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Identification and antimicrobial activity of *Micromonospora* strains from Thai peat swamp forest soils

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ABSTRACT

The identification and antimicrobial activity of thirteen actinomycete strains isolated from peat swamp forest soils collected from Narathiwat, Patthaloong and Yala provinces, the southern part of Thailand were carried out. Based on the phenotypic and chemotaxonomic characteristics, all isolates were belonged to genus *Micromonospora*. They were separated into six groups based on 16S ribosomal RNA gene sequence analysis and were identified as *M. narathiwatensis* (Group 1, 5 isolates), *M. humi* (Group 4, 3 isolates), *M. aurantiaca* (Group 5, 2 isolates), one of each isolate as *M. chalcea* (Group 2) and *M. maritima* (Group 6). The isolate LK6-12 (Group 3) showed low similarity (99.16%) with the type strains of *Micromonospora siamensis* that will be the novel species of the genus *Micromonospora*. *Meso*-diaminopimelic acid (cell wall type II), xylose and arabinose (pattern D) were detected in their whole-cell hydrolysates. The major polar lipid was phosphatidylethanolamine (type II). The predominant cellular fatty acids were $C_{17:0}$, $C_{17:1}\omega8c$, iso-$C_{16:0}$, iso-$C_{15:0}$, iso-$C_{17:0}$, anteiso-$C_{15:0}$, and anteiso-$C_{17:0}$. The predominant menaquinones were MK-9(H_4), MK-9(H_6), or MK-10(H_4). The DNA G+C contents of the isolates ranged from 71.6-72.6 mol%. On the primary screening, 2 isolates exhibited the antimicrobial activity against *Bacillus subtilis* ATCC 6633 and *Kocuria rhizophila* ATCC 9341.

INTRODUCTION

Micromonospora strains are Gram-positive, aerobic, mesophilic, non-motile actinomycete which produces single non-motile spore directly from substrate hyphae and their mycelial pigments generally are in orange, red or brown color. Cell wall contained *meso*-diaminopimelic acid, glutamic acid, glycine, alanine, and glycolylmuramic acid. The diagnostic whole-cell sugars were xylose and arabinose. The phospholipid patterns were diphosphatidylglycerol, phosphatidylinositol, phosphatidylethanolamine, and phosphatidylinositolmannosides, but not phosphatidylcholine. Predominant cellular fatty acids were iso-$C_{16:0}$ and iso-$C_{15:0}$ and small amount of iso-$C_{17:0}$, anteiso-$C_{17:0}$ and anteiso-$C_{15:0}$ were also present. The major menaquinones were MK-9, MK-10, and MK-12. The range of G+C content of the DNA was 71-73 mol% (Kawamoto, 1989; Kasai *et al.*, 2000).

Micromonospora strains have been reported to distribute in different environments such as in soils, sandstone, water, root nodules and mangrove sediment (Kawamoto, 1989; Hirsch *et al.*, 2004; Ara and Kudo, 2007; Trujillo *et al.*, 2007; Huang *et al.*, 2008; Garcia *et al.*, 2010; Wang *et al.*, 2011). In addition, the novel species of *M. auratinigra*, *M. eburnea, M. narathiwatensis*, *M. chaiyaphumensis, M. krabiensis*, *M. marina*, *M. humi*, *M. maritima* and *M. sediminis* strains are isolated (Thawai *et al.*, 2005a; 2007; 2008; Jongrungruangchok *et al.* 2008; Tanasupawat *et al.*, 2010; Songsumanus *et al.*, 2013; Phongsopitanun *et al.*, 2016) were reported in Thailand. These actinomycetes are potential producers for secondary metabolites with diverse chemical structures, and biological activities, e.g. gentamicin (a considered essential to global health), sagamicin, megalomicin, halomicin, mutamicin, everninomicin, and mycinamicin (Glasby, 1993).

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Peat swamp forest is a very special type of the evergreen forests that occurs in fresh-water marshy land (Posa *et al.*, 2011) and was an interested source for soil sampling because it was different from the normal soil. Nowadays, *Micromonospora* strains from tropical peat swamp forests are still limited in isolation and the research on taxonomy and antibiotic production of them have so far received little attention. Thus, the attempt to sampling the soil samples in the unique sources is focused. In this study, *Micromonospora* strains isolated from peat swamp forest soils in the southern areas of Thailand were characterized and identified by both classical and molecular techniques, and the investigation of antimicrobial activity is also performed.

