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TAXONOMY AND SECONDARY METABOLITES OF SELECTED ACTINOMYCETE  
STRAINS AND *MONASCUS KAOLIANG KB20M10.2*

Miss Suchada Jongrungruangchok

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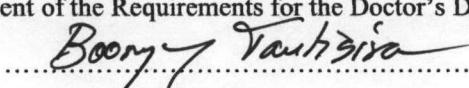
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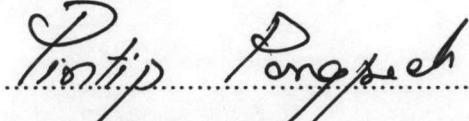
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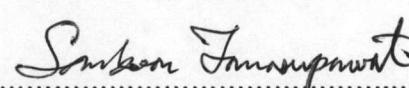
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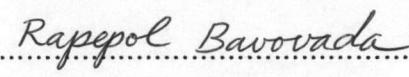
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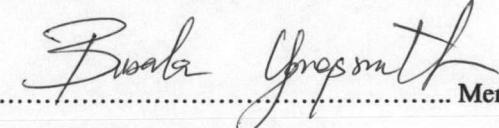
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ໃນການສຶກໝາເພື່ອຄັດເລືອກເຊື່ອແອຄຕິໂນມັຍເຊີ້ທສີ່ສາມາຮັດພິລິດສາຣຖຸດິຍຸນົມແລະການສຶກໝາອຸນົມວິຈານຂອງເຊື່ອ ພບວ່າເຊື່ອ 12 ໄອໂໂລເລມືຖືທີ່ຕ້ານຈຸລື້ບ ແລະສາມາຮັດພິສູງນີ້ເອົກລັກພື້ນຂອງເຊື່ອ PNK1-3, PNK1-5, TT2-9 ແລະ KN-6 ເປັນແບບທີ່ເຮັດໃນສຸກຸລ ສເຕຣບໂຕມັຍຊືສ FLM-2 ເປັນ ຄືຕາສໂຕສປ່ອຮາ MA-1, MA-2, JSM1-1, JSM1-3, MC5-1, MC7-1 ແລະ R1-1 ເປັນໄມໂຄຣໂນໂສປ່ອຮາໂດຍໃຊ້ຜົດການສຶກໝາລັກພະທາງພິໂນໄທປ່າແລະລັກພະທາງອຸນົມວິຈານເຄມື່ຽວມ່ວນທີ່ກາວິເຄຣະໜໍ ດຳລັບເບສໃນໜ່ວຍ 16S rDNA ພບວ່າເຊື່ອເຫັນນີ້ມີ phospholipids ຂົນດີ phosphatidylethanolamine ເປັນອົງປະກອບຫລັກ ຮວມທີ່ມີກຣຳໄຟມັນສ່ວນໃໝ່ແບບ iso-C<sub>16:0</sub>, iso-C<sub>15:0</sub>, iso-C<sub>17:0</sub>, anteiso-C<sub>16:0</sub>, anteiso-C<sub>15:0</sub>, ແລະ anteiso-C<sub>17:0</sub> ແລະມີ menaquinones ຂົນດີ MK-9(H<sub>4</sub>), MK-9(H<sub>6</sub>) ຢ່ວ່າ MK-10(H<sub>4</sub>) ນອກຈາກນີ້ພບວ່າມີປົມາລ G+C ຂອງສາຍ DNA ອູ້ໃນໜ່ວຍ 71-73 mol% ສເຕຣບໂຕມັຍຊືສ ຄືຕາສໂຕສປ່ອຮາ ແລະ ໄມໂຄຣໂນໂສປ່ອຮາ ມີກຣຳ LL, LL ແລະ meso-diaminopimelic ແລະ meso-diaminopimelic ໃນພັນຈຳເຊື່ອຕົ້ນດຳລັບ ໄມໂຄຣໂນໂສປ່ອຮາມີນໍາຕາລ xylose ແລະ arabinose ເປັນສ່ວນປະກອບຂອງພັນຈຳເຊື່ອຕົ້ນດຳລັບ ຈາກຜົດການຄ້າຍຄື່ງຂອງດຳລັບເບສໃນໜ່ວຍ 16S rDNA (99.9%) ສາມາຮັດພິສູງນີ້ວ່າ PNK1-3 ເປັນ *S. hygroscopicus* ຂະໜາທີ່ FLM-2 ມີຄວາມຄ້າຍຄື່ງຂອງດຳລັບເບສໃນໜ່ວຍ 16S rDNA (97.1%) ໄກລີ້ເຄີຍກັບ *K. melanogena* ຈາກຜົດການຄ້າຍຄື່ງທາງ DNA ແລະລັກພະທາງພິໂນໄທປ່າສາມາຮັດແບ່ງແຍກເຊື່ອໄມໂຄຣໂນໂສປ່ອຮາ 7 ສາຍພັນຫຼູກເປັນ 3 ກຸ່ມ ແລະແສດງຜົດການຄ້າຍຄື່ງທາງ DNA ໃນຮະດັບຕໍ່າ (28.7-43.1%) ແລະດຳລັບເບສໃນໜ່ວຍ 16S rDNA (97.5-99.3%) ຮວມທີ່ມີລັກພະທາງພິໂນໄທປ່າແຕກຕ່າງໄປຈາກເຊື່ອໄມໂຄຣໂນໂສປ່ອຮາທີ່ເຄຍມີຮາຍງານໄວ້ ຈຶ່ງສາມາຮັດຈັດເປັນເຊື່ອໜົນໃໝ່ ແລະໄດ້ເສັນອໍ້ສໍາຫັນເຊື້ອກຸ່ມທີ່ 1 (2 ສາຍພັນຫຼູກ) ເປັນ *Micromonospora krabiensis* ກຸ່ມທີ່ 2 (2 ສາຍພັນຫຼູກ) ເປັນ *Micromonospora marinus* ແລະກຸ່ມທີ່ 3 (3 ສາຍພັນຫຼູກ) ເປັນ *Micromonospora chaiyaphumensis* ຕາມດຳລັບ ຈາກການສຶກໝາສາຣຖຸດິຍຸນົມຂອງເຊື່ອທີ່ຄັດເລືອກສາຍພັນຫຼູກ PNK1-3 ພບວ່າສາມາຮັດແຍກສິ່ງສັກດີທີ່ພົບເອົນລະຫິຕະທາງການໜ້າໜັກເຊື່ອຊື່ແສດງຖົງທີ່ຕ້ານເຊື້ອຈຸດິນທຣີຢ່ອຍ່າງມືນຍຳດຳຄັ້ງ ໄດ້ສາຍໃນກຸ່ມ ansamycin 1 ຂົນດີ ອື່ອ geldanamycin ແສດງຖົງທີ່ທາງຮົວກາພໄດ້ແກ່ ຖົກທີ່ຕ້ານເຊື້ອຮາ (*Candida albicans* ATCC 10231) ເຊື້ອນາລາເຮີຍ (*Plasmodium falciparum*) ເຊື້ວັນໂຣຄ (*Mycobacterium tuberculosis*) ປຸກທີ່ຄວາມເປັນພິຍຕ່ອ human epidermoid ແລະ breast cancer cell lines

