	-	+	-	-	-
Streptomyces sp. GMKU 342	-	+	+	-	-
Streptomyces sp. GMKU 343	-	+	+	-	-
Streptomyces sp. GMKU 344	+	+	-	+	+
Streptomyces sp. GMKU 345	+	+	-	+	+
Streptomyces sp. GMKU 352	-	+	+	+	-
Streptomyces sp. GMKU 354	-	+	+	+	+
Streptomyces sp. GMKU 355	-	+	-	+	+

Table 16 (continued)

Isolates	ACCD production	Ammonia production	IAA production	Siderophore production	Phosphate solubilization
Streptomyces sp. GMKU 360	-	+	-	+	-
Streptomyces sp. GMKU 361	-	+	+	+	-
Streptomyces sp. GMKU 365	-	-	+	-	-
Actinoallomurus sp. GMKU 359	-	-	-	+	+
Actinoallomurus oryzae GMKU 370	-	-	-	+	-
Actinomadura sp. GMKU 329	-	-	-	+	-
Actinomadura sp. GMKU 331	-	-	-	+	-
Actinomadura sp. GMKU 351	-	-	-	+	-
Actinomadura sp. GMKU 356	-	-	-	-	-
Actinophytocola oryzae GMKU 367	+	-	-	+	-
Microbispora sp. GMKU 317	-	-	-	-	-
Microbispora sp. GMKU 318	-	-	-	-	+
Microbispora sp. GMKU 324	-	-	-	-	+
Microbispora sp. GMKU 340	-	-	-	-	-
Microbispora sp. GMKU 347	-	-	-	-	-
Microbispora sp. GMKU 350	-	-	-	-	-
Microbispora sp. GMKU 357	-	-	-	-	-
Microbispora sp. GMKU 363	+	+	-	+	-
Microbispora sp. GMKU 364	+	-	-	+	-
Microbispora sp. GMKU 366	-	-	-	+	-
Microbispora sp. GMKU 368	-	-	-	-	-
Microbispora sp. GMKU 369	-	-	-	-	-
Microbispora sp. GMKU 372	-	-	-	-	-
Microbispora sp. GMKU 378	-	-	-	-	-
Micromonospora sp. GMKU 326	-	+	-	-	-
Micromonospora sp. GMKU 328	-	-	-	+	-
Micromonospora sp. GMKU 330	-	+	-	+	-
Micromonospora sp. GMKU 348	-	+	-	-	-
Micromonospora sp. GMKU 353	-	-	-	-	-
Micromonospora sp. GMKU 358	-	+	-	+	-
Micromonospora sp. GMKU 362	+	+	-	+	-
Nocardia sp. GMKU 327	-	-	-	-	-

Table 16 (continued)

Isolates	ACCD production	Ammonia production	IAA production	Siderophore production	Phosphate solubiliza-tion
Nocardiopsis sp. GMKU 346	-	-	-	+	+
Nonomuraea sp. GMKU 325	+	-	-	-	-
Promicromonospora sp. GMKU 303	-	+	-	-	-
Saccharopolyspora sp. GMKU 377	-	-	+	+	+

For the properties of the plant growth promoting agents, the results revealed that 20% (13 isolates) could produce auxin, 30% (20 isolates) could solubilize phosphate, 50% (33 isolates) showed siderophore production, 52% (34 isolates) could produce ammonia and 24% (16 isolates) produced ACC deaminase as shown in Table 17. In summary, there were one isolate, *Streptomyces* sp. GMKU 344 showed ability of all five characters while 9 isolates of *Streptomyces* sp. GMKU 306, GMKU 308, GMKU 314, GMKU 316, GMKU GMKU 335, GMKU 336, GMKU 345, GMKU 354 and GMKU 355 carried four characters. There were seven isolates including *Streptomyces* sp. GMKU 307, GMKU 315, GMKU 319, GMKU 337, *Micromonospora* sp. GMKU 362, *Microbispora* sp. GMKU 363 and *Saccharopolyspora* sp. GMKU 377 showed ability of three characters and 16 isolates carried two characters (Table 17). Interestingly, there were 11 isolates showed no activity of those five plant growth promoting traits (Table 17). A part from that, two isolates, produced only IAA, three isolates could only solubilize phosphate, seven isolates produced only siderophore, nine isolates produced only ammonia and an isolate, *Nonomuraea* sp. GMKU 325, produced only ACC deaminase (Table 17). In this study, it is clear that endophytic actinomycetes can carry one or more characters of plant growth promoting properties. Endophytic actinomycetes isolated from endemic plants of Thailand should be a novel valuable source of plant growth promoting agents because they possess many properties such as production of auxins phytohormone, siderophore, ammonia and ACC deaminase (Nimnoi *et al.,* 2010; Qin *et al*., 2011).

Table 17 Plant growth promoting agents test from endophytic actinomycetes. +, Positive; –, negative

IAA production	Phosphate solubilization	Siderophore production	NH_3 production	ACCD production	Number of isolates
+	+	+	+	+	1
+	+	+	+	-	3
+	+	+	-	+	1
-	+	+	+	+	4
+	-	+	+	+	1
+	+	+	-	-	2
-	-	+	+	+	3
-	+	-	+	+	1
+	+	-	+	-	1
-	+	-	+	-	3
-	-	+	-	+	3
-	-	-	+	+	1
-	-	+	+	-	6
+	-	+	-	-	1
+	-	-	+	-	1
-	+	+	-	-	1
+	-	-	-	-	2
-	+	-	-	-	3
-	-	+	-	-	7
-	-	-	+	-	9
-	-	-	-	+	1
-	-	-	-	-	11
					Total = 66

4. Identification of *acdS* gene

To identify ACC deaminase gene (*acdS*) of endophytic actinomycetes, amino acid sequences of putative ACC deaminases including those of *Actinosynnema mirum* DSM 43827^T, *Brevibacterium linens* BL2, *Kribbella flavida* DSM 17836^T, *Mycobacterium abscessus* ATCC 19977^T, *M. smegmatisstr* MC 2155, *Nakamurella multipartita* DSM 44233^T, *Streptomyces sviceus* ATCC 29083^T, *S. ghanaensis* ATCC 14672^T, *S. griseus* NBRC 13350^T, *S. albus* J1074 and *Saccharopolyspora erythraea* NRRL 2338^T were retrieved from the GenBank database. Multiple sequence alignment was conducted and the specific ATT082 sense primer (5'- CAGTCCAAC CACACSGCSCAG -3') and ATT082 anti-sense primer (5'- GCCATSGACTTSCCC TCGTASAC -3') based on conserved amino acid regions with minimal degeneracy were successfully designed for PCR amplification of *acdS* gene as shown in Figure 36.

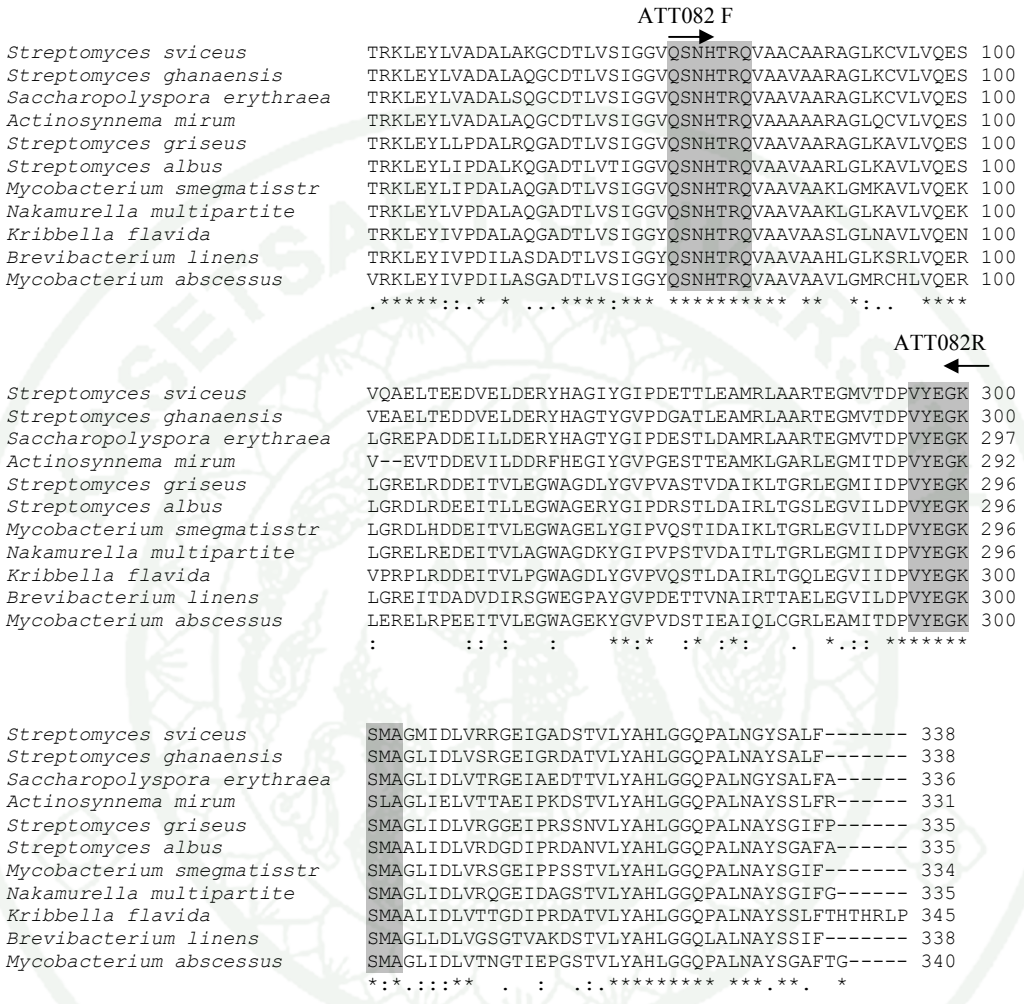


Figure 36 Multiple alignment of amino acid sequences of putative ACC deaminases from 11 species of actinobacteria. Black shading indicates conserved amino acids used for designing of ATT082 F sense primer and ATT 082R anti-sense primer.

For identification of *acdS* gene in the genome of endophytic actinomycetes, all isolates was amplified a partial *acdS* gene with ATT 082F sense primer and ATT 082R anti-sense primer. After PCR amplification of *acdS* gene, 16 isolates including, *Streptomyces* sp. GMKU 308, GMKU 313, GMKU 314, GMKU 315, GMKU 316, GMKU 319, GMKU 320, GMKU 335, GMKU 336, GMKU 344, and GMKU 345), *Microbispora* sp. GMKU 363 and GMKU 364, *Nonomuraea* sp. GMKU 325 and *Micromonospora* sp. GMKU 362 and a new genus *Actinophytocola oryzae* GMKU 367 showed unique bands approximately 650 bp (Figure 37).

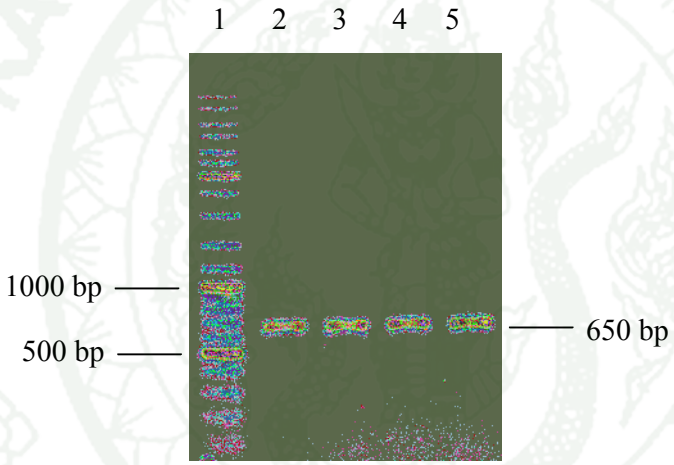


Figure 37 PCR amplification of *acdS* gene (~650 bp) using specific primers, ATT082F and ATT082R. Lane 1, 1 Kb DNA ladder; Lane 2-5 GMKU 314, GMKU 315, GMKU 316 and GMKU 336, respectively.

After direct sequencing of *acdS* gene PCR products, the sequences were compared with *acdS* genes from database and the results showed that the sequence of 16 isolates were highly conserved with level of identity 80-93% at the nucleotide level. When the nucleotide sequences were conceptually translated into amino acid sequences, about 200 residues were obtained. The ACC deaminase were then compared at amino acid level and the isolates showed 83-99% identity and 90-100% similarity to those of actinobacterial ACC deaminase available in GenBank database (Table 18).

Table 18 The identity and similarity values of 16 isolates when compared to the ACC deaminase amino acid sequences in the GenBank database using blastP.

Strains	ACC deaminase of actinobacteria	Identity/ Similarity (%)	Accession no.
Streptomyces sp. GMKU 308	*S. sviceus* ATCC 29083	90/95	ZP_06916233
Streptomyces sp. GMKU 313	*S. ghanaensis* ATCC 14672	87/93	ZP_06579762
Streptomyces sp. GMKU 314	*S. ipomoeae* 91-03	93/95	ZP_10450903
Streptomyces sp. GMKU 315	*S. ghanaensis* ATCC 14672	99/100	ZP_06579762
Streptomyces sp. GMKU 316	*S. ghanaensis* ATCC 14672	89/93	ZP_06579762
Streptomyces sp. GMKU 319	*S. sviceus* ATCC 29083	92/96	ZP_06916233
Streptomyces sp. GMKU 320	*S. ipomoeae* 91-03	91/94	ZP_10450903
Streptomyces sp. GMKU 335	*S. ipomoeae* 91-03	92/95	ZP_10450903
Streptomyces sp. GMKU 336	*S. sviceus* ATCC 29083	93/95	ZP_05016722
Streptomyces sp. GMKU 344	*S. ipomoeae* 91-03	92/95	ZP_10450903
Streptomyces sp. GMKU 345	*S. ipomoeae* 91-03	91/93	ZP_10450903
Micromonospora sp. GMKU 362	*S. violaceusniger* Tu 4113	88/94	YP_004814895
Microbispora sp. GMKU 363	*S. violaceusniger* Tu 4113	88/94	YP_004814895
Microbispora sp. GMKU 364	*S. violaceusniger* Tu 4113	88/94	YP_004814895
Actinophytocola oryzae GMKU 367	*Saccharopolyspora erythraea* NRRL 2338	87/93	YP001104984
Nonomuraea sp. GMKU 325	*S. violaceusniger* Tu 4113	83/90	YP_004814895

For phylogenetic tree analysis of ACC deaminases, multiple sequence alignment of amino acid sequences were determined and constructed with CLUSTAL X software. The topology tree was inferred by the neighbor joining and maximum likelihood methods and the result was shown in Figure 38.

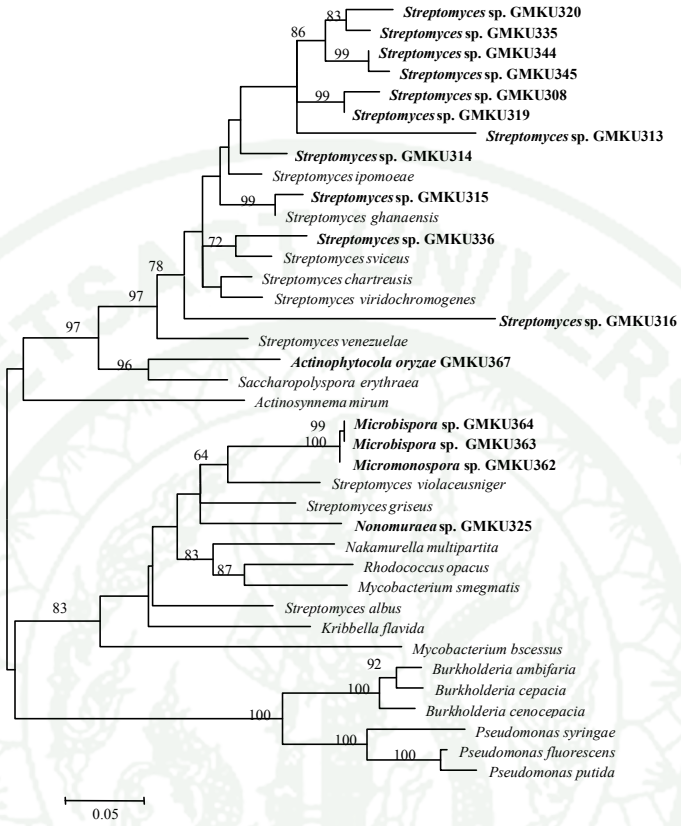


Figure 38 Phylogenetic tree based on amino acid sequences of ACC deaminase constructed using the neighbor-joining method to display the relationship of 16 isolates with the positive and putative strains of ACC deaminase activity. Numbers above branches indicate levels of bootstrap values (%) based on neighbor-joining analysis of 1,000 replicates; only values above 50 % are shown. Bar, 0.2 substitutions per site.

The result from phylogenetic tree analysis revealed that ACC deaminases of endophytic actinomycetes separated from those of *Pseudomonas* sp. (Gammaproteobacteria) and *Burkholderia* sp. (Betaproteobacterium). Glick *et al*. (2007) took 154 available putative bacterial ACC deaminase gene (*acdS*) sequences to construct phylogenetic tree and six *acdS* groups were defined. GroupI, Group II and III contained sequences from Gammaproteobacteria, Betaproteobacteria and other two strains of Gammaproteobacteria respectively. Actinobacteria was in Group V while Group IV included Alphaproteobacteria and a small group of Betaproteobacteria. And

the Group VI consisted of Beta- and Gammaproteobacteria. From this tree, ACC deaminases from actinomycetes were clustered into two groups (Figure 38). The first group included most of *Streptomyces* spp. and some rare actinomycetes namely, *Actinophytocola oryzae* GMKU 367, *Saccharopolyspora erythraea* and *Actinosynnema mirum*. The second group included most of rare actinomycetes namely, *Kribbella flavida*, *Microbispora* spp., *Mycobacterium bscessus*, *M. smegmatis*, *Nakamurella multipartite*, *Nonomuraea* sp., and *Rhodococcus opacus*. There were some streptomycetes included in this group namely, *Streptomyces albus*, *S. griseus* and *S. violaceusniger*.

5. Screening of anti-microbial activities

5.1 Antibacterial activities

Antibacterial activities from endophytic actinomycetes were determined using agar plug assay method (Jeffry, 1994). Some results of antibacterial activities showed in Figure 39. Thirty isolates (45%) inhibited *B. cereus*, 12 isolates (18%) inhibited *E. coli*, 15 isolates (23%) inhibited *S. aureus*, 7 isolates (11%) inhibited *E. carotovora* pv.*carotovora*, 30 isolates (45%) inhibited *R. solanacearum* and 3 isolates (5%) inhibited *X. campestris* pv. *glycine*. Interestingly, *Streptomyces* sp. GMKU 306 and GMKU 311 showed activity against all of the test bacteria (Table 19).

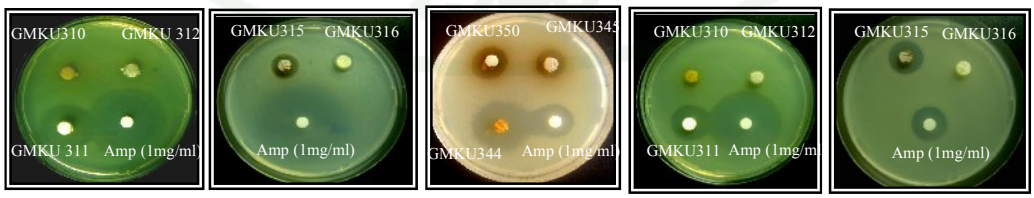


Figure 39 Antibacterial activities of some endophytic actinomycetes.

Table 19 Antibacterial activities of endophytic actinomycetes.

Isolates	*B.cereus*	*E.coli*	*S.aureus*	*E.carotovora*	*R.solanacearum*	*X.campestris*
GMKU301	+	+	+	+	+	-
GMKU302	-	-	-	-	-	-
GMKU303	-	+	-	-	-	-
GMKU305	+	-	-	-	++	-
GMKU306	+	+	+	+	+	+
GMKU307	+	+	+	+	+	-
GMKU308	-	-	-	-	-	-
GMKU309	+	++	-	-	++	-
GMKU311	++	+++	+++	++	++	+++
GMKU312	-	++	+	-	+	+
GMKU313	+	-	-	-	+	-
GMKU314	+	-	-	-	+	-
GMKU315	+	+	-	-	+	-
GMKU316	+	++	-	-	++	-
GMKU317	-	-	-	+	-	-
GMKU318	-	+	-	-	-	-
GMKU319	++	-	++	-	+	-
GMKU320	+	-	-	-	-	-
GMKU322	-	-	-	-	-	-
GMKU323	-	-	-	-	+	-
GMKU324	-	+	-	-	-	-
GMKU325	-	-	-	-	-	-
GMKU326	-	-	-	-	-	-
GMKU327	-	-	-	-	-	-
GMKU328	-	-	-	-	-	ND
GMKU329	+	-	-	-	+	-
GMKU330	-	-	-	-	-	-
GMKU331	-	-	-	-	-	-
GMKU333	-	-	-	-	-	ND
GMKU334	-	-	-	-	-	ND
GMKU335	+++	-	+++	-	+	-
GMKU336	-	-	-	-	-	ND

Table 19 (continued)

Isolates	*B.cereus*	*E.coli*	*S.aureus*	*E.carotovora*	*R.solanacearum*	*X.campestris*
GMKU337	+	-	+	-	+	-
GMKU340	-	-	-	-	-	-
GMKU341	+	-	+	-	+	-
GMKU342	-	-	-	-	+	-
GMKU343	+	-	-	-	+	-
GMKU344	++	-	-	-	+	-
GMKU345	++	-	-	-	-	-
GMKU346	-	-	-	-	+	-
GMKU347	+	-	-	-	-+	-
GMKU348	-	-	-	-	-	ND
GMKU350	+++	-	-	-	+	-
GMKU351	-	-	+	-	+	ND
GMKU352	++	-	-	-	+	ND
GMKU353	-	-	+	-	+	ND
GMKU354	+++	-	+++	-	+++	ND
GMKU355	+	-	+	-	+	-
GMKU356	+	-	-	-	-	ND
GMKU357	-	-	-	-	-	ND
GMKU358	-	-	-	-	-	ND
GMKU359	-	-	-	-	-	ND
GMKU360	-	-	-	-		-
GMKU361	+	-	+	-	+	-
GMKU362	-	-	-	-	-	ND
GMKU363	-	-	-	-	-	ND
GMKU364	-	-	-	-	-	ND
GMKU365	+	-	+	-	+	-
GMKU366	-	-	-	-	-	-
GMKU367	-	-	-	-		-
GMKU368	+	-	-	-	-	-
GMKU369	-	-	-	-	-	ND
GMKU370	-	-	-	-	-	-
GMKU372	+	-	-	-	-	ND

Table 19 (continued)

Isolates	*B.cereus*	*E.coli*	*S.aureus*	*E.carotovora*	*R.solanacearum*	*X.campestris*
GMKU377	-	-	-	-	-	-
GMKU378	-	-	-	-	-	-

ND = not determined

5.2 Antifungal activities

The ability of individual isolate to inhibit the growth of test fungi was carried out by the modified protocol of Crawford *et al*. (1993). For determination of antifungal activity against *C. albicans*, the protocol of Jeffry, (1994) was used. The results revealed that all isolates were active against *A. niger* and *Collectotrichum* sp., while 39 isolates (66 %) inhibited *Fusarium* sp. and 5 isolates (9%) showed activity against *C. albicans* (Table 19). There were five isolates namely, *Streptomyces* sp. GMKU 319, GMKU 335, GMKU 337, GMKU 341 and GMKU 361 active against all test fungi.