MATERIALS AND METHODS

Isolation and identification of isolates

Micromonospora strains were isolated from 11 soil samples (peat, muck) collected from peat swamp forest in Narathiwat, Patthaloong and Yhala provinces, the southern areas of Thailand. The soil suspension was diluted to a ten folds dilution series (10^{-1} to 10^{-3}). The aliquots (100 µL) were spread onto Starch-Casein Nitrate agar (SCA) plates supplemented with antibiotics, nystatin, novobiocin, and tetracycline (Brock *et al.*, 1993), and were incubated at 30°C for 7-21 days. The moist, pale yellow, orange, brown and black colonies of isolates were picked up and streaked for purification on ISP2 agar plates at 30°C for 7-21 days. The single colony was cultivated on ISP2 agar slants at 30°C for 14 days. The soil samples were dried under room temperature for a week. Dried soil (1 g) was put into a test tube which then 2.5 mL of distilled water was added and shaked for 30 sec. The soil solution was measured for pH value.

The morphological characteristics of *Micromonospora* isolates were determined by using simple inclined coverslip technique (Williams and Cross, 1971). Cultural characteristics were studied on the colors of mature substrate mycelium, spore, and diffusible pigment using crosshatch streak (Shirling and Gottlieb, 1966). The strains were cultivated on yeast extract malt extract agar (International *Streptomyces* Project, ISP2 medium) at 30°C for 21 days. The color of the upper and reverse surface mycelium and the soluble pigment color were observed by using the NBS/ISCC colour system (Kelly, 1964). Spore was observed by light microscope or scanning electron microscope. Utilization of various carbon sources and several biochemical tests were determined using standard methods as recommended by Shirling and Gottlieb (1966) and Arai (1975). NaCl tolerance was tested on ISP2 agar plates supplemented with 1.5, 3, 4, 5, 6 or 7% (w/v) NaCl. Growth at 30, 40, and 45°C, at pH 4, 4.5, 5, 6, 7, and 8 were performed on ISP2 agar plates, incubated for 7-14 days. Standard thin-layer chromatography (TLC) procedures were used to determine the isomers of diaminopimelic acid and sugars in whole-cell hydrolysates (Staneck and Roberts, 1974). The *N*-acyl type of muramic acids in the cell wall was determined as described by Uchida and Aida (1984). Menaquinones were extracted as reported by Collins *et al.* (1977) and analysed using high-performance liquid chromatography (HPLC). Cellular fatty acids methyl esters were prepared as described by Sasser (1990) and were analysed using gas chromatography according to the instructions of the Sherlock Microbial Identification System (MIDI). Polar lipids were extracted and analysed by two-dimensional TLC (Minnikin *et al.* 1984).

16S rRNA gene sequence and phylogenetic analyses

The genomic DNA of *Micromonospora* isolates was extracted from the cultures grown in yeast extract-glucose broth on a rotary shaker as described by Tamaoka and Komagata (1984). The DNA G+C content was determined by reversed phase HPLC (Tamaoka and Komagata, 1984). The 16S rRNA gene was amplified using the universal primers 27F [5'-AGAGTTTGATC(AC) TGGCTCAG-3'] and 1492R [5'-ACGG(CT)TACCTTG TTACGACTT-3'] as described by Weisburg *et al.* (1991). The PCR products were sequenced using universal primers (Lane, 1991). The closest phylogenetic neighbours were identified by BLAST searches using the EzBioCloud database (www.ezbiocloud.net/) (Yoon *et al.*, 2017). The 16S rRNA gene sequence was manually verified and multiple-aligned with selected sequences. Phylogenetic tree was generated using the neighbour-joining (Saitou and Nei, 1987) in the MEGA 6.0 software (Tamura *et al.*, 2013). Gaps and ambiguous nucleotides were completely eliminated before the calculations. Evolutionary distances among the strains were analysed based on Kimura's two-parameter method (Kimura, 1980) for neighbour-joining tree. The confidence values of nodes were evaluated by the bootstrap resampling method with 1000 replications (Felsenstein, 1985).