ຈາກການສຶກໝາສາຣຖຸດິຍຸນົມຂອງເຊື່ອກາລາຍພັນຫຼູກ *Monascus kaoliang* KB20M10.2 ທີ່ເຈີ່ງມີພິຍຕ່ອ ພບວ່າສາມາຮັດແຍກສິ່ງສັກດີທີ່ໄດ້ສາຍໃນກຸ່ມ azaphilones 2 ຂົນດີ ອື່ອ monascusone A (6,7-dihydroxy-3(2-hydroxypropyl)-7- methyl-1,5,6,7-tetrahydroisochromen-8-one) ແລະ monascusone B ແລະສາຍ azaphilones ທີ່ເຄຍມີຮາຍງານ 2 ຂົນດີ monascin A ແລະ FK17-P2b2 ການພິສູງໂຄຮງສ່າງທາງເຄມື່ຽວມ່ວນທີ່ໃໝ່ UV, IR, MS ແລະ NMR spectroscopy ສາຍ monascusone A ນີ້ ໄມແສດງຖົງທີ່ຕ້ານເຊື້ອຈຸດິນທຣີທົດສອນ

ສາຂາວິຊາ ແກສ້ຂເຄມື່ອງແລະພິລິດກັນທົງຮ່ວມໝາດ  
ປັກການສຶກໝາ 2547

ລາຍມື້ອໍ້ອື່ອນິສິຕ.....  
ລາຍມື້ອໍ້ອື່ອອາຈາຣຍ໌ທີ່ປຶກຂາ.....  
ລາຍມື້ອໍ້ອື່ອອາຈາຣຍ໌ທີ່ປຶກຂາຮ່ວມ.....

# # 44769708 33 MAJOR: PHARMACEUTICAL CHEMISTRY AND NATURAL PRODUCTS

KEY WORDS: ACTINOMYCETES / MICROMONOSPORA / MONASCUS / ANSAMYCIN / AZAPHILONE

SUCHADA JONGRUNGRUANGCHOK: TAXONOMY AND SECONDARY METABOLITES OF SELECTED ACTINOMYCETE STRAINS AND *MONASCUS KAOLIANG* KB20M10.2. THESIS ADVISOR: ASSOC. PROF. SOMBOON TANASUPAWAT Ph.D., THESIS COADVISOR: ASSOC. PROF. RAPEPOL BAVOVADA. Ph.D., 216 pp. ISBN 974-53-1231-2.