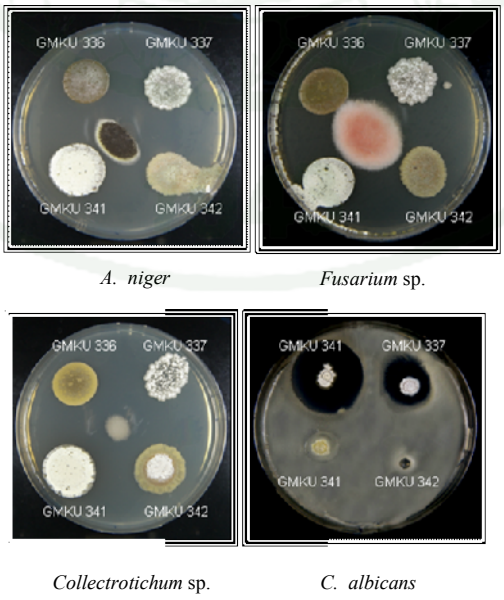


Figure 40 Anti-fungal activities of some endophytic actinomycetes.

In this study, 45% of isolates inhibited *R. solanacearum* which is one of the most important bacteria that causes wilt disease in over 200 families of plant. All isolates were active against *Collectotrichum* sp and *A. niger* that causes anthracnose and black mold diseases, respectively on numerous plant species worldwide . These results of the screening of anti-microbial activities are in agreement with previous reports that endophytic actinomycetes are potential candidates for biocontrol agents that have high potential in medicine and agricultural biotechnology in the future. Nowadays, endophytic actinomycetes have been recognized as new members of plant growth promoting bacterial community due to their properties of plant growth enhancement and protection of plants from infectious diseases (Taechowisan *et al*., 2003).

6. Identification of a novel compound

All of endophytic actinomycetes in this study had been preliminary screening for potential production of novel compounds. This early work was done by Associate Professor Arinthip Thamchaipenet with collaboration with Professor Yasuhiro Igarashi, Toyama Prefecture University, Japan. The results indicated that the endophytic *Microbispora* sp. GMKU 363 isolated from a root of Thai medicinal plant 'Lin Ngu Hao' (*Clinacanthus siamensis* Bremek.) had high potential to produce new metabolites. Strain GMKU 363 was then identified using 16S rRNA gene sequenceing and showed that it was a member of the genus *Microbispora* on the basis of 99.9% 16S rRNA gene sequence (1387 nucleotides; GenBank accession number GU459171; Appendix) with the *Microbispora mesophila* JCM 3151^T type strain (accession number AF002266). *Microbispora* sp. GMKU 363 was cultured in A-11M medium at 30 ˚C for 6 days, and the whole culture was extracted with 1-butanol. The crude extract was screened for novel compounds using reversed-phase column HPLC. The chromatogram (Figure 41) showed 5 target peaks, A, B, C, D and E. The target B was compared its UV spectrum to chemical data base (provided by Professor Yasuhiro Igarashi) and showed high potential to be a novel compound. To get high yield of target B, strain GMKU 363 was cultured again in 1.5 l of A-11M medium and the whole culture broth was extracted with 1-butanol. The organic solvent was pooled and taken to dryness under rotary evaporation to give a dark brown solid (3g).

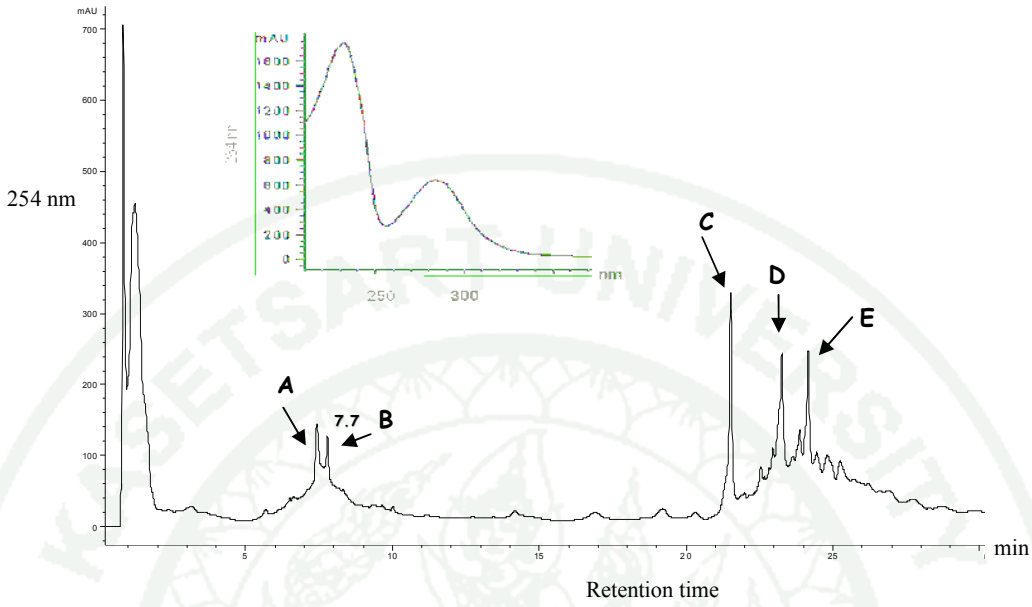


Figure 41 HPLC chromatogram of crude extract of *Microbispora* sp. GMKU 363 revealed 5 targets, A, B, C, D and E. UV spectrum of target B was indicated.

The solid was separated by flash column chromatography using siliga gel 60 (3 cm diameter). The compounds were eluted with chloroform, chloroform: MeOH (20:1, 10:1, 4:1, 2:1 and 1:1) and MeOH, respectively to give eight fractions C1-C8 (Table 20).

Table 20 Fractions obtained from the crude extract of *Microbispora* sp. GMKU 363.

Fraction No.	Weight (mg)	Physiological characters
C1	-	Yellow
C2	40	Colorless
C3	-	Pale yellow
C4	696	Red
C5	229	Yellow
C6	-	Yellow
C7	-	Yellow
C8	500	Dark brown

Each fraction was analyzed by HPLC and the target B was detected. Fraction C5, a yellow solvent (229 mg) was separated by HPLC purification using a C18 column, followed by dryness under rotary evaporation to give a colorless solid (0.7 mg). Fraction C5 (a colorless solid, 0.7 mg) was identified as a new compound designated linfuranone A.

Linfuranone A was obtained as an optically active, colorless amorphous powder that gave an $[M + Na]^+$ peak at *m*/*z* 417.2257 in the high resolution ESITOFMS appropriate for a molecular formula of $C_{22}H_{34}O_6$, (calcd for $C_{22}H_{34}O_6Na$, 417.2248), which was consistent with the ^1H and ^{13}C NMR data (Table 21). The IR spectrum of linfuranone A showed absorption bands for hydroxyl (3333 cm^{-1}) and carbonyl (1691 cm^{-1}) functionalities. ^{13}C NMR and HMQC spectral data confirmed the presence of 22 carbons, which were assigned to two oxygenated quaternary sp^2 carbons, seven olefinic carbons (five are proton-bearing), one quaternary sp^3 carbon, four sp^3 methines (three are oxygen-bearing), three sp^3 methylenes, five methyl carbons.

Table 21 ^{1}H and ^{13}C NMR data for linfuranone A in CD_3OD.

No.	δ_C[a]	δ_H mult (*J* in Hz)[b]	HMBC[b,c]
1	22.3/22.4, CH_3	1.43/1.44, s	2, 3
2	104.37/104.39, qC		
3	205.5, qC		
4	110.3, qC		
5	187.2, qC		
6	38.5, CH_2	2.65, m	4, 5, 7, 8
		2.72, m	4, 5, 7, 8
7	69.6, CH	3.96, m	
8	38.2/38.3, CH_2	1.61, m	7, 9, 10
9	29.9, CH_2	2.18, m	7, 8, 10
		2.24, m	7, 8, 10
10	132.74/132.79,[d] CH	5.59, m	11, 12
11	132.68/132.70,[d] CH	6.06, m	
12	132.00/132.02, CH	6.06, m	
13	136.3/136.4, CH	5.58, m	11, 12
14	41.8, CH	2.31, m	13, 15, 21
15	83.1, C	3.63, d (8.4)	13, 14, 17, 21, 22
16	138.0, qC		
17	133.5, CH	5.35, d (8.4)	18, 22
18	65.2, CH	4.56, dq (8.4, 6.4)	16
19	22.9, CH_3	1.19, d (6.4)	17, 18
20	6.0, CH_3	1.67, s	3, 4, 5
21	18.0, CH_3	0.86, d (7.0)	13, 14, 15
22	11.6, CH_3	1.64, d (1.1)	15, 16, 17

[a] Recorded at 100 MHz.

[b] Recorded at 500 MHz.

[c] HMBC correlations are from proton(s) stated to the indicated carbon.

[d] Interchangeable signals.

Analysis of the COSY spectrum led to four proton-bearing fragments, H-17 to H-19, H-15/H-14/H-21, H-12/H-13, and H-6 to H-11 (Figure 42). The first and second fragments were joined through the quaternary sp^2 carbon C-16 by HMBC correlations from a vinyl methyl H-22 to C-15, C-16, and C-17. This fragment was expanded to include the third fragment on the basis of an HMBC correlation from H-21 to C-13, providing an eight-carbon fragment bearing two oxygen substitutions at C-15 and C-18. The last COSY-defined fragment was connected to the unsaturated ketone substructure bearing an oxygen substitution at position on the basis of a series of HMBC correlations from H-6 to C-4 and C-5, and from a vinyl methyl H-20 to C-3, C-4, and C-5. The carbonyl carbon C-3 was correlated with H-1, which also showed a correlation to the oxygenated carbon C-2 (104.4), establishing the attachment of the two-carbon fragment C-1/C-2 to C-3. The chemical shift of C-2 was suggestive of the bonding of two oxygen atoms to this carbon. Finally, consideration of the molecular formula and the remaining unsaturation degree provided the connectivity between C-11 and C-12 and the placement of an oxygen atom between C-2 and C-5 to establish this molecule as a new member of furanone-containing polyketides. *E* configuration for the double bond between C-16 and C-17 was confirmed by NOEs between H-18 and H-22, and H-15 and H-17. The geometry of C-10/C-11 and C-12/C-13 double bonds could not be assigned due to the proton signal overlapping. With respect to the four stereocenters in the linear chain part, there exist two possible configurations at the hemiketal C-2 carbon. Two signals corresponding to the two diastereomers were observed for H-1 and several carbons. Absolute stereochemistry of the compound is under investigation.

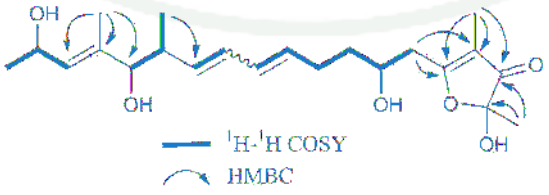


Figure 42 Analysis of the COSY spectrum led to four proton-bearing fragments.

Linfuraone A is a relatively rare 3-furanone derived from polyketide with a hemiketal at C-2 and an unsaturated alkyl chain at C-5 (Figure 43). There are a few known structurally close metabolites isolated from *Streptomyces* spp., which are E837, E492, E975 and actinofuranones A and B (Banskota *et al*., 2006 and Cho *et al.,* 2006) as shown in Figure 55. Further related structures are not from actinobacterial origin, but were isolated from myxobacteria, fungi and marine mollusks (Kuroda, *et al*., 1984; Cimino, *et al.,* 1987; Capon *et al*., 1993; Kunze *et. al.,* 2005). E837, E492 and E975 exhibited inhibition against helminth NADH-fumarate reductase and bovine heart NADH oxidase, while actinofuranones had no display of biological activity (Table 22). Biological screening of linfuranone A in diverse bioassays indicated that this compound was active in an assay designed to screen antidiabetic and antiatherogenic activities using mouse ST-13 preadiopocytes. By the treatment with 50 μM the compound, about 47% of preadipocytes were differentiated into the matured adipocytes and accumulated the lipid droplets. The compound displayed no appreciable activities in antimicrobial and cytotoxic assays.

Since Castillo *et al*. (2002) had isolated an antibiotic, munumbicin, from endophytic *Streptomyces munumbicin* NRRL 30562, there were several novel bioactive compounds have been discovered from endophytic actinomycetes (Qin *et al*., 2011). Recently, a new spirotetronic acid-containing polycyclic polyketide, maklamicin, has been identified from an endophytic actinomycete, *Micromonospora* sp. GMKU 326, in this GMKU culture collection (Igarashi *et al*., 2011). *Micromonospora* sp. GMKU 326 was isolated from root of a leguminous plant, Maklam Phueak (*Abrus pulchellus* Wall. Ex *Thwaites* subsp. *pulchellus*). Maklamicin showed modest antimicrobial activity against Gram-positive bacteria. In this study, a novel compound, linfuraone A, was isolated from an endophytic actinomycete, *Microbispora* sp. GMKU 363, from the same culture collection. It is convinced that endophytic actinomycetes are high potential resources to discover new bioactive compounds and these compounds can be further investigate for drug candidates in the future.

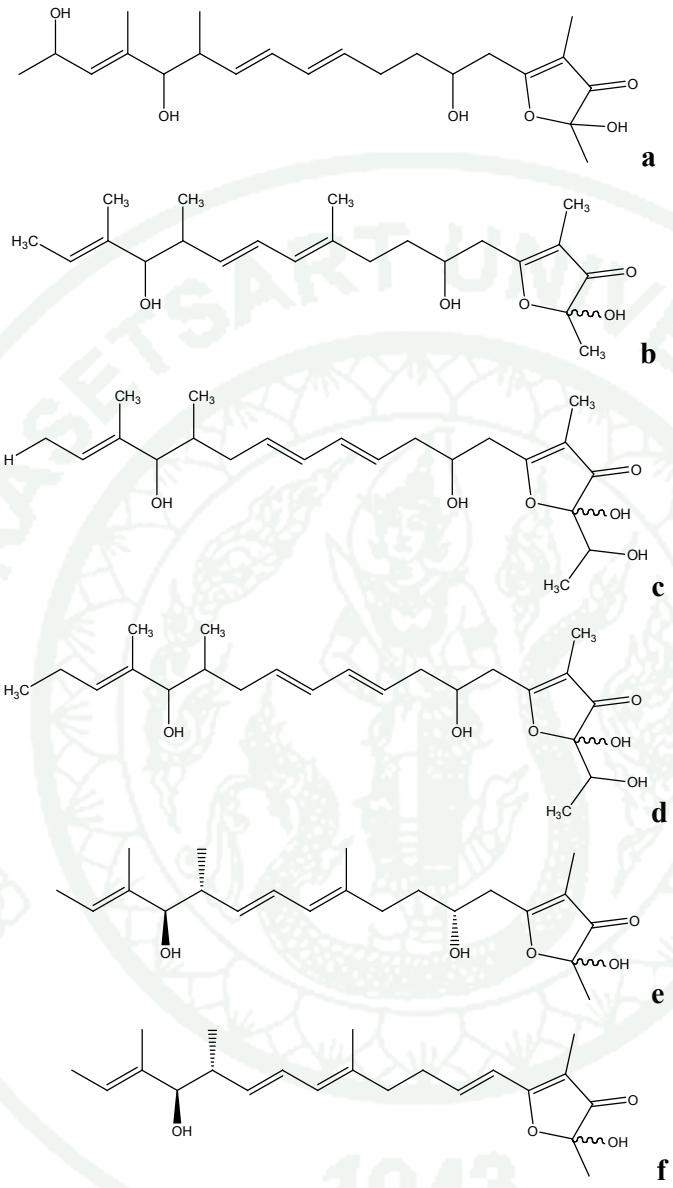


Figure 43 Structures of linfuranone A (a) and related compound; 5-alkenyl-3,3*(2H)* -furanones E-837 (b), 5-alkenyl-3,3*(2H)* -furanones E-492 (c), 5-alkenyl-3,3*(2H)* -furanones E-975 (d), actinofuranone A (e) and actinofuranone B (f).

Table 22 The activities of linfuraone A and related compounds.

Compounds	Sources	Bioactivities	References
Linfuranone A	*Microbispora* sp. GMKU 363	Antidiabetic and antiatherogenic activities using mouse ST-13 preadiopocytes	In this study
Furanones E-837	*Streptomyces aculeolatus* NRRL 18422	1. Antifungal activity: against *Saccharomyces cerevisiae* 2. Electron transport enzyme inhibition: against NADH-fumarate reductase of *Ascaris suum* (roundworm of pig) and NADH oxidase from bovine heart	Banskota A. H. *et al*., 2006
Furanones E-492	*Streptomyces* sp. Eco86	Electron transport enzyme inhibition: against helminth NADH-fumarate reductase of *Ascaris suum* (roundworm of pig) and NADH oxidase from bovine heart	Banskota A. H. *et al*., 2006
Furanones E-975	*Streptomyces* sp. Eco86	1. Antifungal activity: against *Saccharomyces cerevisiae* and *Aspergillus fumigatus* 2. Electron transport enzyme inhibition: against helminth NADH-fumarate reductase of *Ascaris suum* (roundworm of pig) and NADH oxidase from bovine heart	Banskota A. H. *et al*., 2006
Actinofuranone A and B	Marine *streptomyces* CNQ 766	None	Cho *et al.,* 2006

CONCLUSION

In this study endophytic actinomycetes were isolated from endemic medicinal and agricultural Thai plants using surface-sterilized method. A total of 66 endophytic actinomycetes were obtained from root and leaf tissues. The majority of endophytic actinomycetes (83%) was obtained from root tissues while the rest gained from leaf tissues. All isolates were identified using the distinguished basis of characteristic colonial morphology notably the ability to form substrate and aerial mycelia as well as spore indicated that there were two distinguished groups including *Streptomyces* and rare actinomycetes. Identification by using partial sequence of 16S ribosomal RNA gene, revealed that two isolates, GMKU 367 and GMKU 370, of rare actinomycetes were potentially novel strains. On the basis of polyphasic taxonomy, the results showed the significant difference data of phylogenetic, chemotaxonomic and morphological analyses of both strains. Strain GMKU 367 was assigned as a novel genus in the family *Pseudonocardiaceae*, for which the name *Actinophytocola oryzae* gen. nov., sp. nov. was proposed, and strain GMKU370 represented a novel species of the genus *Actinoallomurus*, for which the name *Actinoallomurus oryzae* sp. nov. was proposed. It was concluded that endophytic actinomycetes isolated from this work were very diverse which revealed *Streptomyces* as the majority members (50%), while the remainder belonged to 10 genera including *Microbispora* (n=14), *Micromonospora* (n=7), *Actinomadura* (n=4), *Actinoallomurus* (n=2), *Nocardia* (n=1), *Nocardiopsis* (n=1), *Nonomuraea* (n=1), *Promicromonospora* (n=1), *Saccharopolyspora* (n=1) and a new genus *Actinophytocola* (n=1).

For characterization of plant growth promoting properties from all of 66 strains, the results revealed that 34 strains produced NH_3, 20 strains (34%) could solubilize phosphate, 33 strains (52%) produced siderophores, 13 strains (22%) produced plant hormone (indole-3-acetic acid, IAA) and 16 strains (15%) produced aminocyclopropane-1-carboxylic acid (ACC) deaminase, while eleven strains showed none of the properties. There was one strain, *Streptomyces* sp. GMKU 344 showed ability of all five characters. Sixteen strains that produced ACC deaminase were *Streptomyces* (n=11), *Microbispora* (n=2), *Nonomuraea* (n=1) and *Micromonospora*

(n=1) and a new genus *Actinophytocola oryzae* GMKU 367 were identified ACC deaminase gene (*acdS*) with specific primers, ATT 082F and ATT 082R. 650 bp PCR product of each strain revealed nucleotide sequence identity with the level between 80 and 93%. When ACC deaminases were compared to those of actinobacterial ACC deaminases available in GenBank database at amino acid level, highly conserve values were obtained at 83-99% identity and 90-100% similarity. All endophytic actinomycetes were also tested for anti-bacterial and anti-fungal activities, 30 strains (45%) inhibited *B. cereus*, 12 strains (18%) inhibited *E. coli*, 15 strains (23%) inhibited *S. aureus*, 7 strains (11%) inhibited *E. carotovora* pv. *carotovora*, 30 strains (45%) inhibited *R. solanacearum* and 3 strains (5%) inhibited *X. campestris* pv.*glycine*. For antifungal activities, all strains were active against *A. niger* and *Collectotrichum* sp., while 39 strains (66%) inhibited *Fusarium* sp. and 5 strains (9%) showed activity against *C. albicans*. *Streptomyces* sp. GMKU 306 and GMKU 311, showed activity against all test bacteria while *Streptomyces* sp. GMKU 319, GMKU 335, GMKU 337, GMKU 341 and GMKU 361 were active against all test fungi.

For determination of a novel bioactive compound produced by an endophytic actinomycete, *Microbispora* sp. GMKU 363 isolated from a root of Thai medicinal plant 'Lin Ngu Hao' (*Clinacanthus siamensis* Bremek.) was extracted, purified and determined the novel structure. The new compound is a relatively rare 3-furanone derived from polyketide with a hemiketal at C-2 and an unsaturated alkyl chain at C-5 and was designated Linfuranone A. Linfuranone A was active in an assay designed to screen antidiabetic and antiatherogenic activities by differentiated mouse ST-13 preadiopocytes into the matured adipocytes. In addition, Linfuranone A displayed no activities in antimicrobial and cytotoxic assays.

The results obtained from this study significantly indicated that endophytic actinomycetes isolated from endemic plants of Thailand are a novel valuable source for discovery new taxa, bioactive compounds and plant growth promoting agents that can be used for medicinal and agricultural applications in the future.

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APPENDICES

Appendix A

source of plant samples and endophytic actinomycetes

Appendix Table 1 Type of medicinal and agricultural plants for isolation of actinobacterial endophytes.