Screening of antimicrobial activity of isolates

Each isolate was cultured in 50 mL of seed medium conposing of glucose (0.4%), yeast extract (0.4%), malt extract (1.0%), pH 7.3 (in a 250 mL Erlenmeyer flask) and cultivated on shaker (200 rpm) at room temperature for 4 days. One percent of seed culture was transferred into 200 mL of production medium which comprised glucose (0.4%), yeast extract (0.4%), malt extract (1.0%), and $CaCO_3$ (0.1%), pH 7.3, incubated on a rotary shaker (200 rpm) at room temperature for 10 days. The antimicrobial activity of the isolated fractions was examined by the agar disc diffusion method (Acar and Goldstein, 1991) against *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 25922, *Kocuria rhizophila* ATCC 9341, *Pseudomonas aeruginosa* ATCC 27853, and *Candida albicans* ATCC 10231 on Mueller-Hinton agar plates at 37°C for 24 h, but the yeast strain was cultivated on Sabouraud's dextrose agar plates at 30°C for 24 h.

RESULTS AND DISCUSSION

Isolation and identification of isolates

The actinomycete strains, BTG1-1, KM1-9, BTG2-3, LK2-6, BTG3-4, LK5-4, LK6-12, KM4-24, BTG7-3, BTG10-2, BTG3-2, KM3-14 and KM3-1 were isolated from 11 samples of peat swamp forest soils in Narathiwat, Pattaloong and Yala provinces, Thailand (Table 1). The pH of the soil samples ranged from 3.39-6.37. They produced single non-motile spores on the substrate hyphae. Aerial mycelium was absent. All were aerobic, Gram-positive, aerobic, mesophilic, non-motile actinomycete. They grew well at pH 5–8 and at 25–30°C but did not grow at above 40°C. All isolates liquefied gelatin (Table 2). Their cell wall contained glutamic acid, glycine, alanine, *meso*-diaminopimlic

acid and xylose and arabinose as whole-cell sugars. The *N*-acyl group of muramic acid in the cell wall was glycolyl type. Hydrolysis of starch and milk peptonization were positive. They contained diphosphatidylglycerol, phosphatidylinositolmannosides, phosphatidylinositol and phosphatidylethanolamine, but not phosphatidylcholine as phospholipid profiles. On the basis of their morphological, cultural, physiological, biochemical and chemotaxonomic characteristics including the 16S rRNA gene sequence analysis, 13 isolates were divided into 6 groups as described below.

Table 1: Isolate number, sources of isolation, pH of soil, cultural characteristics, similarity (%) of 16S rRNA gene sequence similarity and identification of *Micromonospora* strains.

Isolate No.	Sources	Province	Soil pH	Group	Upper surface color	Reverse surface color	Soluble pigment color	% similarity	Identification
BTG1-1	Peat	Narathiwat	3.44	1	Black	Dull orange	Pale yellow	100.00	*M. narathiwatensis*
KM1-9	Muck	Patthaloong	4.92	1	Dull orange	Dull orange	Pale yellow	100.00	*M. narathiwatensis*
BTG2-3	Peat	Narathiwat	3.39	1	Black strong yellowish orange	Grayish yellow strong yellowish orange	Pale yellow	100.00	*M. narathiwatensis*
LK2-6	Soil	Yala	6.37	1	Yellowish brown	Pale yellowish pink	Pale yellow	100.00	*M. narathiwatensis*
BTG3-4	Muck	Narathiwat	4.25	1	Black	Grayish yellow	Pale yellow	99.79	*M. narathiwatensis*
LK5-4	Muck	Yala	4.36	2	Black	Deep orange	-	99.71	*M. chalcea*
LK6-12	Peat	Yala	4.64	3	Dark grayish brown	Deep orange	Pale yellow	99.16	*M. siamensis*
KM4-24	Peat	Patthaloong	3.41	4	Deep orange	Deep orange	Pale yellow	100.00	*M. humi*
BTG7-3	Soil	Narathiwat	4.05	4	Dark grayish brown	Vivid orange	Pale yellow	99.72	*M. humi*
BTG10-2	Soil	Narathiwat	4.29	4	Vivid orange	Vivid orange	Pale yellow	99.72	*M. humi*
BTG3-2	Muck	Narathiwat	4.25	5	Brownish black to black	Deep orange	-	100.00	*M. aurantiaca*
KM3-14	Soil	Patthaloong	4.16	5	Dark yellowish brown	Light yellowish	-	100.00	*M. aurantiaca*
KM3-1	Soil	Patthaloong	4.16	6	Dark brown to brownish black	Deep orange	Yellow	99.79	*M. maritima*

Table 2: Characteristics of *Miromonospora* strains.