In the course of our investigation for bioactive metabolites and taxonomy of 12 actinomycete strains isolated from soils, all possessed antimicrobial activities, PNK1-3, PNK1-5, TT2-9, KN-6 were identified as *Streptomyces*; FLM-2 as *Kitasatospora*; and MA-1, MA-2, JSM1-1, JSM1-3, MC5-1, MC7-1 and R1-1 as *Micromonospora* based on the phenotypic and chemotaxonomic characteristics including the phylogenetic analysis using 16S rDNA sequences. The tested strains contained phosphatidylethanolamine as characteristic phospholipids (type II); iso-C<sub>16:0</sub>, iso-C<sub>15:0</sub>, iso-C<sub>17:0</sub>, anteiso-C<sub>16:0</sub>, anteiso-C<sub>15:0</sub>, and anteiso-C<sub>17:0</sub> as major fatty acids; and MK-9(H<sub>4</sub>), MK-9(H<sub>6</sub>), or MK-10(H<sub>4</sub>) as major menaquinones. The range of G+C content of the DNA was 71-73 mol%. *Streptomyces*, *Kitasatospora*, and *Micromonospora* are LL, LL and meso, and meso-diaminopimelic acid in cell wall, respectively. Whole-cell sugar of *Micromonospora* contained xylose and arabinose (pattern D). The potent selected strain, PNK1-3 was identified as *S. hygroscopicus* (99.9% 16S rDNA similarity), while FLM-2 was closed to *K. melanogena* (97.1% 16S rDNA similarity). On the basis of DNA-DNA similarity and phenotypic characteristics, 7 strains of *Micromonospora* could be separated into three groups. A low level of DNA-DNA similarity (28.7-43.1%) and 16S rDNA similarity (97.5-99.3%) including the phenotypic characteristics indicated that these strains were distinguished from all of validly described *Micromonospora* species. The names *Micromonospora krabiensis* sp. nov., *Micromonospora marinus* sp. nov and *Micromonospora chaiyaphumensis* sp. nov. are proposed for Group I (2 strains), Group II (2 strains) and Group III (3 strains), respectively. PNK1-3 was selected for secondary metabolite production and elucidated chemical structure. The ETOAc extract from fermentation broth yielded known ansamycin, geldanamycin which exhibited antifungal activity (*Candida albicans* ATCC10231), antimalarial (*Plasmodium falciparum*), antituberculosis activity (*Mycobacterium tuberculosis*), cytotoxicity activity against human epidermoid and breast cancer cell lines.

For exploration the secondary metabolites of a yellow mutant *Monascus kaoliang* KB20M10.2 grown on rice, CH<sub>2</sub>Cl<sub>2</sub> extract yielded two new azaphilone pigments, monascusone A (6,7-dihydroxy-3(2-hydroxypropyl)-7-methyl-1,5,6,7-tetrahydroisochromen-8-one) and monascusone B, together with two known azaphilones, monascin A and FK17-P2b2. Structures of isolated compounds were elucidated through extensive analyses of their UV, IR, MS, and NMR spectroscopic data. Monascusone A showed no antimicrobial activity.

Field of study Pharmaceutical Chemistry

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### LIST OF ABBREVIATIONS AND SYMBOLS

$[\alpha]^{28}D$	=	Specific rotation at 28° and sodium D line (589 nm)
$\alpha$	=	Alpha
Ala	=	Alanine
Ara	=	Arabinose
ATCC	=	American Type Culture Collection, Maryland, U.S.A.
Ba(OH) <sub>2</sub>	=	Barium hydroxide
BC	=	Breast cancer cell line
° C	=	Degree Celsius
<sup>13</sup> C-NMR	=	Carbon-13 nuclear magnetic resonance
CDCl <sub>3</sub>	=	Deuterated chloroform
CFU	=	Colony forming unit
CHCl <sub>3</sub>	=	Chloroform
cm	=	Centimeter
COSY	=	Correlation spectroscopy
Cz. Sucrose	=	Czapek's sucrose
$\delta$	=	Chemical shift
$d$	=	Doublet
DDBJ	=	DNA Data Bank of Japan
DEPT	=	Distortionless enhancement by polarization transfer
DMF	=	Dimethyl formamide
DMSO- <i>d</i> <sub>6</sub>	=	Deuterated dimethylsulphoxide
DON	=	2,7-dihydroxynaphthalene
DPG	=	Diphosphatidylglycerol
DSMZ	=	Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH
EDTA	=	Disodiummethylenediamine tetraacetate
EMBL	=	European Molecular Biology Laboratory
ESI-TOF-MS	=	Electrospray Ionization-Time of Flight Mass Spectrometry
EtOAc	=	Ethyl acetate
$\epsilon$	=	Molar absorptivity