Family	Scientific name	Thai names	Common names	Locations
Acanthaceae	*Adhatoda vasica* Nees	Sa-nead	Malabar Nut Tree, Vasica, Adhatoda,	2
Acanthaceae	*Barleria strigosa* Willd.	Sang-kor-ra-nee	–	2
Acanthaceae	*Climacanthus nutans* Lindau	Pha-ya-plong-thong, Sa-led-pang-pon (tua-mea)	–	2
Acanthaceae	*Climacanthus siamensis* Brem.	Lin-ngu-how	–	2
Araliaceae	*Polyscias fruticosa* Harms var. deleauanum N.E. Br.	Leb-krut	–	2
Araliaceae	*Schefflera venulosa* Harms	Ha-un-man-para-san-kai, Wan-oil-chang	–	2
Combretaceae	*Terminalia chebula* Retz.	Sa-more-thai	Myrobalan wood	1
Compositae	*Eupatorium stoechadosmum* Hance	Yha-sua-mob, Sun-pra-hom	–	2

Appendix Table 1 (Continued)

Family	Scientific name	Thai names	Common names	Locations
Compositae	*Ageratum conyzoides* Linn.	Sab-rang-sab-ka	Tropical ageratum	2
Convolvulaceae	*Ipomoea pes-caprae* Sweet	Pak-bong-ta-lay	Beach morning glory, Railroad vine	1
Ebenaceae	*Diospyros mollis* Griff.	Ma-grea	Ebony tree	1
Euphorbiaceae	Croton caudatus Geisel	Kra-door-hod-bai-khon, Ko-klan	–	2
Euphorbiaceae	*Phyllanthus pulcher* Wall. ex Muell. Arg.	Wan-thor-ra-nee-san	Tropical leaf flower	1
Guttiferae	*Garcinia mangostana* Linn.	Mang-kud	Mangosteen	4
Lauraceae	*Cinnamomum camphora* Th. Fries	Ka-ra-boon Op-choei-yuan	Camphor tree	1 and 2
Lauraceae	*Cinnamomum bejolghota* Sweet	Op-chey	Cinnamon	1
Leguminosae	*Abrus pulchellus* Wall. ex Thwaites subsp. *pulchellus*	Ma-klaum-puak		2
Menispermaceae	*Tinospora cordifolia* Miers	Ching-cha-cha-lee	–	2

Appendix Table 1 (Continued)

Family	Scientific name	Thai names	Common names	Locations
Mimosaceae	*Parkia speciosa* Hassk.	Sa-tor	Stink Beam	4
Moraceae	*Streblus asper* Lour.	Koi	Siamese rough bush, Tooth brush tree	2
Myristicaceae	*Myristica fragrans* Linn.	Chan-ted	Nutmeg tree	4
Myrsinaceae	*Ardisia polycephala* Wall.	Pi-lang-ka-sa	–	2
Palmae	*Elaeis guineensis* Jacq.	Palm	Oil palm*	4
Phytolaccaceae	*Rivina humilis* Linn.	Prick-fha-rung	Rouge plant	2
Plumbaginaceae	*Plumbago zeylanica* Linn.	Jed-ta-mul-pleung-kaw	Wild leadwort	2
Poaceae	*Oryza sativa* L. cv KDML 105	Khao-hom-ma-li	Jasmine rice*	3
Poaceae	*Oryza sativa* L. cv RD 6	Khao-heaw	Glutinous rice*	3
Rutaceae	*Hesperethusa crenulata* Roem.	Ka-jae	–	1
Sapindaceae	*Lepisanthes senegalensis* Leenh.	Mak-wor	–	2
Verbenaceae	*Clerodendrum serratum* Moon var. wallichii Clarke	Tree-cha-wa	–	2
Zingiberaceae	*Boesenbergia pandurata* Holtt.	Kra-chai	–	2

Appendix Table 1 (Continued)

Family	Scientific name	Thai names	Common names	Locations
Zygophyllaceae	*Tribulus cistoides* Linn.	Kok-ka-soon	Jamaican feverplant, Ground bur-nut, Puncture vine	1

Note: Locations: 1, Chonburi province; 2, Chachoengsao province; 3, Pathum Thani province; 4, Chumphon province

*, agricultural plants

Appendix Table 2 A total of actinobacterial endophytes isolated from plant tissues was used in this study.

No.	Isolates	Sources	No.	Isolates	Sources
1	GMKU 301	GR, Ka-jae	17	GMKU 319	GR, Tree-cha-wa
2	GMKU 302	R, Ka-jae	18	GMKU 320	GR, Op-chey
3	GMKU 303	GR, Ka-jae	19	GMKU 322	R, Op-chey
4	GMKU 305	L, Kok-ka-soon	20	GMKU 323	R, Op-chey
5	GMKU 306	R, Kok-ka-soon	21	GMKU 324	L, Wan-thor-ra-nee-san
6	GMKU 307	R, Kok-ka-soon	22	GMKU 325	GR, Ma-klaum-puak
7	GMKU 308	R, Ka-ra-boon	23	GMKU 326	GR, Ma-klaum-puak
8	GMKU 309	GR, Pak-bong-ta-lay	24	GMKU 327	GR, Ma-klaum-puak
9	GMKU 311	R, Pak-bong-ta-lay	25	GMKU 328	GR, Ma-klaum-puak
10	GMKU 312	L, Pak-bong-ta-lay	26	GMKU 329	GR, Ma-klaum-puak
11	GMKU 313	L, Pak-bong-ta-lay	27	GMKU 330	GR, Ha-nu-man-pra-san-kai
12	GMKU 314	GR, Pak-bong-ta-lay	28	GMKU 331	GR, Ha-nu-man-pra-san-kai
13	GMKU 315	R, Ma-grea	29	GMKU 333	GR, Tree-cha-wa
14	GMKU 316	GR, Ma-grea	30	GMKU 334	R, Tree-cha-wa
15	GMKU 317	GR, Ma-grea	31	GMKU 335	R, Tree-cha-wa
16	GMKU 318	GR, Ma-grea	32	GMKU 336	GR, Tree-cha-wa

Appendix Table 2 (Continued)

No.	Isolates	Sources	No.	Isolates	Sources
33	GMKU 337	GR, Tree-cha-wa	50	GMKU 357	GR, Sang-kor-ra-nee
34	GMKU 340	L, Tree-cha-wa	51	GMKU 358	GR, Sang-kor-ra-nee
35	GMKU 341	R, Ko-klan	52	GMKU 359	GR, Jed-ta-mul-pleung-kaw
36	GMKU 342	R, L, Ko-klan	53	GMKU 360	R, Lin-ngu-how
37	GMKU 343	GR, Pi-lang-ka-sa	54	GMKU 361	R, Lin-ngu-how
38	GMKU 344	R, Pi-lang-ka-sa	55	GMKU 362	L, Lin-ngu-how
39	GMKU 345	GR, Pi-lang-ka-sa	56	GMKU 363	L, Lin-ngu-how
40	GMKU 346	L, Sa-led-pang-pon-tua-mea	57	GMKU 364	R, Koi
41	GMKU 347	GR, Ka-ra-boon	58	GMKU 365	GR, Tree-cha-wa
42	GMKU 348	L, Sab-rang-sab-ka	59	GMKU 366	GR, RD 6, glutinous rice plant
43	GMKU 350	R, San-pra-hom	60	GMKU 367	GR, RD 6, glutinous rice plant
44	GMKU 351	GR, Ma-klaum-puak	61	GMKU 368	GR, Khao Dawk Mali 105, rice plant
45	GMKU 352	L, Ko-klan	62	GMKU 369	GR, Khao Dawk Mali 105, rice plant

Appendix Table 2 (Continued)

No.	Isolates	Sources	No.	Isolates	Sources
46	GMKU 353	GR, Ko-klan	63	GMKU 370	GR, Khao Dawk Mali 105, rice plant
47	GMKU 354	L, Sang-kor-ra-nee	64	GMKU 372	GR, Khao Dawk Mali 105, rice plant
48	GMKU 355	GR, Sang-kor-ra-nee	65	GMKU 377	R, Chan-ted
49	GMKU 356	GR, Sang-kor-ra-nee	66	GMKU 378	GR, Plam

Appendix B

Nucleotide sequences of 16S rRNA gene

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GMKU 301      1  GTCGAACGAT GAACCACCTT CGGGTGGGGA TTAGTGGCGA ACGGGTGAGT
             51  AACACGTGGG CAATCTGCCC TGCACTCTGG GACAAGCCCT GGAAACGGGG
            101  TCTAATACCG GATACTGACC TGCCAAGGCA TCTTGGCGGG TCGAAAGCTC
            151  CGGCGGTGCA GGATGAGCCC GCGGCCTATC AGCTTGTTGG TGAGGTAATG
            201  GCTCACCAAG GCGACGACGG GTAGCCGGCC TGAGAGGGCG ACCGGCCACA
            251  CTGGGACTGA GACACGGCCC AGACTCCTAC GGGAGGCAGC AGTGGGGAAT
            301  ATTGCACAAT GGGCGAAAGC CTGATGCAGC GACGCCGCGT GAGGGATGAC
            351  GGCCTTCGGG TTGTAAACCT CTTTCAGCAG GGAAGAAGCG AAAGTGACGG
            401  TACCTGCAGA AGAAGCGCCG GCTAACTACG TGCCAGCAGC CGCGGTAATA
            451  CGTAGGGCGC AAGCGTTGTC CGGAATTATT GGGCGTAAAG AGCTCGTAGG
            501  CGGCTTGTCG CGTCGGTTGT GAAAGCCCGG GGCTTAACCC CGGGTCTGCA
            551  GTCGATACGG GCAGGCTAGA GTTCGGTAGG GGAGATCGGA ATTCCTGGTG
            601  TAGCGGTG
GMKU 302      1  GGATTAGTGG CGAACGGGTG AGTAACACGT GGGCAATCTG CCCTGCACTC
             51  TGGGACAAGC CCTGGAAACG GGGTCTAATA CCGGATACTG ACCCGCTTGG
            101  GCATCCAAGC GGTTCGAAAG CTCCGGCGGT GCAGGATGAG CCCGCGGCCT
            151  ATCAGCTTGT TGGTGAGGTA ATGGCTCACC AAGGCGACGA CGGGTAGCCG
            201  GCCTGAGAGG GCGACCGGCC ACACTGGGAC TGAGACACGG CCCAGACTCC
            251  TACGGGAGGC AGCAGTGGGG AATATTGCAC AATGGGCGAA AGCCTGATGC
            301  AGCGACGCCG CGTGAGGGAT GACGGCCTTC GGGTTGTAAA CCTCTTTCAG
            351  CAGGGAAGAA GCGAAAGTGA CGGTACCTGC AGAAGAAGCG CCGGCTAACT
            401  ACGTGCCAGC AGCCGCGGTA ATACGTAGGG CGCGAGCGTT GTCCGGAATT
            451  ATTGGGCGTA AAGAGCTCGT AGGCGGCTTG TCACGTCGGT TGTGAAAGCC
            501  CGGGGCTTAA CCCCGGGTCT GCAGTCGATA CGGGCAGGCT AGAGTTCGGT
            551  AGGGGAGATC GGAATTCCTG GTGTAGCGGT GAAATGCGCA GATATCAGGA
            601  GGAACACCGG TGGCGAAGGC GGATCTCTGG GCCGATACTG ACGCTGAAGA
            651  GCGAAAGCGT GGGGAGCGAA CAGGATTAGA TACCCTGGTA GTCCACGCCG
GMKU 303      1  CTTGCTGGGT GGATCAGTGG CGAACGGGTG AGTAACACGT GAGCAACCTG
             51  CCCTTCACTT CGGGATAAGC CTTGGAAACG GGGTCTAATA CCGGATAGGA
            101  GCACCTGCTG CATGGTGGGT GTTGGAAAGT TTTTCGGTGA GGGATGGGCT
            151  CGCGGCCTAT CAGCTTGTTG GTGGGGTAAC GGCCTACCAA GGCGTCGACG
            201  GGTAGCCGGC CTGAGAGGGC GACCGGCCAC ACTGGGACTG AGACACGGCC
            251  CAGACTCCTA CGGGAGGCAG CAGTGGGGAA TATTGCACAA TGGGCGAAAG
            301  CCTGATGCAG CGACGCCGCG TGAGGGATGA CGGCCTTCGG GTTGTAAACC
            351  TCTTTCAGCA GGGAAGAAGC GCAAGTGACG GTACCTGCAG AAGAAGCGCC
            401  GGCTAACTAC GTGCCAGCAG CCGCGGTAAT ACGTAGGGCG CAAGCGTTGT
            451  CCGGAATTAT TGGGCGTAAA GAGCTCGTAG GCGGTCTGTC GCGTCTGGTG
            501  TGAAATCCCA TGGCTCAACT ATGGGCTTGC ATCGGGTACG GGCAGACTAG
            551  AGTGCTGTAG GGGAGACTGG AATTCCTGGT GTAGCGGTGG AATGCGCAGA
            601  TATCAGGAGG AACACCGATG GCGAAGGCAG GTCTCTGGGC AGTCACTGAC
            651  GCTGAGGAGC GAAAGCATGG GGAGCGAACA GGATTAGATA CCCTGGTAGT
            701  CCATGCCGTA AACGTTGGGC ACTAGGTGTG GGGCCAGTTC CACTGGTTCT
            751  GTGCCGTAGC TAACGCATTA AGTGCCCCGC CTGGGGAGTA CGGCCGCAAG
            801  GCTAAAACTC
GMKU 305      1  GGCGAACGGG TGAGTAACAC GTGGGCAATC TGCCCTGCAC TCTGGGACAA
             51  GCCCTGGAAA CGGGGTCTAA TACCGGATAT GACCACCGGC CGCATGGTCT
            101  GGTGGTGGAA AGCTCCGGCG GTGCAGGATG AGCCCGCGGC CTATCAGCTT
            151  GTTGGTGGGG TGATGGCCTA CCAAGGCGAC GACGGGTAGC CGGCCTGAGA
            201  GGGCGACCGG CCACACTGGG ACTGAGACAC GGCCCAGACT CCTACGGGAG
            251  GCAGCAGTGG GGAATATTGC ACAATGGGCG CAAGCCTGAT GCAGCGACGC
            301  CGCGTGAGGG ATGACGGCCT TCGGGTTGTA AACCTCTTTC AGCAGGGAAG
            351  AAGCGCAAGT GACGGTACCT GCAGAAGAAG CACCGGCTAA CTACGTGCCA
            401  GCAGCCGCGG TAATACGTAG GGTGCGAGCG TTGTCCGGAA TTATTGGGCG
            451  TAAAGAGCTC GTAGGCGGCT TGTCGCGTCG GATGTGAAAG CCCGGGGCTT
            501  AACCCCGGGT CTGCATTCGA TACGGGCAGG CTGGAGTTCG GCAGGGGAGA
            551  TTGGAATTCC TGGTGTAGCG GTGAAATGCG CAGATATCAG GAGGAACACC
            601  GGTGGCGAA
GMKU 306      1  AACCACTTCG GTGGGGATTA GTGGCGAACG GGTGAGTAAC ACGTGGGCAA
             51  TCTGCCCTGC ACTCTGGGAC AAGCCCTGGA AACGGGGTCT AATACCGGAT
            101  ACTGATCCTC GCAGGCATCT GCGAGGTTCG AAAGCTCCGG CGGTGCAGGA
            151  TGAGCCCGCG GCCTATCAGC TAGTTGGTGA GGTAACGGCT CACCAAGGCG
            201  ACGACGGGTA GCCGGCCTGA GAGGGCGACC GGCCACACTG GGACTGAGAC
            251  ACGGCCCAGA CTCCTACGGG AGGCAGCAGT GGGGAATATT GCACAATGGG
            301  CGAAAGCCTG ATGCAGCGAC GCCGCGTGAG GGATGACGGC CTTCGGGTTG
            351  TAAACCTCTT TCAGCAGGGA AGAAGCGAAA GTGACGGTAC CTGCAGAAGA
            401  AGCGCCGGCT AACTACGTGC CAGCAGCCGC GGTAATACGT AGGGCGCAAG
            451  CGTTGTCCGG AATTATTGGG CGTAAAGAGC TCGTAGGCGG CTTGTCACGT
            501  CGGTTGTGAA AGCCCGGGGC TTAACCCCGG GTCTGCAGTC GATACGGGCA
            551  GGCTAGAGTT CGGTAGGGGA GATCGGAATT CCTGGTGTAG CGGTGAAATG
            601  CGCAGATATC AGGAGGAACA CCGGTGGCGA AGGCGGATCT CTGGGCCGAT
            651  ACTGACGCTG AGGAGCGAAA GCGTGGGGAG CGAACAGGAT TAGATACCCT
            701  GGTAGTCCAC GCCGTAAACG GTGGGCACTA GGTGTGGGCA ACATTCCACG
            751  TTGTCCGTGC CGCAGCTAAC GCATTAAGTG CCCCGCCTGG GGAGTACGGC
            801  CGCAAGGCTA AAACTCA
GMKU 307      1  GCAAGTCGAA CGATGAACCA CTTCGGTGGG GATTAGTGGC GAACGGGTGA
             51  GTAACACGTG GGCAATCTGC CCTGCACTCT GGGACAAGCC CTGGAAACGG
            101  GGTCTAATAC CGGATACTGA TCCTCTTGGG CATCTTGGAT GATCGAAAGC
            151  TCCGGCGGTG CAGGATGAGC CCGCGGCCTA TCAGCTTGTT GGTGAGGTAA
            201  TGGCTCACCA AGGCGACGAC GGGTAGCCGG CCTGAGAGGG CGACCGGCCA
            251  CACTGGGACT GAGACACGGC CCAGACTCCT ACGGGAGGCA GCAGTGGGGA
            301  ATATTGCACA ATGGGCGAAA GCCTGATGCA GCGACGCCGC GTGAGGGATG
            351  ACGGCCTTCG GGTTGTAAAC CTCTTTCAGC AGGGAAGAAG CGAAAGTGAC
            401  GGTACCTGCA GAAGAAGCGC CGGCTAACTA CGTGCCAGCA GCCGCGGTAA
            451  TACGTAGGGC GCGAGCGTTG TCCGGAATTA TTGGGCGTAA AGAGCTCGTA
            501  GGCGGCTTGT CACGTCGGTT GTGAAAGCCC GGGGCTTAAC CCCGGGTCTG
            551  CAGTCGATAC GGGCAGGCTA GAGTTCGGTA GGGGAGATCG GAATTCCTGG
            601  TGTAGCGGTG AAATGCGCAG ATATCAGGAG GAACACCGGT GGCGAAGGCG
            651  GATCTCTGGG CCGATACTGA CGCTGAGGAG CGAAAGCGTG GGGAGCGAAC
            701  AGGATTAGAT ACCCTGGTAG TCCACGCCGT AAACGGTGGG CACTAGGTGT
            751  GGGCG
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GMKU 308      1  CGAACGATGA ACCACTTCGG TGGGGATTAG TGGCGAACGG GTGAGTAACA
             51  CGTGGGCAAT CTGCCCTTCA CTCTGGGACA AGCCCTGGAA ACGGGGTCTA
            101  ATACCGGATA ACACTCCTGT CCTCCTGGAC GGGGGTTAAA AGCTCCGGCG
            151  GTGAAGGATG AGCCCGCGGC CTATCAGCTT GTTGGTGAGG TAATGGCTCA
            201  CCAAGGCGAC GACGGGTAGC CGGCCTGAGA GGGCGACCGG CCACACTGGG
            251  ACTGAGACAC GGCCCAGACT CCTACGGGAG GCAGCAGTGG GGAATATTGC
            301  ACAATGGGCG AAAGCCTGAT GCAGCGACGC CGCGTGAGGG ATGACGGCCT
            351  TCGGGTTGTA AACCTCTTTC AGCAGGGAAG AAGCGAAAGT GACGGTACCT
            401  GCAGAAGAAG CGCCGGCTAA CTACGTGCCA GCAGCCGCGG TAATACGTAG
            451  GGCGCAAGCG TTGTCCGGAA TTATTGGGCG TAAAGAGCTC GTAGGCGGCT
            501  TGTCACGTCG GGTGTGAAAG CCCGGGGCTT AACCCCGGGT CTGCATTCGA
            551  TACGGGCTAG CTAGAGTGTG GTAGGGGAGA TCGGAATTCC TGGTGTAGCG
            601  GTGAAATGCG CAGATATCAG GAGGAACACC GGTGGCGAAG GCGGATCTCT
            651  GGGCCATTAC TGACGCTGAG GAGCGAAAGC GTGGGGAGCG AACAGGATTA
            701  GATACCCTGG TAGTCCACGC CGTAAACGGT GGGAACTAGG TGTTGGCGAC
            751  ATTCCACGTC GTCGGTGCCG CAGCTAACGC ATTAAGTTCC CCGCCTGGGG
            801  AGTACGGCCG CAAGGCTAAA ACTCA
GMKU 311      1  TCGAACGATG AACCTCCTTC GGGAGGGGAT TAGTGGCGAA CGGGTGAGTA
             51  ACACGTGGGC AATCTGCCCT GCACTCTGGG ACAAGCCCTG GAAACGGGGT
            101  CTAATACCGG ATACTGACCC GCTTGGGCAT CCAAGCGGTT CGAAAGCTCC
            151  GGCGGTGCAG GATGAGCCCG CGGCCTATCA GCTTGTTGGT GAGGTAATGG
            201  CTCACCAAGG CGACGACGGG TAGCCGGCCT GAGAGGGCGA CCGGCCACAC
            251  TGGGACTGAG ACACGGCCCA GACTCCTACG GGAGGCAGCA GTGGGGAATA
            301  TTGCACAATG GGCGAAAGCC TGATGCAGCG ACGCCGCGTG AGGGATGACG
            351  GCCTTCGGGT TGTAAACCTC TTTCAGCAGG GAAGAAGCGA AAGTGACGGT
            401  ACCTGCAGAA GAAGCGCCGG CTAACTACGT GCCAGCAGCC GCGGTAATAC
            451  GTAGGGCGCG AGCGTTGTCC GGAATTATTG GGCGTAAAGA GCTCGTAGGC
            501  GGCTTGTCAC GTCGGTTGTG AAAGCCCGGG GCTTAACCCC GGGTCTGCAG
            551  TCGATACGGG CAGGCTAGAG TTCGGTAGGG GAGATCGGAA TTCCTGGTGT
            601  AGCGGTGAAA TGCGCAGATA TCAGGAGGAA CACCGGTGGC GAAGGCGGAT
            651  CTCTGGGCCG ATACTGACGC TGAGGAGCGA AAGCGTGGGG AGCGAACAGG
            701  ATTAGATACC CTGGTAGTCC ACGCCGTAAA CGGTGGGCAC TAGGTGTGGG
            751  CGACATTCCA CGTCGTCCGT GCCGCAGCTA ACGCATTAAG TGCCCCGCCT
            801  GGGGAGTACG GCCGCAAGGC TAAAA
GMKU 313      1  CAAGTTCGAA CGATGAACCA CCTTCGGGTG GGGATTAGTG GCGAACGGGT
             51  GAGTAACACG TGGGCAATCT GCCCTGCACT CTGGGACAAG CCCTGGAAAC
            101  GGGGTCTAAT ACCGGATACT GAACCACTTG GGCATCCAGG CGGTTCGAAA
            151  GCTCCGGCGG TGCAGGATGA GCCCGCGGCC TATCAGCTTG TTGGTGAGGT
            201  AACGGCTCAC CAAGGCGACG ACGGGTAGCC GGCCTGAGAG GGCGACCGGC
            251  CACACTGGGA CTGAGACACG GCCCAGACTC CTACGGGAGG CAGCAGTGGG
            301  GAATATTGCA CAATGGGCGA AAGCCTGATG CAGCGACGCC GCGTGAGGGA
            351  TGACGGCCTT CGGGTTGTAA ACCTCTTTCA GCAGGGAAGA AGCGAAAGTG
            401  ACGGTACCTG CAGAAGAAGC GCCGGCTAAC TACGTGCCAG CAGCCGCGGT
            451  AATACGTAGG GCGCGAGCGT TGTCCGGAAT TATTGGGCGT AAAGAGCTCG
            501  TAGGCGGCTT GTCGCGTCGG TTGTGAAAGC CCGGGGCTTA ACCCCGGGTC
            551  TGCAGTCGAT ACGGGCAGGC TAGAGTTCGG TAGGGGAGAT CGGAATTCCT
            601  GGTGTAGCGG TGAAATGCGC AGATATCAGG AGGAACACCG GTGGCGAAGG
            651  CGGATCTCTG GGCCGATACT GACGCTGAGG AGCGAAAGCG TGGGGAGCGA
            701  ACAGGATTAG ATACCCTGGT AGTCCACGCC GTAAACGGTG GGCACTAGGT
            751  GTGGGCGACA TTCCACGTCG TCCGTGCCGC AGCTAACGCA TTAAGTGCCC

GMKU 309      1  ATTAGTGGCG AACGGGTGAG TAACACGTGG GCAATCTGCC CTGCACTCTG
             51  GGACAAGCCC TGGAAACGGG GTCTAATACC GGATACTGAC CCTCACGGGC
            101  ATCTGTGAGG TTCGAAAGCT CCGGCGGTGC AGGATGAGCC CGCGGCCTAT
            151  CAGCTTGTTG GTGAGGTAAT GGCTCACCAA GGCGACGACG GGTAGCCGGC
            201  CTGAGAGGGC GACCGGCCAC ACTGGGACTG AGACACGGCC CAGACTCCTA
            251  CGGGAGGCAG CAGTGGGGAA TATTGCACAA TGGGCGAAAG CCTGATGCAG
            301  CGACGCCGCG TGAGGGATGA CGGCCTTCGG GTTGTAAACC TCTTTCAGCA
            351  GGGAAGAAGC GAAAGTGACG GTACCTGCAG AAGAAGCGCC GGCTAACTAC
            401  GTGCCAGCAG CCGCGGTAAT ACGTAGGGCG CGAGCGTTGT CCGGAATTAT
            451  TGGGCGTAAA GAGCTCGTAG GCGGCTTGTC GCGTCGGTTG TGAAAGCCCG
            501  GGGCTTAACC CCGGGTCTGC AGTCGATACG GGCAGGCTAG AGTTCGGTAG
            551  GGGAGATCGG AATTCCTGGT GTAGCGGTGA AATGCGCAGA TATCAGGAGG
            601  AACACCGGTG GCGAAGGCGG ATCTCTGGGC CGATACTGAC GCTGAGGAGC
            651  GAAAGCGTGG GGAGCGAACA GGATTAGATA CCCTGGTAGT CCACGCCGTA
            701  AACGGTGGGC ACTAGGTGTG GGCAACATTC CACGTTGTCC GTGCCGCAGC
            751  TAACGCATTA AGTGCCCCGC CTGGGGAGTA CGGCCGCAAG GCTAAAACTC
            801  AAAGGAATT
GMKU 312      1  ACATGCAAGA CGAACGATGA ACCACTTCGT ATGGGGATTA GTGGCGAACG
             51  GGTGAGTAAC ACGTGGGCAA TCTGCCCTGC ACTCTGGGAC AAGCCCTGGA
            101  AACGGGGTCT AATACCGGAT ACTGACCCGC TTGGGCATCC AAGCGGTTCG
            151  AAAGCTCCGG CGGTGCAGGA TGAGCCCGCG GCCTATCAGC TTGTTGGTGA
            201  GGTAATGGCT CACCAAGGCG ACGACGGGTA GCCGGCCTGA GAGGGCGACC
            251  GGCCACACTG GGACTGAGAC ACGGCCCAGA CTCCTACGGG AGGCAGCAGT
            301  GGGGAATATT GCACAATGGG CGAAAGCCTG ATGCACCGAC GCCCCGTGAG
            351  GGATGACGGC CTTCGGGTTG TAAACCTCTT TCAGCAGGGA AGAAGCGAAA
            401  GTGACGGTAC CTGCAGAAGA AGCGCCGGCT AACTACGTGC CAGCAGCCGC
            451  GGTAATACGT AGGGCGCGAG CGTTGTCCGG AATTATTGGG CGTAAAGAGC
            501  TCGTAGGCGG CTTGTCACGT CGGTTGTGAA AGCCCGGGGC TTAACCCCGG
            551  GTCTGCAGTC AATACGGGCA GGCTAGAGTT CGGTAGGGGA GATCGGAATT
            601  CCTGGTGTAG CGGTGAAATG CGCAGATATC AGGAGGAACA CCGGTGGC
GMKU 314      1  AACGATGAAC CACTTCGGTG GGGATTAGTG GCGAACGGGT GAGTAACACG
             51  TGGGCAATCT GCCCTGCACT CTGGGACAAG CCCTGGAAAC GGGGTCTAAT
            101  ACCGGATACT GATCTGCTTG GGCATCCAAG TGGTTCGAAA GCTCCGGCGG
            151  TGCAGGATGA GCCCGCGGCC TATCAGCTTG TTGGTGAGGT AACGGCTCAC
            201  CAAGGCGACG ACGGGTAGCC GGCCTGAGAG GGCGACCGGC CACACTGGGA
            251  CTGAGACACG GCCCAGACTC CTACGGGAGG CAGCAGTGGG GAATATTGCA
            301  CAATGGGCGA AAGCCTGATG CAGCGACGCC GCGTGAGGGA TGACGGCCTT
            351  CGGGTTGTAA ACCTCTTTCA GCAGGGAAGA AGCGAAAGTG ACGGTACCTG
            401  CAGAAGAAGC GCCGGCTAAC TACGTGCCAG CAGCCGCGGT AATACGTAGG
            451  GCGCAAGCGT TGTCCGGAAT TATTGGGCGT AAAGAGCTCG TAGGCGGCTT
            501  GTCGCGTCGG TTGTGAAAGC CCGGGGCTTA ACCCCGGGTC TGCAGTCGAT
            551  ACGGGCAGGC TAGAGTTCGG TAGGGGAGAT CGGAATTCCT GGTGTAGCGG
            601  TGAAATGCGC AGATATCAGG AGGAACACCG GTGGCGAAGG CGGATCTCTG
            651  GGCCGATACT GACGCTGAGG AGCGAAAGCG TGGGGAGCGA ACAGGATTAG
            701  ATACCCTGGT AGTCCACGCC GTAAACGGTG GGCACTAGGT GTGGGCAACA
            751  TTCCACGTTG TCCGTGCCGC AGCTAACGCA TTAAGTGCCC CGCCTGGGGA
            801  GTACGGCCGC AAGGCT
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GMKU 315       1  GCAAGTCGAA CGATGAACCA CCTTCGGGTT GGGGATTAGT GGCGAACGGG
              51  TGAGTAACAC GTGGGCAATC TGCCCTGCAC TTCGGGACAA GCCCTGGAAA
             101  CGGGGTCTAA TACCGGATAC GACGCGCTCG GGCATCCGAT GCGCGTGGAA
             151  AGCTCCGGCG GTGCAGGATG GGCCCGCGGC CTATCAGCTG GTTGGTGAGG
             201  TAACGGCTCA CCAAGGCGAC GACGGGTAGC CGGCCTGAGA GGGCGACCGG
             251  CCACACTGGG ACTGAGACAC GGCCCAGACT CCTACGGGAG GCAGCAGTGG
             301  GGAATATTGC ACAATGGGCG CAAGCCTGAT GCAGCGACGC CGCGTGAGGG
             351  ATGACGGCCT TCGGGTTGTA AACCTCTTTC AGCAGGGAAG AAGCGCGAGT
             401  GACGGTACCT GCAGAAGAAG CGCCGGCTAA CTACGTGCCA GCAGCCGCGG
             451  TAATACGTAG GGCGCGAGCG TTGTCCGGAA TTATTGGGCG TAAAGAGCTC
             501  GTAGGCGGCC TGTCGCGTCG GTTGTGAAAG CCCGGGGCTC AACCCCGGGT
             551  CTGCAGTCGA TACGGGCAGG CTAGAGTTCG GTAGGGGAGA TCGGAATTCC
             601  TGGTGTAGCG GTGAAATGCG CAGATATCAG GAGGAACACC GGTGGCGAAG
             651  GCGGATCTCT GGGCCGACAC TGACGCTGAG GAGCGAAAGC GTGGGGAGCG
             701  AACAGGATTA GATACCCTGG TAGTCCACGC CGTAAACGGT GGGCACTAGG
             751  TGTGGGCGAC ATTCCACGTC GTCCGTGCCG CAGCTAACG

GMKU 317       1  GTCGAGCGGA AAGGCCCTTC GGGGTACTCG AGCGGCGAAC GGGTGAGTAA
              51  CACGTGAGTA ACCTGCCCCT GACTCTGGGA TAAGCCTGGG AAACCGGGTC
             101  TAATACCGGA TACGACACTC CTCCGCATGG TGTGGGTGTG GAAAGTTTTT
             151  TCGGTTGGGG ATGGGCTCGC GGCCTATCAG CTTGTTGGTG GGGTGATGGC
             201  CTACCAAGGC GACGACGGGT AGCCGGCCTG AGAGGGCGAC CGGCCACACT
             251  GGGACTGAGA CACGGCCCAG ACTCCTACGG GAGGCAGCAG TGGGGAATAT
             301  TGCGCAATGG GCGGAAGCCT GACGCAGCGA CGCCGCGTGG GGGATGACGG
             351  CCTTCGGGTT GTAAACCTCT TTCAGCAGGG ACGAAGTTGA CGTGTACCTG
             401  TAGAAGAAGC GCCGGCTAAC TACGTGCCAG CAGCCGCGGT AATACGTAGG
             451  GCGCGAGCGT TGTCCGGAAT TATTGGGCGT AAAGAGCTCG TAGGTGGCTT
             501  GTTGCGTCTG CCGTGAAAGC CCGTGGCTTA ACTACGGGTC TGCGGTGGAT
             551  ACGGGCAGGC TAGAGGCTGG TAGGGGCAAG CGGAATTCCT GGTGTAGCGG
               1  TTCGGGGTGG ATTAGTGGCG AACGGGTGAG TAACACGTGG GCAATCTGCC

GMKU 319      51  CTGCACTCTG GGACAAGCCC TGGAAACGGG GTCTAATACC GGATATCACT
             101  CTTGCAGGCA TCTGTGAGGG TCGAAAGCTC CGGCGGTGCA GGATGAGCCC
             151  GCGGCCTATC AGCTTGTTGG TGAGGTAATG GCTCACCAAG GCGACGACGG
             201  GTAGCCGGCC TGAGAGGGCG ACCGGCCACA CTGGGACTGA GACACGGCCC
             251  AGACTCCTAC GGGAGGCAGC AGTGGGGAAT ATTGCACAAT GGGCGAAAGC
             301  CTGATGCAGC GACGCCGCGT GAGGGATGAC GGCCTTCGGG TTGTAAACCT
             351  CTTTCAGCAG GGAAGAAGCG AAAGTGACGG TACCTGCAGA AGAAGCGCCG
             401  GCTAACTACG TGCCAGCAGC CGCGGTAATA CGTAGGGCGC AAGCGTTGTC
             451  CGGAATTATT GGGCGTAAAG AGCTCGTAGG CGGCTTGTCA CGTCGGGTGT
             501  GAAAGCCCGG GGCTTAACCC CGGGTCTGCA TTCGATACGG GCTAGCTAGA
             551  GTGTGGTAGG GGAGATCGGA ATTCCTGGTG TAGCGGTGAA ATGCGCAGAT
             601  ATCAGGAGGA ACACCGGTGG CGAAGGCGGA TCTCTGGGCC ATTACTGACG
             651  CTGAGGAGCG AAAGCGTGGG GAGCGAACAG GATTAGATAC CCTGGTAGTC
             701  CACGCCGTAA ACGGTGGGAA CTAGGTGTTG GCGACATTCC ACGTCGTCGG
             751  TGCCGCAGCT AACGCATTAA GTTCCCCGCC TGGGGAGTAC GGCCGCAAGG
             801  CTAAAAACTC

GMKU 316       1  GATGAACCAC TTCGGTGGGG ATTAGTGGCG AACGGGTGAG TAACACGTGG
              51  GCAATCTGCC CTTCACTCTG GGACAAGCCC TGGAAACGGG GTCTAATACC
             101  GGATACCACT CGCGCAGGCA TCTGTGCGGG TTGAAAGCTC CGGCGGTGAA
             151  GGATGAGCCC GCGGCCTATC AGCTTGTTGG TGAGGTAACG GCTCACCAAG
             201  GCGACGACGG GTAGCCGGCC TGAGAGGGCG ACCGGCCACA CTGGGACTGA
             251  GACACGGCCC AGACTCCTAC GGGAGGCAGC AGTGGGGAAT ATTGCACAAT
             301  GGGCGAAAGC CTGATGCAGC GACGCCGCGT GAGGGATGAC GGCCTTCGGG
             351  TTGTAAACCT CTTTCAGCAG GGAAGAAGCG AAAGTGACGG TACCTGCAGA
             401  AGAAGCGCCG GCTAACTACG TGCCAGCAGC CGCGGTAATA CGTAGGGCGC
             451  AAGCGTTGTC CGGAATTATT GGGCGTAAAG AGCTCGTAGG CGGCTTGTCA
             501  CGTCGGGTGT GAAAGCCCGG GGCTTAACCC CGGGTCTGCA TTCGATACGG
             551  GCTAGCTAGA GTGTGGTAGG GGAGATCGGA ATTCCTGGTG TAGCGGTGAA
             601  ATGCGCAGAT ATCAGGAGGA ACACCGGTGG CGAAGGCGGA TCTCTGGGCC
             651  ATTACTGACG CTGAGGAGCG AAAGCGTGGG GAGCGAACAG GATTAGATAC
             701  CCTGGTAGTC CACGCCGTAA ACGGTGGGAA CTAGGTGTTG GCGACATTCC
             751  ACGTCGTCGG TGCCGCAGCT AACGCATTAA GTTCCCCGCC TGGGG

GMKU 318       1  ACATGCAAGT CGAGCGGAAA GGCCCTTCGG GGTACTCGAG CGGCGAACGG
              51  GTGAGTAACA CGTGAGTAAC CTGCCCCTGA CTCTGGGATA AGCCTGGGAA
             101  ACCGGGTCTA ATACCGGATA TGACACTCCT CCGCATGGTG TGGGTGTGGA
             151  AAGTTTTTTC GGTTGGGGAT GGACTCGCGG CCTATCAGCT TGTTGGTGGG
             201  GTGATGGCCT ACCAAGGCGA CGACGGGTAG CCGGCCTGAG AGGGCGACCG
             251  GCCACACTGG GACTGAGACA CGGCCCAGAC TCCTACGGGA GGCAGCAGTG
             301  GGGAATATTG CGCAATGGGC GAAAGCCTGA CGCAGCGACG CCGCGTGGGG
             351  GATGACGGCC TTCGGGTTGT AAACCTCTTT CAGCAGGGAC GAAGTTGACG
             401  TGTACCTGTA GAAGAAGCGC CGGCTAACTA CGTGCCAGCA GCCGCGGTAA
             451  TACGTAGGGC GCGAGCGTTG TCCGGAATTA TTGGGCGTAA AGAGCTCGTA
             501  GGTGGCTTGT TGCGTCTGCC GTGAAAGCCC GTGGCTTAAC TACGGGTCTG
             551  CGGTGGATAC GGGCAGGCTA GAGGCTGGTA GGGGCAAGCG GAATTCCTGG
             601  TGTAGCGGTG AAATGCGCAG ATATCAGGAG GAACACCGGT GGCGAAGGCG
             651  GCTTGCTGGG CCAGTTCTGA CGCTGAGGAG CGAAAGCGTG GGGAGCGAAC
             701  AGGATTAGAT ACCCTGGTAG TCCACGCTGT AAACGTTGGG CGCTAGGTGT
             751  GGGGGTCTTC CACGATCTCT GTGCCGTAAC TAACGCATTA AGCGCCCCCC
             801  CCTGGGGGAG T

GMKU 320       1  ACTTCGGTGG GGATTAGTGG CGAACGGGTG AGTAACACGT GGGCAATCTG
              51  CCCTTCACTC TGGGACAAGC CCTGGAAACG GGGTCTAATA CCGGATAACA
             101  CTCTTACGGG CATCTGTGAG GGTTAAAAGC TCCGGCGGTG AAGGATGAGC
             151  CCGCGGCCTA TCAGCTTGTT GGTGAGGTAA TGGCTCACCA AGGCGACGAC
             201  GGGTAGCCGG CCTGAGAGGG CGACCGGCCA CACTGGGACT GAGACACGGC
             251  CCAGACTCCT ACGGGAGGCA GCAGTGGGGA ATATTGCACA ATGGGCGAAA
             301  GCCTGATGCA GCGACGCCGC GTGAGGGATG ACGGCCTTCG GGTTGTAAAC
             351  CTCTTTCAGC AGGGAAGAAG CGAAAGTGAC GGTACCTGCA GAAGAAGCGC
             401  CGGCTAACTA CGTGCCAGCA GCCGCGGTAA TACGTAGGGC GCAAGCGTTG
             451  TCCGGAATTA TTGGGCGTAA AGAGCTCGTA GGCGGCTTGT CACGTCGATT
             501  GTGAAAGCCC GAGGCTTAAC CTCGGGTCTG CAGTCGATAC GGGCTAGCTA
             551  GAGTGTGGTA GGGGAGATCG GAATTCCTGG TGTAGCGGTG AAATGCGCAG
             601  ATATCAGGAG GAACACCGGT GGCGAAGGCG GATCTCTGGG CCATTACTGA
             651  CGCTGAGGAG CGAAAGCGTG GGGAGCGAAC AGGATTAGAT ACCCTGGTAG
             701  TCCACGCCGT AAACGGTGGG AACTAGGTGT TGGCGACATT CCACGTCGTC
             751  GGTGCCGCAG CTAACGCATT AAGTTCCCCG CCTGGGGAGT ACGGCCGCAG
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GMKU 322

```
   1  CGGTGGGGAT TAGTGGCGAA CGGGTGAGTA ACACGTGGGC AATCTGCCCT
  51  GCACTCTGGG ACAAGCCCTG GAAACGGGGT CTAATACCGG ATACTGATCT
 101  TCTTGGGCAT CCTGGATGAT CGAAAGCTCC GGCGGTGCAG GATGAGCCCG
 151  CGGCCTATCA GCTAGTTGGT GAGGTAATGG CTCACCAAGG CGACGACGGG
 201  TAGCCGGCCT GAGAGGGCGA CCGGCCACAC TGGGACTGAG ACACGGCCCA
 251  GACTCCTACG GGAGGCAGCA GTGGGGAATA TTGCACAATG GGCGAAAGCC
 301  TGATGCAGCG ACGCCGCGTG AGGGATGACG GCCTTCGGGT TGTAAACCTC
 351  TTTCAGCAGG GAAGAAGCGA AAGTGACGGT ACCTGCAGAA GAAGCGCCGG
 401  CTAACTACGT GCCAGCAGCC GCGGTAATAC GTAGGGCGCG AGCGTTGTCC
 451  GGAATTATTG GGCGTAAAGA GCTCGTAGGC GGCTTGTCAC GTCGGTTGTG
 501  AAAGCCCGGG GCTTAACCCC GGGTCTGCAG TCGATACGGG CAGGCTAGAG
 551  TTCGGTAGGG GAGATCGGAA TTCCTGGTGT AGCGGTGAAA TGCGCAGATA
 601  TCAGGAGGAA CACCGGTGGC GAAGGCGGAT CTCTGGGCCG ATACTGACGC
 651  TGAGGAGCGA AAGCGTGGGG AGCGAACAGG ATTAGATACC CTGGTAGTCC
 701  ACGCCGTAAA CGGTGGGCAC TAGGTGTGGG CGACATTCCA CGTCGTCCGT
 751  GCCGCAGCTA ACGCATTAAG TGCCCCGCCT GGGGAGTACG GCCGCAAGGC
 801  TAAAACTCA
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GMKU 323

```
   1  TTCGGTGGGG ATTAGTGGCG AACGGGTGAG TAACACGTGG GCAATCTGCC
  51  CTGCACTCTG GGACAAGCCC TGGAAACGGG GTCTAATACC GGATACTGAC
 101  CCGCTTGGGC ATCCAAGCGG TTCGAAAGCT CCGGCGGTGC AGGATGAGCC
 151  CGCGGCCTAT CAGCTTGTTG GTGAGGTAAT GGCTCACCAA GGCGACGACG
 201  GGTAGCCGGC CTGAGAGGGC GACCGGCCAC ACTGGGACTG AGACACGGCC
 251  CAGACTCCTA CGGGAGGCAG CAGTGGGGAA TATTGCACAA TGGGCGAAAG
 301  CCTGATGCAG CGACGCCGCG TGAGGGATGA CGGCCTTCGG GTTGTAAACC
 351  TCTTTCAGCA GGGAAGAAGC GAAAGTGACG GTACCTGCAG AAGAAGCGCC
 401  GGCTAACTAC GTGCCAGCAG CCGCGGTAAT ACGTAGGGCG CGAGCGTTGT
 451  CCGGAATTAT TGGGCGTAAA GAGCTCGTAG GCGGCTTGTC ACGTCGGTTG
 501  TGAAAGCCCG GGGCTTAACC CCGGGTCTGC AGTCGATACG GGCAGGCTAG
 551  AGTTCGGTAG GGGAGATCGG AATTCCTGGT GTAGCGGTGA AATGCGCAGA
 601  TATCAGGAGG AACACCGGTG GCGAAGGCGG ATCTCTGGGC CGATACTGAC
 651  GCTGAGGAGC GAAAGCGTGG GGAGCGAACA GGATTAGATA CCCTGGTAGT
 701  CCACGCCGTA AACGGTGGGC ACTAGGTGTG GGCGACATTC CACGTCGTCC
 751  GTGCCGCAGC TAACGCATTA AGTGCCCCGC CTGGGGAGTA CGGCCGCAAG
 801  GCTAAAACTC
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GMKU 324

```
   1  AAGTCGAGCG GAAAGGCCCT TCGGGGTACT CGAGCGGCGA ACGGGTGAGT
  51  AACACGTGAG TAACCTGCCC CTGACTCTGG GATAAGCCTG GGAAACCGGG
 101  TCTAATACCG GATATGACAC TCCTTCGCAT GGTGTGGGTG TGGAAAGTTT
 151  TTTCGGTTGG GGATGGGCTC GCGGCCTATC AGCTTGTTGG TGGGGTGATG
 201  GCCTACCAAG GCGACGACGG GTAGCCGGCC TGAGAGGGCG ACCGGCCACA
 251  CTGGGACTGA GACACGGCCC AGACTCCTAC GGGAGGCAGC AGTGGGGAAT
 301  ATTGCGCAAT GGGCGGAAGC CTGACGCAGC GACGCCGCGT GGGGGATGAC
 351  GGCCTTCGGG TTGTAAACCT CTTTCAGCAG GGACGAAGTT GACGTGTACC
 401  TGTAGAAGAA GCGCCGGCTA ACTACGTGCC AGCAGCCGCG GTAATACGTA
 451  GGGCGCGAGC GTTGTCCGGA ATTATTGGGC GTAAAGAGCT CGTAGGTGGC
 501  TTGTTGCGTC TGCCGTGAAA GCCCGTGGCT TAACTACGGG TCTGCGGTGG
 551  ATACGGGCAG GCTAGAGGCT GGTAGGGGCA AGCGGAATTC CTGGTGTAGC
 601  GGTGAAATGC GCAGATATCA GGAGGAACAC CGGTGGCGAA GGCGGCTTGC
 651  TGGGCCAGTT CTGACGCTGA GGAGCGAAAG CGTGGGGAGC GAACAGGATT
 701  AGATACCCTG GTAGTCCACG CTGTAAACGT TGGGCGCTAG GTGTGGGGGT
 751  CTTCCACGAT TCCTGTGCCG TAGCTAACGC ATTAAGCGCC C
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GMKU 325

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   1  GCAAGTCGAG CGGAAAGGCC CTTCGGGGTA CTCGAGCGGC GAACGGGTGA
  51  GTAACACGTG AGCAACCTGC CCCTGACTCT GGGATAAGCC CGGGAAACTG
 101  GGTCTAATAC CGGATATGAC CACCGTCCGC ATGGTCTGGT GGTGGAAAGT
 151  TTTTCGGTTG GGGATGGGCT CGCGGCCTAT CAGCTTGTTG GTGGGGTAGT
 201  GGCCTACCAA GGCGACGACG GGTAGCCGGC CTGAGAGGGC GACCGGCCAC
 251  ACTGGGACTG AGACACGGCC CAGACTCCTA CGGGAGGCAG CAGTGGGGAA
 301  TATTGCGCAA TGGGCGGAAG CCTGACGCAG CGACGCCGCG TGGGGGATGA
 351  CGGCCTTCGG GTTGTAAACC TCTTTCAGCA GGGACGAAGT TGACGTGTAC
 401  CTGCAGAAGA AGCGCCGGCT AACTACGTGC CAGCAGCCGC GGTAATACGT
 451  AGGGCGCAAG CGTTGTCCGG AATTATTGGG CGTAAAGAGC TCGTAGGTGG
 501  CTGGTCGCGT CTGCCGTGAA AGCCCGCAGC TTAACTGCGG GTCTGCGGTG
 551  GATACGGGCC GGCTAGAGGT AGGCAGGGGC AAGTGGAATT CCTGGTGTAG
 601  CGGTGAAATG CGCAGATATC AGGAGGAACA CCGGTGGCGA AGGCGGCTTG
 651  CTGGGCCTTA CCTGACGCTG AGGAGCGAAA GCGTGGGGAG CGAACAGGAT
 701  TAGATACCCT GGTAGTCCAC GCTGTAAACG TTGGGCGCTA GGTGTGGGGG
 751  TCTTCCACGA CTTCCGTGCC GGAGCTAACG CATTAAGCGC CCCGCCTGGG
 801  GAGTACGGCC GCAAGGCTA
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GMKU 326