<b>g</b>	=	<b>Gram</b>
<b>G</b>	=	<i>N</i> -acetylglucosamine
<b>Gal</b>	=	Glalactose
<b>GC</b>	=	Gas chromatography
<b>GenBank</b>	=	National Institute of Health genetic sequence database
<b>Glu</b>	=	Glutamic acid
<b>GluNu</b>	=	Phaspholipids of unknown structure containing glucosamine
<b>Gly</b>	=	Glycerine
<b>h</b>	=	Hour
<b>HCl</b>	=	Hydrochloric acid
<b>HMBC</b>	=	<sup>1</sup> H-detected heteronuclear multiple bond correlation
<b>HMQC</b>	=	<sup>1</sup> H-detected heteronuclear multiple quantum coherence
<b><sup>1</sup>H-NMR</b>	=	Proton nuclear magnetic resonance
<b>HPLC</b>	=	High performance liquid chromatography
<b>HPTLC</b>	=	High performance thin layer chromatography
<b>H<sub>2</sub>O</b>	=	Water
<b>H<sub>2</sub>SO<sub>4</sub></b>	=	Sulfuric acid
<b>Hz</b>	=	Hertz
<b>IC<sub>50</sub></b>	=	Inhibition concentration
<b>IR</b>	=	Infrared
<b>ISP</b>	=	International <i>Streptomyces</i> Project
<b>J</b>	=	Coupling constant
<b>JCM</b>	=	Japan Collection of Microorganisms
<b>KB</b>	=	A human epidermoid carcinoma cell line of the nasopharynx
<b>KNO<sub>3</sub></b>	=	Potassium nitrate
<b>KOH</b>	=	Potassium hydroxide
<b>L</b>	=	Liter
<b>L-DA</b>	=	L-diamino acid
<b>Lyso-PE</b>	=	Lyso-phosphatidylethanolamine
<b>m</b>	=	Multiplet
<b>mm<sup>3</sup></b>	=	Cubic meter
<b>M</b>	=	Molar

[M+H] <sup>+</sup>	=	Protonated molecular ion
MeCN	=	Methyl cyanide
MEGA	=	Molecular Evolutionary Genetics Analysis
MeOH	=	Methanol
<i>meso</i> -DAP	=	<i>meso</i> -Diaminopimelic acid
Methyl-PE	=	methyl-Phosphatidylethanolamine
μg	=	Microgram
mg	=	Milligram
MgCl <sub>2</sub>	=	Magnesium chloride
MHA	=	Mueller-hinton agar
MHz	=	Mega hertz
MK	=	Menaquinone
μl	=	Microliter
ml	=	Milliliter
μm	=	Micrometer
mm	=	Millimeter
MS	=	Mass spectroscopy
NaCl	=	Sodium chloride
NaOH	=	Sodium hydroxide
N <sub>2</sub> gas	=	Nitrogen gas
nm	=	Nanometer
NMR	=	Nuclear magnetic resonance
NOESY	=	Nuclear overhouser effect correlation spectroscopy
NSS	=	Normal saline solution
nt	=	Nucleotide
OH-PE	=	Hydroxyl-phosphatidylethanolamine
PBS	=	Phosphate buffer saline
PC	=	Phosphatidylcholine
PCR	=	Polymerase chain reaction
Pd	=	Palladium
PE	=	Phosphatidylethanolamine
PG	=	Phosphatidylglycerol

PI	=	Phosphatidylinositol
PIMs	=	Phosphatidylinostolmannoside
PME	=	Phosphatidyl- <i>N</i> -methyllethylethanolamine
ppm	=	Part per million
rDNA	=	Ribosomal deoxynucleic acid
rRNA	=	Ribosomal ribonucleic acid
rpm	=	Round per minute
<i>s</i>	=	Singlet
SCA	=	Starch caseinate agar
SDA	=	Sabouraud's dextrose agar
sec	=	Second
Si Gel	=	Silica gel
sp.	=	Species
SSC	=	Standard sodium citrate
<i>t</i>	=	Triplet
TAE	=	Tris-acetate
TBE	=	Tris-borate
TCA	=	Trichloroacetic acid
UV	=	Ultraviolet
Vero cell line	=	African monkey kidney cell line
Xyl	=	Xylose