```
     1  AAGGCCCTTC GGGGTACTCG AGCGGCGAAC GGGTGAGTAA CACGTGAGTA
    51  ACCTGCCCTA GGCTTTGGGA TAACCCTCGG AAACGGGGGC TAATACCGGA
   101  TATGACCGCT GATCGCATGG TTGGTGGTGG AAAGTTTTTC GGCCTGGGAT
   151  GGGCTCGCGG CCTATCAGCT TGTTGGTGGG GTGATGGCCT ACCAAGGCGA
   201  CGACGGGTAG CCGGCCTGAG AGGGCGACCG GCCACACTGG GACTGAGACA
   251  CGGCCCAGAC TCCTACGGGA GGCAGCAGTG GGGAATATTG CACAATGGGC
   301  GGAAGCCTGA TGCAGCGACG CCGCGTGAGG GATGACGGCC TTCGGGTTGT
   351  AAACCTCTTT CAGCAGGGAC GAAGCGTAAG TGACGGTACC TGCAGAAGAA
   401  GCGCCGGCCA ACTACGTGCC AGCAGCCGCG GTAAGACGTA GGGCGCGAGC
   451  GTTGTCCGGA TTTATTGGGC GTAAAGAGCT CGTAGGCGGC TTGTCGCGTC
   501  GACTGTGAAA ACCCGCAGCT CAACTGCGGG CCTGCAGTCG ATACGGGCAG
   551  GCTAGAGTTC GGTAGGGGAG ACTGGAATTC CTGGTGTAGC GGTGAAATGC
   601  GCAGATATCA GGAGGAACAC CGGTGGCGAA GGCGGGTCTC TGGGCCGATA
   651  CTGACGCTGA GGAGCGAAAG CGTGGGGAGC GAACAGGATT AGATACCCTG
   701  GTAGTCCACG CTGTAAACGT TGGGCGCTAG GTGTGGGGGG CCTCTCCGGT
   751  TCTCTGTGCC GCAGCTAACG CATTAAGCGC CCCGCCTGGG GAGTACGGCC
   801  GCAAGGCTAA AACTCAAAGG AATTGACGGG GGCCCGCACA AGCGGCGGAG
   851  CATGCGGATT AATTCGATGC AACGCGAAGA ACCTTACCTG GGTTTGACAT
   901  GGCCGCAAAA CTGTCAGAGA TGGCAGGTCC TTCGGGGGCG GTCACAGGTG
   951  GTGCATGGCT GTCGTCAGCT CGTGTCGTGA GATGTTGGGT TAAGTCCCGC
  1001  AACGAGCGCA ACCCTCGTTC GATGTTGCCA GCGCGTTATG GCGGGGACTC
  1051  ATCGAAGACT GCCGGGGTCA ACTCGGAGGA AGGTGGGGAT GACGTCAAGT
  1101  CATCATGCCC CTTATGTCCA GGGCTTCACG CATGCTACAA TGGCCGGTAC
  1151  AATGGGCTGC GATACCGTGA GGTGGAGCGA ATCCCAAAAA GCCGGTCTCA
  1201  GTTCGGATCG GGGTCTGCAA CTCGACCCCG TGAAGTCGGA GTCGCTAGTA
  1251  ATCGCAGATC AGCAACGCTG CGGTGAATAC GTTCCCGGGC CTTGTACACA
  1301  CCGCCCGTCA CGTCACGAAA GTCGGCAACA CCCGAAGCCG GTGGCCCAAC
  1351  CCTTGTGGAG GGAGCCGT
```

GMKU 328

```
     1  ACATGCAAGT CGAGCGGAAA GGCCCTTCGG GGTACTCGAG CGGCGAACGG
    51  GTGAGTAACA CGTGAGCAAC CTGCCCCAGG CTTTGGGATA ACCCCGGGAA
   101  ACCGGGGCTA ATACCGAATA TGACCTTGCA CCGCATGGTG TTTGGTGGAA
   151  AGTTTTTCGG CTTGGGATGG GCTCGCGGCC TATCAGCTTG TTGGTGGGGT
   201  GATGGCCTAC CAAGGCGACG ACGGGTAGCC GGCCTGAGAG GGCGACCGGC
   251  CACACTGGGA CTGAGACACG GCCCAGACTC CTACGGGAGG CAGCAGTGGG
   301  GAATATTGCA CAATGGGCGG AAGCCTGATG CAGCGACGCC GCGTGAGGGA
   351  TGACGGCCTT CGGGTTGTAA ACCTCTTTCA GCAGGGACGA AGCGTAAGTG
   401  ACGGTACCTG CAGAAGAAGC GCCGGCCAAC TACGTGCCAG CAGCCGCGGT
   451  AAGACGTAGG GCGCGAGCGT TGTCCGGATT TATTGGGCGT AAAGAGCTCG
   501  TAGGCGGCTT GTCGCGTCGA CCGTGAAAAC CTGGGGCTCA ACCCCAGGCC
   551  TGCGGTCGAT ACGGGCAGGC TAGAGTTCGG TAGGGGAGAC TGGAATTCCT
   601  GGTGTAGCGG TGAAATGCGC AGATATCAGG AGGAACACCG GTGGCGAAGG
   651  CGGGTCTCTG GGCCGATACT GACGCTGAGG AGCGAAAGCG TGGGGAGCGA
   701  ACAGGATTAG ATACCCTGGT AGTCCACGCT GTAAACGTTG GGCGCTAGGT
   751  GTGGGGGGCC TCTCCGGTTC CCTGTGCCGC AGCTAACGCA TTAAGCGCCC
   801  CGCCTGGGGA GTACGGCCGC AAGGC
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GMKU 327

```
     1  TGCAAGTCGA GCGGTAAGGC CCTTCGGGGT ACACGAGCGG CGAACGGGTG
    51  AGTAACACGT GGGTGATCTG CCTTGCACTC TGGGATAAGC CTGGGAAACT
   101  GGGTCTAATA CCGGATATGA CCATTGGTTG CATGATCGGT GGTGGAAAGA
   151  TTTATCGGTG CGAGATGGGC CCGCGGCCTA TCAGCTTGTT GGTGGGGTAA
   201  TGGCCTACCA AGGCGACGAC GGGTAGCCGA CCTGAGAGGG TGACCGGCCA
   251  CACTGGGACT GAGACACGGC CCAGACTCCT ACGGGAGGCA GCAGTGGGGA
   301  ATATTGCACA ATGGGCGGAA GCCTGATGCA GCGACGCCGC GTGAGGGATG
   351  ACGGCCTTCG GGTTGTAAAC CTCTTTCGAC AGGGACGAAG CGTGAGTGAC
   401  GGTACCTGTA GAAGAAGCAC CGGCCAACTA CGTGCCAGCA GCCGCGGTAA
   451  TACGTAGGGT GCGAGCGTTG TCCGGAATTA CTGGGCGTAA AGAGCTTGTA
   501  GGCGGTTCGT CGCGTCGATT GTGAAAACTT GCAGCTCAAC TGCAAGCTTG
   551  CAGTCGATAC GGGCGGACTA GAGTACTTCA GGGGAGACTG GAATTCCTGG
   601  TGTAGCGGTG AAATGCGCAG ATATCAGGAG GAACACCGGT GGCGAAGGCG
   651  GGTCTCTGGG AAGTAACTGA CGCTGAGAAG CGAAAGCGTG GGTAGCGAAC
   701  AGGATTAGAT ACCCTGGTAG TCCACGCCGT AAACGGTGGG TACTAGGTGT
   751  GGGTTTCCTT CCACGGGATC CGTGCCGTAG CTAACGCATT AAGTACCCCG
   801  CCTGGGGAGT ACGG
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GMKU 329

```
     1  CACATGCAAG TCGAGCGGAA AGGCCCCTTC GGGGGTACTC GAGCGGCGAA
    51  CGGGTGAGTA ACACGTGAGC AACCTGCCCC TGACTCTGGG ATAAGCCCGG
   101  GAAACTGGGT CTAATACCGG ATATGACCAC GGGTCGCATG GCCTTGTGGT
   151  GGAAAGTTTT TCGGTTGGGG ATGGGCTCGC GGCCTATCAG CTTGTTGGTG
   201  GGGTGATGGC CTACCAAGGC GACGACGGGT AACCGGCCTG AGAGGGCGAC
   251  CGGTCACACT GGGACTGAGA CACGGCCCAG ACTCCTACGG GAGGCAGCAG
   301  TGGGGAATAT TGCGCAATGG GCGGAAGCCT GACGCAGCGA CGCCGCGTGG
   351  GGGATGACGG CCTTCGGGTT GTAAACCTCT TTCAGCAGGG ACGAAGCGGA
   401  AGTGACGGTA CCTGCAGAAG AAGCGCCGGC TAACTACGTG CCAGCAGCCG
   451  CGGTAATACG TAGGGCGCAA GCGTTGTCCG GAATTATTGG GCGTAAAGAG
   501  CTCGTAGGCG GTTTGTCGCG TCTGTCGTGA AAGCCCACGG CTTAACTGTG
   551  GGTCTGCGGT GGATACGGGC AGACTAGAGG CAGGTAGGGG AGAATGGAAT
   601  TCCCGGTGTA GCGGTGAAAT GCGCAGATAT CGGGAGGAAC ACCGGTGGCG
   651  AAGGCGGTTC TCTGGGCCTG TACTGACGCT GAGGAGCGAA AGCGTGGGGA
   701  GCGAACAGGA TTAGATACCC TGGTAGTCCA CGCCGTAAAC GTTGGGCGCT
   751  AGGTGTGGGG TTCTTCCACG GATTCCGCGC CGTAGCTAAC GCATTAAGCG
   801  CCCCGCCTGG GGAGTACGGC CGCAAGG
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GMKU 330        1  ACACATGCAA GTCGAGCGGA AAGGCCCTTC GGGGTACTCG AGCGGCGAAC
               51  GGGTGAGTAA CACGTGAGCA ACCTGCCCCA GGCTTTGGGA TAACCCCGGG
              101  AAACCGGGGC TAATACCGAA TATGACCTCT GACCGCATGG TTGGTGGTGG
              151  AAAGTTTTTC GGCTTGGGAT GGGCTCGCGG CCTATCAGCT TGTTGGTGGG
              201  GTGATGGCCT ACCAAGGCGA CGACGGGTAG CCGGCCTGAG AGGGCGACCG
              251  GCCACACTGG GACTGAGACA CGGCCCAGAC TCCTACGGGA GGCAGCAGTG
              301  GGGAATATTG CACAATGGGC GGAAGCCTGA TGCAGCGACG CCGCGTGAGG
              351  GATGACGGCC TTCGGGTTGT AAACCTCTTT CAGCAGGGAC GAAGCGTAAG
              401  TGACGGTACC TGCAGAAGAA GCGCCGGCCA ACTACGTGCC AGCAGCCGCG
              451  GTAAGACGTA GGGCGCGAGC GTTGTCCGGA TTTATTGGGC GTAAAGAGCT
              501  CGTAGGCGGC TTGTCGCGTC GACCGTGAAA ACTTGGGGCT CAACCCCAAC
              551  CCCTGC
GMKU 333        1  TCGGGGTGGA TCAGTGGCGA ACGGGTGAGT AACACGTGGG CAATCTGCCC
               51  TGCACTCTGG GACAAGCCCT GGAAACGGGG TCTAATACCG GATATTACCT
              101  TCCCCTGCAT GGGGGTGGGT GGAAAGCTCC GGCGGTGCAG GATGAGCCCG
              151  CGGCCTATCA GCTTGTTGGT GGGGTAATGG CCTACCAAGG CGACGACGGG
              201  TAGCCGGCCT GAGAGGGCGA CCGGCCACAC TGGGACTGAG ACACGGCCCA
              251  GACTCCTACG GGAGGCAGCA GTGGGGAATA TTGCACAATG GGCGAAAGCC
              301  TGATGCAGCG ACGCCGCGTG AGGGATGACG GCCTTCGGGT TGTAAACCTC
              351  TTTCAGCAGG GAAGAAGCGA AAGTGACGGT ACCTGCAGAA GAAGCACCGG
              401  CTAACTACGT GCCAGCAGCC GCGGTAATAC GTAGGGTGCG AGCGTTGTCC
              451  GGAATTATTG GGCGTAAAGA GCTCGTAGGC GGCTTGTCGC GTCGGTTGTG
              501  AAAGCCCGGG GCTTAACCCC GGGTCTGCAG TCGATACGGG CAGGCTAGAG
              551  TTCGGTAGGG GAGATCGGAA TTCCTGGTGT AGCGGTGAAA TGCGCAGATA
              601  TCAGGAGGAA CACCGGTGGC GAAGGCGGAT CTCTGGGCCG ATACTGACGC
              651  TGAGGAGCGA AAGCGTGGGG AGCGAACAGG ATTAGATACC CTGGTAGTCC
              701  ACGCCGTAAA CGTTGGGAAC TAGGTGTGGG CGACATTCCA CGTCGTCCGT
              751  GCCGCAGCTA ACGCATTAAG TTCCCCGCCT GGGGAGTACG GCCGCAAGGC
              801  TAAAACTCAA
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GMKU 331        1  GAGCGGAAAG GCCCCTTCGG GGGTACTCGA GCGGCGAACG GGTGAGTAAC
               51  ACGTGAGCAA CCTGCCCCTG ACTCTGGGAT AAGCCCGGGA AACTGGGTCT
              101  AATACCGGAT ATGACCACGG GTCGCATGGC CTTGTGGTGG AAAGTTTTTC
              151  GGTTGGGGAT GGGCTCGCGG CCTATCAGCT TGTTGGTGGG GTGATGGCCT
              201  ACCAAGGCGA CGACGGGTAA CCGGCCTGAG AGGGCGACCG GTCACACTGG
              251  GACTGAGACA CGGCCCAGAC TCCTACGGGA GGCAGCAGTG GGGAATATTG
              301  CGCAATGGGC GGAAGCCTGA CGCAGCGACG CCGCGTGGGG GATGACGGCC
              351  TTCGGGTTGT AAACCTCTTT CAGCAGGGAC GAAGCGGAAG TGACGGTACC
              401  TGCAGAAGAA GCGCCGGCTA ACTACGTGCC AGCAGCCGCG GTAATACGTA
              451  GGGCGCAAGC GTTGTCCGGA ATTATTGGGC GTAAAGAGCT CGTAGGCGGT
              501  TTGTCGCGTC TGTCGTGAAA GCCCACGGCT TAACTGTGGG TCTGCGGTGG
              551  ATACGGGCAG ACTAGAGGCA GGTAGGGGAG AATGGAATTC CCGGTGTAGC
              601  GGTGAAATGC GCAGATATCG GGAGGAACAC CGGTGGCGAA GGCGGTTCTC
              651  TGGGCCTGTA CTGACGCTGA GGAGCGAAAG CGTGGGGAGC GAACAGGATT
              701  AGATACCCTG GTAGTCCACG CCGTAAACGT TGGGCGCTAG GTGTGGGGTT
              751  CTTCCACGGA TTCCGCGCCG TAGCTAACGC ATTAAGCGCC CCGCCTGGGG
              801  AGTACGGCCG CAAGGCTAAA ACTCAAAGGA ATTGACGGGG GCCCGCACAA
              851  GCGGCGGAAC ATGTTGCTTA ATTC
GMKU 334        1  CTTACACATG CAAGTCGAAC GGGTGAAGCC TTTCGGGGTG GATCAGTGGC
               51  GAACGGGTGA GTAACACGNT GGGCAATCTG CCCTGCACTC TGGGACAAGC
              101  CCTGGAAACG GGGTCTAATA CCGGATATTA CCTTCCTCTG CATGGGGGTT
              151  GGTGGAAAGC TCCGGCGGTG CAGGATGAGC CCGCGGCCTA TCAGCTTGTT
              201  GGTGGGGTAA TGGCCTACCA AGGCGACGAC GGGTAGCCGG CCTGAGAGGG
              251  CGACCGGCCA CACTGGGACT GAGACACGGC CCAGACTCCT ACGGGAGGCA
              301  GCAGTGGGGA ATATTGCACA ATGGGCGAAA GCCTGATGCA GCGACGCCGC
              351  GTGAGGGATG ACGGCCTTCG GGTTGTAAAC CTCTTTCACC ACGGAAAAAC
              401  CAAAGTGACC GGTACCTGCA AAAGAAACAC CGGTCTAACT ACGTTGCCAT
              451  CACCGCGGTA ATACTAAGGT GCGAGCCGTT GTCCCCGAAT TTTATGGCCG
              501  T
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GMKU 335       1  CGATGAAGCC CTTCGGGGTG GATTAGTGGC GAACGGGTGA GTAACACGTG
              51  GGCAATCTGC CCTTCACTCT GGGACAAGCC CTGGAAACGG GGTCTAATAC
             101  CGGATATCAC TCTCGCAGGC ATCTGTGAGG GTCGAAAGCT CCGGCGGTGA
             151  AGGATGAGCC CGCGGCCTAT CAGCTTGTTG GTGAGGTAAC GGCTCACCAA
             201  GGCGACGACG GGTAGCCGGC CTGAGAGGGC GACCGGCCAC ACTGGGACTG
             251  AGACACGGCC CAGACTCCTA CGGGAGGCAG CAGTGGGGAA TATTGCACAA
             301  TGGGCGAAAG CCTGATGCAG CGACGCCGCG TGAGGGATGA CGGCCTTCGG
             351  GTTGTAAACC TCTTTCAGCA GGGAAGAAGC GAAAGTGACG GTACCTGCAG
             401  AAGAAGCGCC GGCTAACTAC GTGCCAGCAG CCGCGGTAAT ACGTAGGGCG
             451  CAAGCGTTGT CCGGAATTAT TGGGCGTAAA GAGCTCGTAG GCGGCTTGTC
             501  ACGTCGGGTG TGAAAGCCCG GGGCTTAACC CCGGGTCTGC ATTCGATACG
             551  GGCTAGCTAG AGTGTGGTAG GGGAGATCGG AATTCCTGGT GTAGCGGTGA
             601  AATGCGCAGA TATCAGGAGG AACACCGGTG GCGAAGGCGG ATCTCTGGGC
             651  CATTACTGAC GCTGAGGAGC GAAAGCGTGG GGAGCGAACA GGATTAGATA
             701  CCCTGGTAGT CCACGCCGTA AACGGTGGGA ACTAGGTGTT GGCGACATTC
             751  CACGTCGTCG GTGCCGCAGC TAACGCATTA AGTTCCCCGC CTGGGGAGTA
             801  CGGCCGCAAG GCTAAAACTC AA
GMKU 337       1  ACCTCCTTCG GGAGGGGATT AGTGGCGAAC GGGTGAGTAA CACGTGGGCA
              51  ATCTGCCCTT CACTCTGGGA CAAGCCCTGG AAACGGGGTC TAATACCGGA
             101  TACGACCTCC GACCGCATGG TCTGGTGGTG GAAAGCTCCG GCGGTGAAGG
             151  ATGAGCCCGC GGCCTATCAG CTTGTTGGTG GGGTGATGGC CTACCAAGGC
             201  GACGACGGGT AGCCGGCCTG AGAGGGCGAC CGGCCACACT GGGACTGAGA
             251  CACGGCCCAG ACTCCTACGG GAGGCAGCAG TGGGGAATAT TGCACAATGG
             301  GCGAAAGCCT GATGCAGCGA CGCCGCGTGA GGGATGACGG CCTTCGGGTT
             351  GTAAACCTCT TTCAGCAGGG AAGAAGCGAG AGTGACGGTA CCTGCAGAAG
             401  AAGCGCCGGC TAACTACGTG CCAGCAGCCG CGGTAATACG TAGGGCGCAA
             451  GCGTTGTCCG GAATTATTGG GCGTAAAGAG CTCGTAGGCG GCTTGTCACG
             501  TCGGATGTGA AAGCCCGGGG CTTAACCCCG GGTCTGCATT CGATACGGGC
             551  AGGCTAGAGT TCGGTAGGGG AGATCGGAAT TCCTGGTGTA GCGGTGAAAT
             601  GCGCAGATAT CAGGAGGAAC ACCGGTGGCG AAGGCGGATC TCTGGGCCGA
             651  TACTGACGCT GAGGAGCGAA AGCGTGGGGA GCGAACAGGA TTAGATACCC
             701  TGGTAGTCCA CGCCGTAAAC GTTGGGAACT AGGTGTGGGC GACATTCCAC
             751  GTCGTCCGTG CCGCAGCTAA CGCATTAAGT TCCCCGCCTG GGGAGTACGG
             801  CCGCAAGGCT AAAACT
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GMKU336        1  AGTGGCGAAC GGGTGAGTAA CACGTGGGCA ATCTGCCCTT CACTCTGGGA
              51  CAAGCCCTGG AAACGGGGTC TAATACCGGA TACCACTGCT CAAGGCATCT
             101  TGGGTGGTTG AAAGCTCCGG CGGTGAAGGA TGAGCCCGCG GCCTATCAGC
             151  TTGTTGGTGA GGTAACGGCT CACCAAGGCG ACGACGGGTA GCCGGCCTGA
             201  GAGGGCGACC GGCCACACTG GGACTGAGAC ACGGCCCAGA CTCCTACGGG
             251  AGGCAGCAGT GGGGAATATT GCACAATGGG CGCAAGCCTG ATGCAGCGAC
             301  GCCGCGTGAG GGATGACGGC CTTCGGGTTG TAAACCTCTT TCAGCAGGGA
             351  AGAAGCGCAA GTGACGGTAC CTGCAGAAGA AGCGCCGGCT AACTACGTGC
             401  CAGCAGCCGC GGTAATACGT AGGGCGCAAG CGTTGTCCGG AATTATTGGG
             451  CGTAAAGAGC TCGTAGGCGG CTTGTCACGT CGGGTGTGAA AGCCCGGGGC
             501  TTAACCCCGG GTCTGCATTC GATACGGGCA GGCTAGAGTG TGGTAGGGGA
             551  GATCGGAATT CCTGGTGTAG CGGTGAAATG CGCAGATATC AGGAGGAACA
             601  CCGGTGGCGA AGGCGGATCT CTGGGCCATT ACTGACGCTG AGGAGCGAAA
             651  GCGTGGGGAG CGAACAGGAT TAGATACCCT GGTAGTCCAC GCCGTAAACG
             701  GTGGGAACTA GGTGTTGGCG ACATTCCACG TCGTCGGTGC CGCAGCTAAC
             751  GCATTAAGTT CCCCGCCTGG GGAGTACGGC CGCAAGGCTA AAACTCAAAG
             801  GAATTGACGG GGGCCCGCAC AAGCAGCGGA GCATGTGGCT TAATTCGACG
             851  CAACGCGAAG AACCTTACCA AGGCTTGACA TACACCGGAA ACGGCCAGAG
             901  ATGGTCGCCC CCTTGTGGTC GGTGTACAGG TGGTGCATGG CTGTCGTCAG
             951  CTCGTGTCGT GAGATGTTGG GTTAAGTCCC GCAACGAGCG CAACCCTTGT
            1001  CCTGTGTTGC CAGCGTGCCC TTCGGGGTGA CGGGGACTCA CAGGAGACCG
            1051  CCGGGGTCAA CTCGGAGGAA GGTGGGGACG ACGTCAAGTC ATCATGCCCC
            1101  TTATGTCTTG GGCTGCACAC GTGCTACAAT GGCAGGTACA ATGAGCTGCG
            1151  ATACCGTGAG GTGGAGCGAA TCTCAAAAAG CCTGTCTCAG TTCGGATTGG
            1201  GGTCTGCAAC TCGACCCCAT GAAGTCGGAG TTGCTAGTAA TCGCAGATCA
            1251  GCATTGCTGC GGTGAATACG TTCCCGGGCC TTGTACACAC CGCCCGTCAC
            1301  GTCACGAAAG TCGGTAACAC CCGAAGCCGG TGGCCCAACC CCTTGTGGGA
            1351  GGGAGCTGTC GAAGGTGGGA CTGGC
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GMKU 341       1  GGCGTGCCTT ACACATGCAA GTCGAACGAT GAACCGCCTT TCGGGCGGGG
              51  ATTAGTGGCG AACGGGTGAG TAACACGTGG GCAATCTGCC CTGCACTCTG
             101  GGACAAGCCC TGGAAACGGG GTCTAATACC GGATATGACT GTCTGTCGCA
             151  TGGTGGATGG TGTAAAGCTC CGGCGGTGCA GGATGAGCCC GCGGCCTATC
             201  AGCTTGTTGG TGAGGTAGTG GCTCACCAAG GCGACGACGG GTAGCCGGCC
             251  TGAGAGGGCG ACCGGCCACA CTGGGACTGA GACACGGCCC AGACTCCTAC
             301  GGGAGGCAGC AGTGGGGAAT ATTGCACAAT GGGCGAAAGC CTGATGCAGC
             351  GACGCCGCGT GAGGGATGAC GGCCTTCGGG TTGTAAACCT CTTTCAGCAG
             401  GGAAGAAGCG AAAGTGACGG TACCTGCAGA AGAAGCGCCG GCTAACTACG
             451  TGCCAGCAGC CGCGGTAATA CGTAGGGCGC AAGCGTTGTC CGGAATTATT
             501  GGGCGTAAAG AGCTCGTAGG CGGCTTGTCA CGTCGGTTGT GAAAGCCCGG
             551  GGCTTAACCC CGGGTCTGCA GTCGATACGG GCAGGCTAGA GTTCGGTAGG
             601  GGAGATCGGA ATTCCTGGTG TAGCGGTGAA ATGCGCAGAT ATCAGGAGGA
             651  ACACCGGTGG CGAAGGCGGA TCTCTGGGCC GATACTGACG CTGAGGAGCG
             701  AAAGCGTGGG GAGCGAACAG GATTAGATAC CCTGGTAGTC CACGCCGTAA
             751  ACGGTGGGCA CTAGGTGTGG GCAACATTCC ACGTTGTCCG TGCCGCAGCT
             801  AACGCATTAA GTGCCCCGCC TGGGGAGTAC GGCCGCAAGG CTAAAACTCA
             851  AAG
GMKU 343       1  GCTGGCGGCG TGCTTACACA TGCAAGTCGA ACGATGAACC GGTTTCGGCC
              51  GGGGATTAGT GGCGAACGGG TGAGTAACAC GTGGGCAATC TGCCCTGCAC
             101  TCTGGGATAA GCCCGGGAAA CTGGGTCTAA TACCGGATAC GACACATGAG
             151  GGCATCCTCG TGTGTGGAAA GTTCCGGCGG TGCAGGATGA GCCCGCGGCC
             201  TATCAGCTTG TTGGTGGGGT AGTGGCCTAC CAAGGCGACG ACGGGTAGCC
             251  GGCCTGAGAG GGTGACCGGC CACACTGGGA CTGAGACACG GCCCAGACTC
             301  CTACGGGAGG CAGCAGTGGG GAATATTGCA CAATGGGCGA AAGCCTGATG
             351  CAGCGACGCC GCGTGAGGGA TGACGGCCTT CGGGTTGTAA ACCTCTTTCA
             401  GCAGGGAAGA AGCGAAAGTG ACGGTACCTG CAGAAGAAGC ACCGGCTAAC
             451  TACGTGCCAG CAGCCGCGGT AATACGTAGG GTGCAAGCGT TGTCCGGAAT
             501  TATTGGGCGT AAAGAGCTCG TAGGCGGCCT GTCGCGTCGA TTGTGAAAGC
             551  CCAGGGCTTA ACCCTGGGTC TGCAGTCGAT ACGGGCAGGC TAGAGTTCGG
             601  TAGGGGAGAT CGGAATTCCT GGTGTAGCGG TGAAATGCGC AGATATCAGG
             651  AGGAACACCG GTGGCGAAGG CGGATCTCTG GGCCGATACT GACGCTGAGG
             701  AGCGAAAGCG TGGGGAGCGA ACAGGATTAG ATACCCTGGT AGTCCACGCC
             751  GTAAACGGTG GGCACTAGGT GTGGGCAACA TTCCACGTTG TCCGTGCCGC
             801  AGCTAACGCA TTAAGTGCCC CGCCTGGGGA GTACGGCCGC AAGGCTAAAA
             851  CTCAAAGGAA TTGACGGGGG CCCGCACAAG CGGCGGA

GMKU 342       1  CACATGGCAA GTCGAACGAT GAACCACTTC GGTGGGGATT AGTGGCGAAC
              51  GGGTGAGTAA CACGTGGGCA ATCTGCCCTG CACTCTGGGA CAAGCCCTGG
             101  AAACGGGGTC TAATACCGGA TACTGATCC CTTGGGCATC CTAGATGATC
             151  GAAAGCTCCG GCGGTGCAGG ATGAGCCCGC GGCCTATCAG CTAGTTGGTG
             201  AGGTAATGGC TCACCAAGGC GACGACGGGT AGCCGGCCTG AGAGGGCGAC
             251  CGGCCACACT GGGACTGAGA CACGGCCCAG ACTCCTACGG GAGGCAGCAG
             301  TGGGGAATAT TGCACAATGG GCGAAAGCCT GATGCAGCGA CGCCGCGTGA
             351  GGGATGACGG CCTTCGGGTT GTAAACCTCT TTCAGCAGGG AAGAAGCGAA
             401  AGTGACGGTA CCTGCAGAAG AAGCGCCGGC TAACTACGTG CCAGCAGCCG
             451  CGGTAATACG TAGGGCGCGA GCGTTGTCCG GAATTATTGG GCGTAAAGAG
             501  CTCGTAGGCG GCTTGTCACG TCGGTTGTGA AAGCCCGGGG CTTAACCCCG
             551  GGTCTGCAGT CGATACGGGC AGGCTAGAGT TCGGTAGGGG AGATCGGAAT
             601  TCCTGGTGTA GCGGTGAAAT GCGCAGATAT CAGGAGGAAC ACCGGTGGCG
             651  AAGGCGGATC TCTGGGCCGA TACTGACGCT GAGGAGCGAA AGCGTGGGGA
             701  GCGAAC

GMKU 344       1  TACACATGCA AGTCGAACGA TGAAGCCCTT CGGGGTGGAT TAGTGGCGAA
              51  CGGGTGAGTA ACACGTGGGC AATCTGCCCT TCACTCTGGG ACAAGCCCTG
             101  GAAACGGGGT CTAATACCGG ATATCACTTC CATCTGCATG GGTGGGGGTT
             151  GAAAGCTCCG GCGGTGAAGG ATGAGCCCGC GGCCTATCAG CTTGTTGGTG
             201  AGGTAACGGC TCACCAAGGC GACGACGGGT AGCCGGCCTG AGAGGGCGAC
             251  CGGCCACACT GGGACTGAGA CACGGCCCAG ACTCCTACGG GAGGCAGCAG
             301  TGGGGAATAT TGCACAATGG GCGAAAGCCT GATGCAGCGA CGCCGCGTGA
             351  GGGATGACGG CCTTCGGGTT GTAAACCTCT TTCAGCAGGG AAGAAGCGAA
             401  AGTGACGGTA CCTGCAGAAG AAGCGCCGGC TAACTACGTG CCAGCAGCCG
             451  CGGTAATACG TAGGGCGCAA GCGTTGTCCG GAATTATTGG GCGTAAAGAG
             501  CTCGTAGGCG GCTTGTCACG TCGGGTGTGA AAGCCCGGGG CTTAACCCCG
             551  GGTCTGCATT CGATACGGGC TAGCTAGAGT GTGGTAGGGG AGATCGGAAT
             601  TCCTGGTGTA GCGGTGAAAT GCGCAGATAT CAGGAGGAAC ACCGGTGGCG
             651  AAGGCGGATC TCTGGGCCAT TACTGACGCT GAGGAGCGAA AGCGTGGGGA
             701  GCGAACAGGA TTAGATACCC TGGTAGTCCA CGCCGTAAAC GGTGGGAACT
             751  AGGTGTTGGC GACATTCCAC GTCGTCGGTG CCGCA
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GMKU 345

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   1  CATGCAAGTC GAACGATGAA GCCCTTCGGG GTGGATTAGT GGCGAACGGG
  51  TGAGTAACAC GTGGGCAATC TGCCCTTCAC TCTGGGACAA GCCCTGGAAA
 101  CGGGGTCTAA TACCGGATAT CACTTCCATC TGCATGGGTG GGGGTTGAAA
 151  GCTCCGGCGG TGAAGGATGA GCCCGCGGCC TATCAGCTTG TTGGTGAGGT
 201  AACGGCTCAC CAAGGCGACG ACGGGTAGCC GGCCTGAGAG GGCGACCGGC
 251  CACACTGGGA CTGAGACACG GCCCAGACTC CTACGGGAGG CAGCAGTGGG
 301  GAATATTGCA CAATGGGCGA AAGCCTGATG CAGCGACGCC GCGTGAGGGA
 351  TGACGGCCTT CGGGTTGTAA ACCTCTTTCA GCAGGGAAGA AGCGAAAGTG
 401  ACGGTACCTG CAGAAGAAGC GCCGGCTAAC TACGTGCCAG CAGCCGCGGT
 451  AATACGTAGG GCGCAAGCGT TGTCCGGAAT TATTGGGCGT AAAGAGCTCG
 501  TAGGCGGCTT GTCACGTCGG GTGTGAAAGC CCGGGGCTTA ACCCCGGGTC
 551  TGCATTCGAT ACGGGCTAGC TAGAGTGTGG TAGGGGAGAT CGGAATTCCT
 601  GGTGTAGCGG TGAAATGCGC AGATATCAGG AGGAACACCG GTGGCGAAGG
 651  CGGATCTCTG GGCCATTACT GACGCTGAGG AGCGAAAGCG TGGGGAGCGA
 701  ACAGGATTAG ATACCCTGGT AGTCCACGCC GTAAACGGTG GGAACTAGGT
 751  GTTGGCGACA TTCCACGTCG TCGGTGCCGC AGCTAACGCA TTAAGTTCCC
 801  CGCCTGGGGA GTACGGCCGC AAGG
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GMKU 346

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   1  ATTCATGGCT CAGGACGAAC GCTGGCGGCG TGCTTAACAC ATGCAAGTCG
  51  AGCGGTAAGG CCCTTCGGGG TACACGAGCG GCGAACGGGT GAGTAACACG
 101  TGAGCAACCT GCCCCTGACT CTGGGATAAG CGGTGGAAAC GCCGTCTAAT
 151  ACCGGATACG ACCCGCCACC TCATGGTGGA GGGTGGAAAG TTTTTCGGTC
 201  AGGGATGGGC TCGCGGCCTA TCAGCTTGTT GGTGGGGTAA CGGCCTACCA
 251  AGGCGATTAC GGGTAGCCGG CCTGAGAGGG CGACCGGCCA CACTGGGACT
 301  GAGACACGGC CCAGACTCCT GCGGGAGGCA GCAGTGGGGA ATATTGCGCA
 351  ATGGGCGAAA GCCTGACGCA GCGACGCCGC GTGGGGGATG ACGGCCTTCG
 401  GGTTGTAAAC CTCTTTTACC ACCAACGCAG GCTTCCAGTT CTCTGGAGGT
 451  TGACGGTAGG TGGGGAATAA GGACCGGCTA ACTACGTGCC AGCAGCCGCG
 501  GTAATACGTA GGGTCCGAGC GTTGTCCGGA ATTATTGGGC GTAAAGAGCT
 551  CGTAGGCGGC GTGTCGCGTC TGCTGTGAAA GACCGGGGCT TAACTCCGGT
 601  TCTGCAGTGG ATACGGGCAT GCTAGAGGTA GGTAGGGGAG ACTGGAATTC
 651  CTGGTGTAGC GGTGAAATGC GCAGATATCA GGAGGAACAC CGGTGGCGAA
 701  GGCGGGTCTC TGGGCCTTAC CTGACGCTGA GGAGCGAAAG CATGGGGAGC
 751  GAACAGGATT AGATACCCTG GTAGTCCATG CCGTAAACGT TGGGCGCTAG
 801  GTGTGGGGAC TTTCCACGGT TTCCGCGCCG TAGCTAACGC ATTAAGCGCC
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GMKU 347

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   1  GTAGGTGGCT TGTCGCGTCT GCCGTGAAAG CCCGTGGCTT AACTACGGGT
  51  CTGCGGTGGA TACGGGCAGG CTAGAGGCTG GTAGGGGCAA GCGGAATTCC
 101  TGGTGTAGCG GTGAAATGCG CAGATATCAG GAGGAACACC GGTGGCGAAG
 151  GCGGCTTGCT GGGCCAGTTC TGACGCTGAG GAGCGAAAGC GTGGGGAGCG
 201  AACAGGATTA GATACCCTGG TAGTCCACGC TGTAAACGTT GGGCGCTAGG
 251  TGTGGGGGTC TTCCACGATC TCTGTGCCGT AGCTAACGCA TTAAGCGCCC
 301  CGCCTGGGGA GTACGGCCGC AAGGCTAAAA CTCAAAGGAA TTGACGGGGG
 351  CCCGCACAAG CGGCGGAGCA TGTTGCTTAA TTCGACGCAA CGCGAAGAAC
 401  CTTACCAAGG TTTGACATAC ACCGGAAAGC TCTGGAGACA GAGCCCTCCT
 451  TTGGACTGGT GTACAGGTGG TGCATGGCTG TCGTCAGCTC GTGTCGTGAG
 501  ATGTTGGGTT AAGTCCCGCA ACGAGCGCAA CCCTTGTTCC ATGTTGCCAG
 551  CACGCCCTTT GGGGTGGTGG GGACTCATGG GAGACTGCCG GGGTCAACTC
 601  GGAGGAAGGT GGGGATGACG TCAAGTCATC ATGCCCCTTA TGTCTTGGGC
 651  TGCAAACATG CTACAATGGC CGGTACAGAG GGTTGCGATA CCGTGAGGTG
 701  GAGCGAATCC CTAAAAGCCG GTCTCAGTTC GGATTGGGGT CTGCAACTCG
 751  ACCCCATGAA GTCGGAGTCG CTAGTAATCG CAGATCAGCA ACGCTGCGGT
 801  GAATACGTTC CCGGGCCTTG TACACACCGC CCGTCACGTC ACGAAAGTCG
 851  GCAACACCCG AAGCCCGTGG CCCAACCACT TGTGGGGGGA GCGGTCGAAG
 901  GTGGGGCTGG CGATTGGGAC GAAGTCGTAA CAAG
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GMKU 348

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   1  AAAGGCCCTT CGGGGTACTC GAGCGGCGAA CGGGTGAGTA ACACGTGAGC
  51  AACCTGCCCC AGGCTTTGGG ATAACCCCGG GAAACCGGGG CTAATACCGA
 101  ATATGACCTT GCACCGCATG GTGTTTGGTG GAAAGTTTTT CGGCTTGGGA
 151  TGGGCTCGCG GCCTATCAGC TTGTTGGTGG GGTGATGGCC TACCAAGGCG
 201  ACGACGGGTA GCCGGCCTGA GAGGGCGACC GGCCACACTG GGACTGAGAC
 251  ACGGCCCAGA CTCCTACGGG AGGCAGCAGT GGGGAATATT GCACAATGGG
 301  CGGAAGCCTG ATGCAGCGAC GCCGCGTGAG GGATGACGGC CTTCGGGTTG
 351  TAAACCTCTT TCAGCAGGGA CGAAGCGTAA GTGACGGTAC CTGCAGAAGA
 401  AGCGCCGGCC AACTACGTGC CAGCAGCCGC GGTAAGACGT AGGGCGCGAG
 451  CGTTGTCCGG ATTTATTGGG CGTAAAGAGC TCGTAGGCGG CTTGTCGCGT
 501  CGACTGTGAA AACCCGCAGC TCAACTGCGG GCCTGCAGTC GATACGGGCA
 551  GGCTAGAGTT CGGTAGGGGA GACTGGAATT CCTGGTGTAG CGGTGAAATG
 601  CGCAGATATC AGGAGGAACA CCGGTGGCGA AGGCGGGTCT CTGGGCCGAT
 651  ACTGACGCTG AGGAGCGAAA GCGTGGGGAG CGAACAGGAT TAGATACCCT
 701  GGTAGTCCAC GCTGTAAACG TTGGGCGCTA GGTGTGGGGG GCCTCTCCGG
 751  TTCCCTGTGC CGCAGCTAAC GCATTAAGCG CCCCGCCTGG GGAGTACGGC
 801  CGCCAAGGCT AAAACT
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GMKU 350        1  AATCGTACGG TAAGGCCCTT CGGAGACCCT CGAAGCGGCG AACGGGTGAG
               51  TAACACGTGA GTAACCTGCC CCTGACTCTG GGATAAGCCT GGGAAACCGG
              101  GTCTAATACC GGATACGACC ATTTCTCGCA TGTGATGGTG GTGGAAAGTT
              151  TTTCCGGTGG GGAAGGGGCT CGGGGCCTCT CA
GMKU 352        1  CGGCCGGGGA TTAGTGGCGA ACGGGTGAGT AACACGTGGG CAATCTGCCC
               51  TGCACTCTGG GATAAGCCCG GGAAACTGGG TCTAATACCG GATACGACAC
              101  TCCGAGGCAT CTTGGGGTGT GGAAAGTTCC GGCGGTGCAG GATGAGCCCG
              151  CGGCCTATCA GCTTGTTGGT GGGGTAATGG CCTACCAAGG CGACGACGGG
              201  TAGCCGGCCT GAGAGGGTGA CCGGCCACAC TGGGACTGAG ACACGGCCCA
              251  GACTCCTACG GGAGGCAGCA GTGGGGAATA TTGCACAATG GGCGAAAGCC
              301  TGATGCAGCG ACGCCGCGTG AGGGATGACG GCCTTCGGGT TGTAAACCTC
              351  TTTCAGCAGG GAAGAAGCGA AAGTGACGGT ACCTGCAGAA GAAGCACCGG
              401  CTAACTACGT GCCAGCAGCC GCGGTAATAC GTAGGGTGCG AGCGTTGTCC
              451  GGAATTATTG GGCGTAAAGA GCTCGTAGGC GGTTTGTCGC GTCGATTGTG
              501  AAAGCCCGGG GCTTAACCCT GGGTCTGCAG TCGATACGGG CAGGCTAGAG
              551  TTCGGCAGGG GAGACTGGAA TTCCTGGTGT AGCGGTGAAA TGCGCAGATA
              601  TCAGGAGGAA CACCGGTGGC GAAGGCGGGT CTCTGGGCCG ATACTGACGC
              651  TGAGGAGCGA AAGCGTGGGG AGCGAACAGG ATTAGATACC CTGGTAGTCC
              701  ACGCCGTAAA CGGTGGGCAC TAGGTGTGGG CAACATTCCA CGTTGTCCGT
              751  GCCGCAGCTA ACGCATTAAG TGCCCCGCCT GGGGAGTACG GCCGCAAGGC
              801  TAAAACTC
GMKU 354        1  TGCAAGTCGA ACGATGAACC ACTTCGGTGG GGATTAGTGG CGAACGGGTG
               51  AGTAACACGT GGGCAATCTG CCCTGCACTC TGGGACAAGC CCTGGAAACG
              101  GGGTCTAATA CCGGATACTG ACCTTCACGG GCATCTGTGA AGGTCGAAAG
              151  CTCCGGCGGT GCAGGATGAG CCCGCGGCCT ATCAGCTTGT TGGTGAGGTA
              201  ATGGCTCACC AAGGCGACGA CGGGTAGCCG GCCTGAGAGG GCGACCGGCC
              251  ACACTGGGAC TGAGACACGG CCCAGACTCC TACGGGAGGC AGCAGTGGGG
              301  AATATTGCAC AATGGGCGAA AGCCTGATGC AGCGACGCCG CGTGAGGGAT
              351  GACGGCCTTC GGGTTGTAAA CCTCTTTCAG CAGGGAAGAA GCGAAAGTGA
              401  CGGTACCTGC AGAAGAAGCG CCGGCTAACT ACGTGCCAGC AGCCGCGGTA
              451  ATACGTAGGG CGCAAGCGTT GTCCGGAATT ATTGGGCGTA AAGAGCTCGT
              501  AGGCGGCTTG TCACGTCGGT TGTGAAAGCC CGGGGCTTAA CCCCGGGTCT
              551  GCAGTCGATA CGGGCAGGCT AGAGTTCGGT AGGGGAGATC GGAATTCCTG
              601  GTGTAGCGGT GAAATGCGCA GATATCAGGA GGAACACCGG TGGCGAAGGC
              651  GGATCTCTGG GCCGATACTG ACGCTGAGGA GCGAAAGCGT GGGGAGCGAA
              701  CAGGATTAGA TACCCTGGTA GTCCACGCCG TAAACGGTGG GCACTAGGTG
              751  TGGGCAACAT TCCACGTTGT CCGTGCCGCA GCTAACGCAT TAAGTGCCCC
              801  GCCTGGGGAG TACGGCCGCA AG
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GMKU 351        1  TCGAGCGGAA AGGCCCCTTC GGGGGTACTC GAGCGGCGAA CGGGTGAGTA
               51  ACACGTGAGC AACCTGCCCC TGACTTTGGG ATAAGCCTGG GAAACTGGGT
              101  CTAATACCGG ATATGACCAT GTCACGCATG TGGTGTGGTG GAAAGTTTTT
              151  CGGTTGGGGA TGGGCTCGCG GCCTATCAGC TTGTTGGTGG GGTGATGGCC
              201  TACCAAGGCG ACGACGGGTA ACCGGCCTGA GAGGGCGACC GGTCACACTG
              251  GGACTGAGAC ACGGCCCAGA CTCCTACGGG AGGCAGCAGT GGGGAATATT
              301  GCGCAATGGG CGGAAGCCTG ACGCAGCGAC GCCGCGTGAG GGATGACGGC
              351  CTTCGGGTTG TAAACCTCTT TCAGCAGGGA CGAAGCTAAC GTGACGGTAC
              401  CTGCAGAAGA AGCGCCGGCT AACTACGTGC CAGCAGCCGC GGTAATACGT
              451  AGGGCGCAAG CGTTGTCCGG AATTATTGGG CGTAAAGAGC TCGTAGGTGG
              501  TTTGTCGCGT CTGTCGTGAA AGCCCACGGC TTAACTGTGG GTCTGCGGTG
              551  GATACGGGCA GACTAGAGGC AGGTAGGGGA GAATGGAATT CCCGGTGTAG
              601  CGGTGAAATG CGCAGATATC GGGAGGAACA CCGGTGGCGA AGGCGGTTCT
              651  CTGGGCCTGT ACTGACGCTG AGGAGCGAAA GCGTGGGGAG CGAACAGGAT
              701  TAGATACCCT GGTAGTCCAC GCCGTAAACG TTGGGCGCTA GGTGTGGGGT
              751  TCTTCCACGG ATTCCGCGCC GTAGCTAACG CAT
GMKU 355        1  TTCGGTGGGG ATTAGTGGCG AACGGGTGAG TAACACGTGG GCAATCTGCC
               51  CTGCACTCTG GGACAAGCCC TGGAAACGGG GTCTAATACC GGATACTGAC
              101  CTTCACGGGC ATCTGTGAAG GTCGAAAGCT CCGGCGGTGC AGGATGAGCC
              151  CGCGGCCTAT CAGCTTGTTG GTGAGGTAAT GGCTCACCAA GGCGACGACG
              201  GGTAGCCGGC CTGAGAGGGC GACCGGCCAC ACTGGGACTG AGACACGGCC
              251  CAGACTCCTA CGGGAGGCAG CAGTGGGGAA TATTGCACAA TGGGCGAAAG
              301  CCTGATGCAG CGACGCCGCG TGAGGGATGA CGGCCTTCGG GTTGTAAACC
              351  TCTTTCAGCA GGGAAGAAGC GAAAGTGACG GTACCTGCAG AAGAAGCGCC
              401  GGCTAACTAC GTGCCAGCAG CCGCGGTAAT ACGTAGGGCG CAAGCGTTGT
              451  CCGGAATTAT TGGGCGTAAA GAGCTCGTAG GCGGCTTGTC ACGTCGGTTG
              501  TGAAAGCCCG GGGCTTAACC CCGGGTCTGC AGTCGATACG GGCAGGCTAG
              551  AGTTCGGTAG GGGAGATCGG AATTCCTGGT GTAGCGGTGA AATGCGCAGA
              601  TATCAGGAGG AACACCGGTG GCGAAGGCGG ATCTCTGGGC CGATACTGAC
              651  GCTGAGGAGC GAAAGCGTGG GGGAGCGAAC AGGATTAGAT ACCCTGGTAG
              701  TCCACGCCGT AAACGGTGGG CACTAGGTGT GGGCAACATT CCACGTTGTC
              751  CGTGCCGCAG CTAACGCATT AA
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GMKU 356

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   1  CCCCTTCGGG GGTACTCGAG CGGCGAACGG GTGAGTAACA CGTGAGCAAC
  51  CTGCCCCTGA CTCTGGGATA AGCCTGGGAA ACCGGGTCTA ATACCGGATA
 101  CGACCGGTCA CCGCATGGTG TGCCGGTGGA AAGTTTTTTC GGTTGGGGAT
 151  GGGCTCGCGG CCTATCAGCT TGTTGGTGGG GTGATGGCCT ACCAAGGCGA
 201  CGACGGGTAA CCGGCCTGAG AGGGCGACCG GTCACACTGG GACTGAGACA
 251  CGGCCCAGAC TCCTACGGGA GGCAGCAGTG GGGAATATTG CGCAATGGGC
 301  GGAAGCCTGA CGCAGCGACG CCGCGTGGGG GATGACGGCC TTCGGGTTGT
 351  AAACCTCTTT CAGCAGGGAC GAAGCTAACG TGACGGTACC TGCAGAAGAA
 401  GCGCCGGCTA ACTACGTGCC AGCAGCCGCG GTAATACGTA GGGCGCAAGC
 451  GTTGTCCGGA ATTATTGGGC GTAAAGAGCT CGTAGGCGGT TTGTCGCGTC
 501  TGTCGTGAAA GCCCACGGCT TAACCGTGGG TCTGCGGTGG ATACGGGCAG
 551  ACTAGAGGCA GGTAGGGGAG AATGGAATTC CCGGTGTAGC GGTGAAATGC
 601  GCAGATATCG GGAGGAACAC CGGTGGCGAA GGCGGTTCTC TGGGCCTGTA
 651  CTGACGCTGA GGAGCGAAAG CGTGGGGAGC GAACAGGATT AGATACCCTG
 701  GTAGTCCACG CCGTAAACGT TGGGCGCTAG GTGTGGGGTT CTTCCACGGA
 751  TTCCGCGCCG TAGCTAACGC ATTAAGCGCC CCGCCTGGGG AGTACGGCCG
 801  CAAGGCTAAA A
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GMKU 358

```
   1  GCTTTAGGCG TGCTTACACA TGCAAGTCGA GCGGAAAGGC CCTTCGGGGT
  51  ACTCGAGCGG CGAACGGGTG AGTAACACGT GAGCAACCTG CCCCAGGCTT
 101  TGGGATAACC CCGGGAAACC GGGGCTAATA CCGGATATGA CCTTGCACCG
 151  CATGGTGTTT GGTGGAAAGT TTTTCGGCTT GGGATGGGCT CGCGGCCTAT
 201  CAGCTTGTTG GTGGGGTGAT GGCCTACCAA GGCGACGACG GGTAGCCGGC
 251  CTGAGAGGGC GACCGGCCAC ACTGGGACTG AGACACGGCC CAGACTCCTA
 301  CGGGAGGCAG CAGTGGGGAA TATTGCACAA TGGGCGGAAG CCTGATGCAG
 351  CGACGCCGCG TGAGGGATGA CGGCCTTCGG GTTGTAAACC TCTTTCAGCA
 401  GGGACGAAGC GTAAGTGACG GTACCTGCAG AAGAAGCGCC GGCCAACTAC
 451  GTGCCAGCAG CCGCGGTAAG ACGTAGGCGC GAGCGTTGTC CGGATTTATT
 501  GGGCGTAAAG AGCTCGTAGC GGCTTGTCGC GTCACCGTGA AAACCTGGGG
 551  GCTCAACCCC AGGCCTGCGG TCGATACGGG CAGGCTAGAG TTCGGTAGGG
 601  GAGACTGGAA TTCCTGGTGT AGCGGTGAAA TGCGCAATAT CAGGAGGAAC
 651  ACCGGTGGCG AAGCGGGTCT CTGGGCCGAT ACTGAC
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GMKU 357

```
   1  ATGCAAGTCG AGCGGAAAGG CCCTTCGGGG TACTCGAGCG GCGAACGGGT
  51  GAGTAACACG TGAGTAACCT GCCCCTGACT CTGGGATAAG CCTGGGAAAC
 101  CGGGTCTAAT ACCGGATACG ACCATTTCTC GCATGTGATG GTGGTGGAAA
 151  GTTTTTTCGG TTGGGGATGG GCTCGCGGCC TATCAGCTTG TTGGTGGGGT
 201  GATGGCCTAC CAAGGCGACG ACGGGTAGCC GGCCTGAGAG GGCGACCGGC
 251  CACACTGGGA CTGAGACACG GCCCAGACTC CTACGGGAGG CAGCAGTGGG
 301  GAATATTGCG CAATGGGCGG AAGCCTGACG CAGCGACGCC GCGTGGGGGA
 351  TGACGGCCTT CGGGTTGTAA ACCTCTTTCA GCAGGGACGA AGTTGACGTG
 401  TACCTGTAGA AGAAGCGCCG GCTAACTACG TGCCAGCAGC CGCGGTAATA
 451  CGTAGGGCGC GAGCGTTGTC CGGAATTATT GGGCGTAAAG AGCTCGTAGG
 501  TGGCTTGTCG CGTCTGCCGT GAAAGCCCGT GGCTTAACTA CGGTCTGCGG
 551  TGGATACGGG CAGGCTA
```

GMKU 359

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   1  CATGCAAGTC GAGCGGAAAG GCCCTTCGGG GTACTCGAGC GGCGAACGGG
  51  TGAGTAACAC GTGAGTAACC TGCCCTTGGC TCTGGGATAA GCCCGGGAAA
 101  CTGGGTCTAA TACCGGATAT GACCCTCCAC CGCATGGTGT GAGGGTGGAA
 151  AGTTTTTCGG CTGAGGATGG ACTCGCGGTC TATCAGCTTG TTGGTGGGGT
 201  GATGGCCTAC CAAGGCGACG ACGGATAGCC GGCCTGAGAG GGTGACCGGC
 251  CACACTGGGA CTGAGACACG GCCCAGACTC CTACGGGAGG CAGCAGTGGG
 301  GAATATTGCG CAATGGGCGG AAGCCTGACG CAGCGACGCC GCGTGGGGGA
 351  TGAAGGCCTT CGGGTTGTAA ACCTCTTTCA CCACCGACGA AGCTAACGTG
 401  ACGGTAGGTG GGGAAGAAGC GCCGGCTAAC TACGTGCCAG CAGCCGCGGT
 451  AAACGTAGGG CGCAAGCGTT GTCCGGAATT ATTGGGCGTA AAGAGCTCGT
 501  AGGTGGTTTG TCGCGTCTGC CGTGAAAACC CGAGGCTTAA CCTCGGGCCT
 551  GCGGTGGATA CGGGCAGACT AGAAGTAGGT AGGGGAGAAT GGAATTCCCG
 601  GTGTAGCGGT GAAATGCGCA GATATCGGGA GGAACACCGG TGGCGAAGGC
 651  GGTTCTCTGG GCCTTACCTG ACGCTGAGGA GCGAAAGCGT GGGGAGCGAA
 701  CAGGATTAGA TACCCTGGTA GTCCACGCCG TAAACGTTGG GCGCTAGGTG
 751  TGGGGTCTTT CCACGGACTC CGTGCCGTAG CTAACGCATT AAGCGCCCCG
 801  CCTGGGGAGT ACGGCCGCAA GGCTAAAACT CAAAGGAATT GACGGGGGCC
 851  CGCACAAGCG GCGGAGCATG TTGCTTAATT CGACGCAACG CGAAGAACCT
 901  TACCAAGGTT TGACATCACC GGAAAACTCG CAGAGATGCG GGGTCCTTTT
 951  GGGCCGGTGA CAGGTGGTGC ATGGCTGTCG TCAGCTCGTG TCGTGAGATG
1001  TTGGGTTAAG TCCCGCAACG AGCGCAACCC TCGTTCCATG TTGCCAGCAC
1051  GTAATGGTGG GGACTCATGG GAGACTGCCG GGGTCAACTC GGAGGAAGGT
1101  GGGGATGACG TCAAGTCATC ATGCCCCTTA TGTCTTGGGC TGCAAACATG
1151  CTACAATGGC CGGTACAAAG GGCTGCGAAA CCGTGAGGTG GAGCGAATCC
1201  CGGAAAGCCG GTCTCAGTTC GGATTGGGGT CTGCAACTCG ACCCCATGAA
1251  GTCGGAGTCG CTAGTAATCG CAGATCAGCA ACGCTGCGGT GAATACGTTC
1301  CCGGGCCTTG TACACACCGC CCGTCACGTC ACGAAAGTCG GCAACACCCG
1351  AAGTCCGGTG GCCCAACCCT TGTGGGGGGA GCCGTCGAAG GTGGGGCCGG
1401  CGATTGGGAC GAAGTC
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GMKU 360

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   1  CACATGCAAG TCGAACGATG AACCGGTTTC GGCCGGGGAT TAGTGGCGAA
  51  CGGGTGAGTA ACACGTGGGC AATCTGCCCT GCACTCTGGG ATAAGCCCGG
 101  GAAACTGGGT CTAATACCGG ATACGACACT CCGAGGCATC TTGGGGTGTG
 151  GAAAGTTCCG GCGGTGCAGG ATGAGCCCGC GGCCTATCAG CTTGTTGGTG
 201  GGGTAATGGC CTACCAAGGC GACGACGGGT AGCCGGCCTG AGAGGGTGAC
 251  CGGCCACACT GGGACTGAGA CACGGCCCAG ACTCCTACGG GAGGCAGCAG
 301  TGGGGAATAT TGCACAATGG GCGAAAGCCT GATGCAGCGA CGCCGCGTGA
 351  GGGATGACGG CCTTCGGGTT GTAAACCTCT TTCAGCAGGG AAGAAGCGAA
 401  AGTGACGGTA CCTGCAGAAG AAGCACCGGC TAACTACGTG CCAGCAGCCG
 451  CGGTAATACG TAGGGTGCGA GCGTTGTCCG GAATTATTGG GCGTAAAGAG
 501  CTCGTAGGCG GTTTGTCGCG TCGATTGTGA AAGCCCGGGG CTTAACCCTG
 551  GGTCTGCAGT CGATACGGGC AGGCTAGAGT TCGGCAGGGG AGACTGGAAT
 601  TCCTGGTGTA GCGGTGAAAT GCGCAGATAT CAGGAGGAAC ACCGGTGGCG
 651  AAGGCGGGTC TCTGGGCCGA TACTGACGCT GAGGAGCGAA AGCGTGGGGA
 701  GCGAACAGGA TTAGATACCC TGGTAGTCCA CGCCGTAAAC GGTGGGCACT
 751  AGGTGTGGGC AACATTCCAC GTTGTCCGTG CCGCAGCTAA CGCATTAAGT
 801  GCCCCGCCTG GGGAGTACGG CCGCAA
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GMKU 361

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   1  GGCGTGCTTA ACACATGACA AGTCGAACGA TGAACCGCTT TCGGGCGGGG
  51  ATTAGTGGCG AACGGGTGAG TAACACGTGG GCAATCTGCC CTGCACTCTG
 101  GGACAAGCCC TGGAAACGGG GTCTAATACC GGATATGACT GTCCATCGCA
 151  TGGTGGATGG TGTAAAGCTC CGGCGGTGCA GGATGAGCCC GCGGCCTATC
 201  AGCTTGTTGG TGAGGTAGTG GCTCACCAAG GCGACGACGG GTAGCCGGCC
 251  TGAGAGGGCG ACCGGCCACA CTGGGACTGA GACACGGCCC AGACTCCTAC
 301  GGGAGGCAGC AGTGGGGAAT ATTGCACAAT GGGCGAAAGC CTGATGCAGC
 351  GACGCCGCGT GAGGGATGAC GGCCTTCGGG TTGTAAACCT CTTTCAGCAG
 401  GGAAGAAGCG AAAGTGACGG TACCTGCAGA AGAAGCGCCG GCTAACTACG
 451  TGCCAGCAGC CGCGGTAATA CGTAGGGCGC AAGCGTTGTC CGGAATTATT
 501  GGGCGTAAAG AGCTCGTAGG CGGCTTGTCA CGTCGGTTGT GAAAGCCCGG
 551  GGCTTAAACC CCGGGTCTGC AGTCGATACG GGGCAGGCTA GAGTTCGGTA
 601  GGGGAGATCG GAATTCCTGG TGTAGCGGTG AAATGCGCAA TATCAGGAGG
 651  AACACCGGTG GCGAAGCGGA TCTCTGGGCC GATACTGACG CTGAGGAGCG
 701  AAAGCGTGGG GAGCGAACAG GATTAGATAC CCTGGTAGTC CACGCCGTAA
 751  ACGGTGGGCA CTAGGTGTGG GCAACATTCC ACGTTGTCCG TGCCGCAGCT
 801  AACGCATTAA GTGCCCC
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GMKU 362

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   1  CCCTTCGGGG TACTCGAGCG GCGAACGGGT GAGTAACACG TGAGTAACCT
  51  GCCCCTGACT CTGGGATAAG CCTGGGAAAC CGGGTCTAAT ACCGGATACG
 101  ACCATCTTCC GCATGGGATG GTGGTGGAAA GTTTTTCGGT TGGGGATGGG
 151  CTCGCGGCCT ATCAGCTTGT TGGTGGGGTG ATGGCCTACC AAGGCGACGA
 201  CGGGTAGCCG GCCTGAGAGG GCGACCGGCC ACACTGGGAC TGAGACACGG
 251  CCCAGACTCC TACGGGAGGC AGCAGTGGGG AATATTGCGC AATGGGCGGA
 301  AGCCTGACGC AGCGACGCCG CGTGGGGGAT GACGGCCTTC GGGTTGTAAA
 351  CCTCTTTCGG CAGGGACGAA GTTGACGTGT ACCTGTAGAA GAAGCGCCGG
 401  CTAACTACGT GCCAGCAGCC GCGGTAATAC GTAGGGCGCA AGCGTTGTCC
 451  GGAATTATTG GGCGTAAAGA GCTCGTAGGT GGCTTGTCGC GTCTGCCGTG
 501  AAAGCCCGTG GCTTAACTAC GGGTCTGCGG TGGATACGGG CAGGCTAGAG
 551  GCTGGTAGGG GCAAGTGGAA TTCCTGGTGT AGCGGTGAAA TGCGCAGATA
 601  TCAGGAGGAA CACCGGTGGC GAAGGCGGCT TGCTGGGCCA GTTCTGACGC
 651  TGAGGAGCGA AAGCGTGGGG AGCGAACAGG ATTAGATACC CTGGTAGTCC
 701  ACGCTGTAAC GTTGGGCGCT AGGTGTGGGG GTCTTCCATG GTTCCTGTGC
 751  CGTAGCTAAC GCATTAAGCG CCCCGCCTGG GGAGTACGGC CGCAAGGCTA
 801  AAACTCAAAG GAATTGACGG GGGCCCGCAC AAGCGGCGGA GCATGTTGCT
 851  TAATTCGACG CAACGCGAAG AACCTTACCA AGGTT
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GMKU 363

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   1  CCCTTCGGGG TACTCGAGCG GCGAACGGGT GAGTAACACG TGAGTAACCT
  51  GCCCCTGACT CTGGGATAAG CCTGGGAAAC CGGGTCTAAT ACCGGATACG
 101  ACCATCTTCC GCATGGGATG GTGGTGGAAA GTTTTTCGGT TGGGGATGGG
 151  CTCGCGGCCT ATCAGCTTGT TGGTGGGGTG ATGGCCTACC AAGGCGACGA
 201  CGGGTAGCCG GCCTGAGAGG GCGACCGGCC ACACTGGGAC TGAGACACGG
 251  CCCAGACTCC TACGGGAGGC AGCAGTGGGG AATATTGCGC AATGGGCGGA
 301  AGCCTGACGC AGCGACGCCG CGTGGGGGAT GACGGCCTTC GGGTTGTAAA
 351  CCTCTTTCGG CAGGGACGAA GTTGACGTGT ACCTGTAGAA GAAGCGCCGG
 401  CTAACTACGT GCCAGCAGCC GCGGTAATAC GTAGGGCGCA AGCGTTGTCC
 451  GGAATTATTG GGCGTAAAGA GCTCGTAGGT GGCTTGTCGC GTCTGCCGTG
 501  AAAGCCCGTG GCTTAACTAC GGGTCTGCGG TGGATACGGG CAGGCTAGAG
 551  GCTGGTAGGG GCAAGTGGAA TTCCTGGTGT AGCGGTGAAA TGCGCAGATA
 601  TCAGGAGGAA CACCGGTGGC GAAGGCGGCT TGCTGGGCCA GTTCTGACGC
 651  TGAGGAGCGA AAGCGTGGGG AGCGAACAGG ATTAGATACC CTGGTAGTCC
 701  ACGCTGTAAA CGTTGGGCGC TAGGTGTGGG GGTCTTCCAT GGTTCCTGTG
 751  CCGTAGCTAA CGCATTAAGC GCCCCGCCTG GGGAGTACGG CCGCAAGGCT
 801  AAAACTCAAA GGAATTGACG GGGGCCCGCA CAAGCGGCGG AGCATGTTGC
 851  TTAATTCGAC GCAACGCGAA GAACCTTACC AAGGTTTGAC ATACACCGGA
 901  CGCGCTCGGA GACGGGTGTT CCTTTCGGGG CTGGTGTACA GGTGGTGCAT
 951  GGCTGTCGTC AGCTCGTGTC GTGAGATGTT GGGTTAAGTC CCGCAACGAG
1001  CGCAACCCTT GTTCCATGTT GCCAGCGGCG GCTTTCGGGC CGGCCGGGGA
1051  CTCATGGGAG ACTGCCGGGG TCAACTCGGA GGAAGGTGGG GATGACGTCA
1101  AGTCATCATG CCCCTTATGT CTTGGGCTGC AAACATGCTA CAATGGTCGG
1151  TACAGAGGGC TGCTAACCCG TGAGGGGGAG CGAATCCCTA AAAGCCGGTC
1201  TCAGTTCGGA TTGGGGTCTG CAACTCGACC CCATGAAGTC GGAGTCGCTA
1251  GTAATCGCAG ATCAGCAATG CTGCGGTGAA TACGTTCCCG GGCCTTGTAC
1301  ACACCGCCCG TCACGTCACG AAAGTCGGCA ACACCCGAAG CCCGTGGCCC
1351  AACCCGTTTG GGGGGGAGCG GTCGAAGGTG GGGCTGG
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GMKU 364       1  TGCAGTCGAG CGGAAAGGCC CTTCGGGGTA CTCGAGCGGC GAACGGGTGA
              51  GTAACACGTG AGTAACCTGC CCCTGACTCT GGGATAAGCC TGGGAAACCG
             101  GGTCTAATAC CGGATACGAC CATCTTCCGC ATGGGATGGT GGTaGGAAAG
             151  TTTTTCGGTT GGGGATGGGC TCGCGGCCTA TCAGCTTGTT GGTGGGGTGA
             201  TGGCCTACCA AGGCGACGAC GGGTAGCCGG CCTGAGAGGG CGACCGGCCA
             251  CACTGGGACT GAGACACGGC CCAGACTCCT ACGGGAGGCA GCAGTGGGGA
             301  ATATTGCGCA ATGGGCGGAA GCCTGACGCA GCGACGCCGC GTGGGGGATG
             351  ACGGCCTTCG GGTTGTAAAC CTCTTTCGGC AGGGACGAAG TTGACGTGTA
             401  CCTGTAGAAG AAGCGCCGGC TAACTACGTG CCAGCAGCCG CGGTAATACG
             451  TAGGGCGCAA GCGTTGTCCG GAATTATTGG GCGTAAAGAG CTCGTAGGTG
             501  GCTTGTCGCG TCTGCCGTGA AAGCCCGTGG CTTAACTACG GGTCTGCGGT
             551  GGATACGGGC AGGCTAGAGG CTGGTAGGGG CAAGTGGAAT TCCTGGTGTA
             601  GCGGTGAAAT GCGCAGATAT CAGGAGGAAC ACCGGTGGCG AAGGCGGCTT
             651  GCTGGGCCAG TTCTGACGCT GAGGAGCGAA AGCGTGGGGA GCGAACAGGA
             701  TTAGATACCC TGGTAGTCCA CGCTGTAAAC GTTGGGCGCT AGGTGTGGGG
             751  GTCTTCCATG GTTCCTGTGC CGTAGCTAAC GCATTAAGCG CCCCGCCTGG
             801  GGA
GMKU 366       1  CAAGTCGAGC GGAAAGGCCC TTCGGGGTAC TCGAGCGGCG AACGGGTGAG
              51  TAACACGTGA GATAACCTGC CCCTGACTCT GGGATAAGCC TGGGAAACCG
             101  GGTCTAATAC CGGATATGAC ACTCCTCCGC ATGGTGTGGG TGTGGAAAGT
             151  TTTTTCGGTT GGGGATGGAC TCGCGGCCTA TCAGCTTGTT GGTGGGGTGA
             201  TGGCCTACCA AGGCGACGAC GGGTAGCCGG CCTGAGAGGG CGACCGGCCA
             251  CACTGGGACT GAGACACGGC CCAGACTCCT ACGGGAGGCA GCAGTGGGGA
             301  ATATTGCGCA ATGGGCGGAA GCCTGACGCA GCGACGCCGC GTGGGGGATG
             351  ACGGCCTTCG GGTTGTAAAC CTCTTTCAGC AGGGACGAAG TTGACGTGTA
             401  CCTGTAGAAG AAGCGCCGGC TAACTACGTG CCAGCAGCCG CGGTAATACG
             451  TAGGGCGCGA GCGTTGTCCG GAATTATTGG GCGTAAAGAG CTCGTAGGTG
             501  GCTTGTTGCG TCTGCCGTGA AAGCCCGTGG CTTAACTACG GGTCTGCGGT
             551  GGATACGGGC AGGCTAGAGG CTGGTAGGGG CAAGCGGAAT TCCTGGTGTA
             601  GCGGTGAAAT GCGCAGATAT CAGGAGGAAC ACCGGTGGCG AAGGCGGCTT
             651  GCTGGGCCAG TTCTGACGCT GAGGAGCGAA AGCGTGGGGA GCGAACAGGA
             701  TTAGATACCC TGGTAGTCCA CGCTGTAAAC GTTGGGCGCT AGGTGTGGGG
             751  GGTCTTCCAC GATCTCTGTG CCGTAGCTAA CGCATTAAGC GCCCCGCCTG
             801  GGGAGTACGG CCGCAAGGCT AAAACTCAAA GG
GMKU 368       1  CGAGCGGAAA GGCCCTTCGG GGTACTCGAG CGGCGAACGG GTGAGTAACA
              51  CGTGAGATAA CCTGCCCCTG ACTCTGGGAT AAGCCTGGGA AACCGGGTCT
             101  AATACCGGAT ATGACACTCC TCCGCATGGT GTGGGTGTGG AAAGTTTTTT
             151  CGGTTGGGGA TGGACCCCCT CCTTATCAGC TTGTTGGTGG GGTGATGGCC
             201  TACCAAGGCG ACGACGGGTA GCCGGCCTGA GAGGGCGACC GGCCACACTG
             251  GGACTGAGAC ACGGCCCAAA CTCCTACGGG AGGAAGCAGT GGGGAATATT
             301  GCGCAATGGG CGAAAGCCTG ACGCAGCGAC GCCGCGTGGG GGATGACGGC
             351  CTTCGGGTTG TAAACCTCTT TCACCAGGGA CAAAGTTGAC GTGTACCTGT
             401  AGAAGAAGCG CCGGCTAACT ACGTGCCAGC AGCCGCGGTA ATACGTAGGG
             451  CGCGAGCGTT GTCCGGAATT ATTGGGCGTA AAGAGCTCGT AGGTGGCTTG
             501  TTGCGTCTGC CGCGAAAGCC CGTGGCTTAA CTACGGTTCT GCGGTGGATA
             551  CGGGCAGGTT AAAGGCTGGT AGGGGCCAGG C
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GMKU 365       1  CACATGCAAG TCGAACGATG AACCGGTTTC GGCCGGGGAT TAGTGGCGAA
              51  CGGGTGAGTA ACACGTGGGC AATCTGCCCT GCACTCTGGG ATAAGCCCGG
             101  GAAACTGGGT CTAATACCGG ATACGACACA TGAGGGCATC CTCGTGTGTG
             151  GAAAGTTCCG GCGGTGCAGG ATGAGCCCGC GGCCTATCAG CTTGTTGGTG
             201  GGGTAGTGGC CTACCAAGGC GACGACGGGT AGCCGGCCTG AGAGGGTGAC
             251  CGGCCACACT GGGACTGAGA CACGGCCCAG ACTCCTACGG GAGGCAGCAG
             301  TGGGGAATAT TGCACAATGG GCGAAAGCCT GATGCAGCGA CGCCGCGTGA
             351  GGGATGACGG CCTTCGGGTT GTAAACCTCT TTCAGCAGGG AAGAAGCGAA
             401  AGTGACGGTA CCTGCAGAAG AAGCACCGGC TAACTACGTG CCAGCAGCCG
             451  CGGTAATACG TAGGGTGCAA GCGTTGTCCG GAATTATTGG GCGTAAAGAG
             501  CTCGTAGGCG GCCTGTCGCG TCGATTGTGA AAGCCCAGGG CTTAACCCTG
             551  GGTCTGCAGT CGATACGGGC AGGCTAGAGT TCGGTAGGGG AGATCGGAAT
             601  TCCTGGTGTA GCGGTGAAAT GCGCAGATAT CAGGAGGAAC ACCGGTGGCG
             651  AAGGCGGATC TCTGGGCCGA TACTGACGCT GAGGAGCGAA AGCGTGGGGA
             701  GCGAACAGGA TTAGATACCC TGGTAGTCCA CGCCGTAAAC GGTGGGCACT
             751  AGGTGTGGGC AACATTCCAC GTTGTCCGTG CCGCAGCTAA CGCATTAAGT
             801  GCCCCGCCTG GGGAGTACGG CCGCAAGGCT AAAACTCAAA GGAATTGACG
             851  GGGGCCCGCA CAAGCGGCGG AGCATGTGGC
GMKU 367       1  GCTCAGGACG AACGCTGGCG GCGTGCTTAA CACATGCAAG TCGAACGATG
              51  AAGCCTTTCG GGGTGGATTA GTGGCGAACG GGTGAGTAAC ACGTGGGTAA
             101  CCTGCCCTTG GCTTTGGGAT AAGCCCTGGA AACGGGGTCT AATACCGGAT
             151  ATGACTGATT CTCGCATGGG GATTGGTGGA AAGTTCCGGC GGCCAGGGAT
             201  GGGCCCGCGG CCTATCAGCT TGTTGGTGGG GTGATGGCCT ACCAAGGCGA
             251  CGACGGGTAG CCGGCCTGAG AGGGCGACCG GCCACACTGG GACTGAGACA
             301  CGGCCCAGAC TCCTACGGGA GGCAGCAGTG GGGAATATTG CACAATGGGC
             351  GAAAGCCTGA TGCAGCGACG CCGCGTGAGG GATGACGGCC TTCGGGTTGT
             401  AAACCTCTTT CGCCCGGGAC GAAGCGAGAG TGACGGTACC GGGAGAAGAA
             451  GCACCGGCTA ACTACGTGCC AGCAGCCGCG GTAATACGTA GGGTGCGAGC
             501  GTTGTCCGGA ATTATTGGGC GTAAAGAGCT CGTAGGCGGT TTGTCGCGTC
             551  GACTGTGAAA ACCTGGGGCT TAACTCTGGG CTTGCAGTCG ATACGGGCAG
             601  ACTTGAGTTC GGTAGGGGAG ACTGGAATTC CTGGTGTAGC GGTGAAATGC
             651  GCAGATATCA GGAGGAACAC CGGTGGCGAA GGCGGGTCTC TGGGCCGATA
             701  CTGACGCTGA GGAGCGAAAG CGTGGGGAGC GAACAGGATT AGATACCCTG
             751  GTAGTCCACG CTGTAAACGT TGGGCGCTAG GTGTGGGGAT CATTCACGGT
             801  TTCCGTGCCG TAGCTAACGC ATTAAGCGCC CCGCCTGGGG AGTACGGCCG
             851  CAAGGCTAAA ACTCAAAGGA ATTGACGGGG GCCCGCACAA GCGGCGGAGC
             901  ATGTGGATTA ATTCGATGCA ACGCGAAGAA CCTTACCTGG GTTTGACATG
             951  CACCAGACAT CCCTAGAGAT AGGGCTTCCC TTGTGGTTGG TGTGCAGGTG
            1001  GTGCATGGCT GTCGTCAGCT CGTGTCGTGA GATGTTGGGT TAAGTCCCGC
            1051  AACGAGCGCA ACCCTCGTTC CATGTTGCCA GCGCGTAATG GCGGGGACTC
            1101  ATGGGAGACT GCCGGGGTCA ACTCGGAGGA AGGTGGGGAT GACGTCAAGT
            1151  CATCATGCCC CTTATGTCCA GGGCTTCACA CATGCTACAA TGGCCGGTAC
            1201  AGAGGGTTGC GAGACCGTGA GGTGGAGCGA ATCCCTTAAA GCCGGTCTCA
            1251  GTTCGGATCG GGGTCTGCAA CTCGACCCCG TGAAGTCGGA GTCGCTAGTA
            1301  ATCGCAGATC AGCAACGCTG CGGTGAATAC GTTCCCGGGC CTTGTACACA
            1351  CCGCCCGTCA CGTCACGAAA GTCGGTAACA CCCGAAGCCC ATGGCCCAAC
            1401  CCGTAAGGGA GGGAGTGGTC GAAGGTGGGA CTGGCGATTG GGAC
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GMKU 370

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     1  GACGAACGCT GGCGGCGTGC TTAACACATG CAAGTCGAGC GGAAAGGCCC
    51  TTCGGGGTAC TCGAGCGGCG AACGGGTGAG TAACACGTGA GTAACCTGCC
   101  CTTGGCTCTG GGATAAGCCC GGGAAACTGG GTCTAATACC GGATATGACT
   151  CCTCATCGCA TGATGTGGGG GTGGAAAGTT TTTCGGCTGG GGATGGGCTC
   201  GCGGTCTATC AGCTTGTTGG TGGGGTGATG GCCTACCAAG GCGACGACGG
   251  GTAGCCGGCC TGAGAGGGTG ACCGGCCACA CTGGGACTGA GACACGGCCC
   301  AGACTCCTAC GGGAGGCAGC AGTGGGGAAT ATTGCGCAAT GGGCGGAAGC
   351  CTGACGCAGC GACGCCGCGT GGGGGATGAA GGCCTTCGGG TTGTAAACCT
   401  CTTTCACCAC CGACGAAGCT AACGTGACGG TAGGTGGGGA AGAAGCGCCG
   451  GCTAACTACG TGCCAGCAGC CGCGGTAATA CGTAGGGCGC AAGCGTTGTC
   501  CGGAATTATT GGGCGTAAAG AGCTCGTAGG TGGTTTGTCG CGTCTGCCGT
   551  GAAAACCCGA GGCTTAACCT CGTGGCTTGC GGTGGATACG GGCAGACTAG
   601  AGGTAGGTAG GGGAGAATGG AATTCCCGGT GTAGCGGTGA AATGCGCAGA
   651  TATCGGGAGG AACACCGGTG GCGAAGGCGG TTCTCTGGGC CTTACCTGAC
   701  GCTGAGGAGC GAAAGCGTGG GGAGCGAACA GGATTAGATA CCCTGGTAGT
   751  CCACGCCGTA AACGTTGGGC GCTAGGTGTG GGGTCTTTCC ACGGATTCCG
   801  TGCCGTAGCT AACGCATTAA GCGCCCCGCC TGGGGAGTAC GGCCGCAAGG
   851  CTAAAACTCA AAGGAATTGA CGGGGGCCCG CACAAGCGGC GGAGCATGTT
   901  GCTTAATTCG ACGCAACGCG AAGAACCTTA CCAAGGTTTG ACATCACCGG
   951  AAAACTCGCA GAGATGCGGG GTCCTTTTGG GCCGGTGACA GGTGGTGCAT
  1001  GGCTGTCGTC AGCTCGTGTC GTGAGATGTT GGGTTAAGTC CCGCAACGAG
  1051  CGCAACCCTC GTTCCATGTT GCCAGCACGT AGTGGTGGGG ACTCATGGGA
  1101  GACTGCCGGG GTCAACTCGG AGGAAGGTGG GGATGACGTC AAGTCATCAT
  1151  GCCCCTTATG TCTTGGGCTG CAAACATGCT ACAATGGCCG GTACAAAGGG
  1201  CTGCGAAACC GTGAGGTGGA GCGAATCCCG GAAAGCCGGT CTCAGTTCGG
  1251  ATTGGGGTCT GCAACTCGAC CCCATGAAGT CGGAGTCGCT AGTAATCGCA
  1301  GATCAGCAAC GCTGCGGTGA ATACGTTCCC GGGCCTTGTA CACACCGCCC
  1351  GTCACGTCAC GAAAGTCGGC AACACCCGAA GCCGGTGGCC TAACCCTTGT
  1401  GGGAGGAGCC GTCGAAGGTG GGGCCGGCGA TTGGGACGAA GTCGTAACAA
  1451  GGTAGCCGTA CCGGAAGG
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GMKU 369

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     1  CATGCAAGTC GAGCGGAAAG GCCCTTCGGG GTACTCGAGC GGCGAACGGG
    51  TGAGTAACAC GTGAGTAACC TGCCCCTGAC TCTGGGATAA GCCTGGGAAA
   101  CCGGGTCTAA TACCGGATAC GACCATTTCT CGCATGGGAT GGTGGTGGAA
   151  AGTTTTTTCG GTTGGGGATG GGCTCGCGGC CTATCAGCTT GTTGGTGGGG
   201  TGATGGCCTA CCAAGGCGAC GACGGGTAGC CGGCCTGAGA GGGCGACCGG
   251  CCACACTGGG ACTGAGACAC GGCCCAGACT CCTACGGGAG GCAGCAGTGG
   301  GGAATATTGC GCAATGGGCG GAAGCCTGAC GCAGCGACGC CGCGTGGGGG
   351  ATGACGGCCT TCGGGTTGTA AACCTCTTTC AGCAGGGACG AAGTTGACGT
   401  GTACCTGTAG AAGAAGCGCC GGCTAACTAC GTGCCAGCAG CCGCGGTAAT
   451  ACGTAGGGCG CGAGCGTTGT CCGGAATTAT TGGGCGTAAA GAGCTCGTAG
   501  GTGGCTTGTC GCGTCTGCCG TGAAAGCCCG TGGCTTAACT ACGGGTCTGC
   551  GGTGGATACG GGCAGGCTAG AGGCTGGTAG GGGCAAGCGG AATTCCTGGT
   601  GTAGCGGTGA AATGCGCAGA TATCAGGAGG AACACCGGTG GCGAAGGCGG
   651  CTTGCTGGGC CAGTTCTGAC GCTGAGGAGC GAAAGCGTGG GGAGCGAACA
   701  GGATTAGATA CCCTGGTAGT CCACGCTGTA AACGTTGGGC GCTAGGTGTG
   751  GGGGTCTTCC ACGACTTCTG TGCCGTA
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GMKU 372

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     1  ACACATGCAA GTCGAGCGGA AAGGCCCTTC GGGGTACTCG AGCGGCGAAC
    51  GGGTGAGTAA CACGTGAGTA ACCTGCCCCT GACTCTGGGA TAAGCCTGGG
   101  AAACTGGGTC TAATACCGGA TACGACCATT TCTCGCATGG GATGGTGGTG
   151  GAAAGTTTTT TCGGTTGGGG ATGGGCTCGC GGCCTATCAG CTTGTTGGTG
   201  GGGTGATGGC CTACCAAGGC GACGACGGGT AGCCGGCCTG AGAGGGCGAC
   251  CGGCCACACT GGGACTGAGA CACGGCCCAG ACTCCTACGG GAGGCAGCAG
   301  TGGGGAATAT TGCGCAATGG GCGGAAGCCT GACGCAGCGA CGCCGCGTGG
   351  GGGATGACGG CCTTCGGGTT GTAAACCTCT TTCAGCAGGG ACGAAGTTGA
   401  CGTGTACCTG TAGAAGAAGC GCCGGCTAAC TACGTGCCAG CAGCCGCGGT
   451  AATACGTAGG GCGCGAGCGT TGTCCGGAAT TATTGGGCGT AAAGAGCTCG
   501  TAGGTGGCTT GTCGCGTCTG CCGTGAAAGC CCGTGGCTTA ACTACGGGTC
   551  TGCGGTGGAT ACGGGCAGGC TAGAGGCTGG TAGGGGCAAG CGGAATTCCT
   601  GGTGTAGCGG TGAAATGCGC AGATATCAGG AGGAACACCG GTGGCGAAGG
   651  CGGCTTGCTG GGCCAGTTCT GACGCTGAGG AGCGAAAGCG TGGGGAGCGA
   701  ACAGGATTAG ATACCCTGGT AGTCCACGCT GTAAACGTTG GGCGCTAGGT
   751  GTGGGGGTCT TCCACGACTT CTGTGCCGTA
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GMKU 377       1  GGCGTGCTTA ACACATGCAA GTCGAACGCT GAAGCACTTT CGGGTGTGGA
              51  TGAGTGGCGA ACGGGTGAGT AACACGTGGG TAATCTGCCC TGCACTCTGG
             101  GATAAGCCTT GGAAACGGGG TCTAATACCG GATATGACAC TGGATCGCAT
             151  GGTCTGGTGT GGAAAGTTCC GGCGGTGCAG GATGAGCCCG CGGCCTATCA
             201  GCTTGTTGGT GGGGTAGTGG CCTACCAAGG CGACGACGGG TAGCCGGCCT
             251  GAGAGGGTGA CCGGCCACAC TGGGACTGAG ACACGGCCCA GACTCCTACG
             301  GGAGGCAGCA GTGGGGAATC TTGCGCAATG GGCGAAAGCC TGACGCAGCA
             351  ACGCCGCGTG GGGGATGACG GCCTTCGGGT TGTAAACCTC TTTCGACATC
             401  GACGAAGGCC CCTTGGGGTT GACGGTAGGT GTAGAAGAAG CACCGGCTAA
             451  CTACGTGCCA GCAGCCGCGG TAATACGTAG GGTGCAAGCG TTGTCCGGAT
             501  TTATTGGGCG TAAAGAGCTC GTAGGCGGTT TGTTGCGTCG GCCGTGAAAA
             551  CCTGCAGCTT AACTGTGGGC TTGCGGTCGA TACGGGCAGA CTTGAGTTCG
             601  GTAGGGGAGA CTGGAATTCC TGGTGTAGCG GTGAAATGCG CAGATATCAG
             651  GAGGAACACC GGTGGCGAAG GCGGGTCTCT GGGCCGATAC TGACGCTGAG
             701  AGCGAAAGCG TGGGGAGCGA ACAGGATTAG ATACCCTGGT AGTCCACGCC
             751  GTAAACGTTG GGCGCTAGGT GTGGGGATGG GTTCCCTGTC TCCGTGCCGT
             801  A

GMKU 378       1  CTGGCGGCGT GCTTACACAT GCAAGTCGAG CGGAAAGGCC CTTCGGGGTA
              51  CTCGAGCGGC GAACGGGTGA GTAACACGTG AGTAACCTGC CCCTGACTCT
             101  GGGATAAGCC TGGGAAACCG GGTCTAATAC CGGATACGAC CATTTCTCGC
             151  ATGTGATGGT GGTGGAAAGT TTTTTCGGTT GGGGATGGGC TCGCGGCCTA
             201  TCAGCTTGTT GGTGGGGTGA TGGCCTACCA AGGCGACGAC GGGTAGCCGG
             251  CCTGAGAGGG CGACCGGCCA CACTGGGACT GAGACACGGC CCAGACTCCT
             301  ACGGGAGGCA GCAGTGGGGA ATATTGCGCA ATGGGCGGAA GCCTGACGCA
             351  GCGACGCCGC GTGGGGGATG ACGGCCTTCG GGTTGTAAAC CTCTTTCAGC
             401  AGGGACGAAG TTGACGTGTA CCTGTAGAAG AAGCGCCGGC TAACTACGTG
             451  CCAGCAGCCG CGGTAATACG TAGGGCGCAA GCGTTGTCCG GAATTATTGG
             501  GCGTAAAGAG CTCGTAGGTG GCTTGTCGCG TCTGCCGTGA AAGCCCGTGG
             551  CTTAACTACG GGTCTGCGGT GGATACGGGC AGGCTAGAGG CTGGTAGGGG
             601  CAAGCGGAAT TCCTGGTGTA GCGGTGAAAT GCGCAGATAT CAGGAGGAAC
             651  ACCGGTGGCG AAGGCGGCTT GCTGGGCCAG TTCTGACGCT GAGGAGCGAA
             701  AGCGTGGGGA GCGAACAGGA TTAGATACCC TGGTAGTCCA CGCTGTAAAC
             751  GTTGGGCGCT AGGTGTGGGG GTCTTCCACG ATCTCTGTGC CGTAGCTAAC
             801  GCATTAAGCG CCCCGCCTGG GGAGTACGGC CGCAAGGCTA AAACTCAAAG
             851  GAATTGACGG GGGCCCGCAC AAGCGGCGGA CATGTTGCTT AATTCGACCA
             901  CGCGAAGAAC CTTACAGGTT TGACATACAC CGGAAACCTC AGAGATGGAT
             951  GCCTCCTTTG GACTGGTGTA CAGGTGGTGC ATGGCTGTC
```

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PUBLICATION : **Indananda, C.**, Igarashi, Y., Ikeda, M., Oikawa, T. and Thamchaipenet, A. **(2013).** Linfuranone A, a new polyketide from plant-derived *Microbispora* sp. GMKU 363. **J. Antibiot**. (accepted).

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