Characteristics	Group					
	1	2	3	4	5	6
No. of isolate	5	1	1	3	2	1
Gelatin liquefaction	+ (w1)	+	w	+(w1)	+	+
Milk peptonization	w(+2)	+	w	+	+	+
Milk coagulation	+ (-1)	+	-	+	+	+
Starch hydrolysis	+	+	+	+	w	+
Nitrate reduction	-(w2)	+	-	-	+	w
NaCl tolerance (%)	≤ 3	≤ 6	≤ 4	≤ 4	≤ 4	≤ 5
Carbon utilization						
L-Arabinose	-(w1)	-	-	-	w	-
Cellobiose	-(+2)	+	w	+(w1)	+	+
D-Fructose	-(w1)	w	-	-	+	w
D-Galactose	-(+2)	+	-	+(-1)	+	+
Glycerol	w(-2)	w	+	w(+1)	-	+
Lactose	w(-2)	+	w	+	-	+
D-Mannitol	w(-2)	w	+	+ (-)	-	w
D-Melibiose	+ (-1)	+	-	+	+	+
D-Raffinose	+	+	+	+	+ (w1)	w
L-Rhamnose	-(+2)	w	w	+(w1)	-	+
D-Ribose	-(+2)	-	-	-	-	-
Salicin	+(w2)	-	+	-(+1)	-	w
D-Xylose	+	+	+	+	+	+
Antibacterial activity						
B. subtilis ATCC 6633	-	-	-	+	-	-
K. rhizophila ATCC 9341	-	-	-	+	-	-
Major menaquinones	MK-10(H_8), MK-10(H_6), MK-10(H_4)	MK-10(H_4), MK-10(H_6)	MK-10(H_4), MK-10(H_6), MK-9(H_4)	MK-10(H_4), MK-10(H_6),	MK-10(H_4), MK-9(H_4)	MK-10(H_4), MK-10(H_6)
G+C (mol%)	71.6	72.3	72.6	72.5	72.4	ND

Groups: 1, BTG1-1, KM1-9, BTG2-3, LK2-6 and BTG3-4; 2, LK5-4; 3, LK6-12; 4, KM4-24, BTG7-3 and BTG10-2; 5, BTG3-2 and KM3-14; 6, KM3-1. Number in parentheses indicates the number of isolate shows positive, weakly positive or negative reaction. ND, not determined. All isolates exhibited no antimicrobial activity against *E. coli* ATCC 25922*, P. aeruginosa* ATCC 27853*, S. aureus* ATCC 25923 and *C albicans* ATCC 10231.

Group 1 comprised five isolates, BTG1-1, KM1-9, BTG2-3, LK2-6 and BTG3-4. Colonies were grayish yellow on ISP2 medium, turning to black after sporulation. Single spores are formed on the substrate hyphae (Figure 2). They utilized D-glucose, D-melibiose, D-raffinose, salicin and D-xylose. Growth occured in the presence of NaCl up to 3%. The predominant cellular fatty acids were $C_{17:0}$, $C_{17:1}\omega8c$, iso-$C_{15:0}$, anteiso-$C_{15:0}$, iso-$C_{16:0}$ and iso-$C_{17:0}$ were also present (Table 3). MK-10(H_8), MK-10(H_6) and MK-10(H_4) were major menaquinone components. The DNA G+C content of strain BTG2-3 was 71.6 mol%. The BLAST analysis of 16S rRNA gene sequence of strains BTG1-1, KM1-9, BTG2-3, LK2-6 and BTG3-4 indicated that the strains were closely related to *Micromonospora narathiwatensis* (99.79–100% similarity) as shown in Table 1. Phylogenetic analysis of strains BTG1-1, KM1-9, BTG2-3, LK2-6 and BTG3-4 revealed that they were identified as *M. narathiwatensis* (Thawai *et al.*, 2007) (Figure 1).

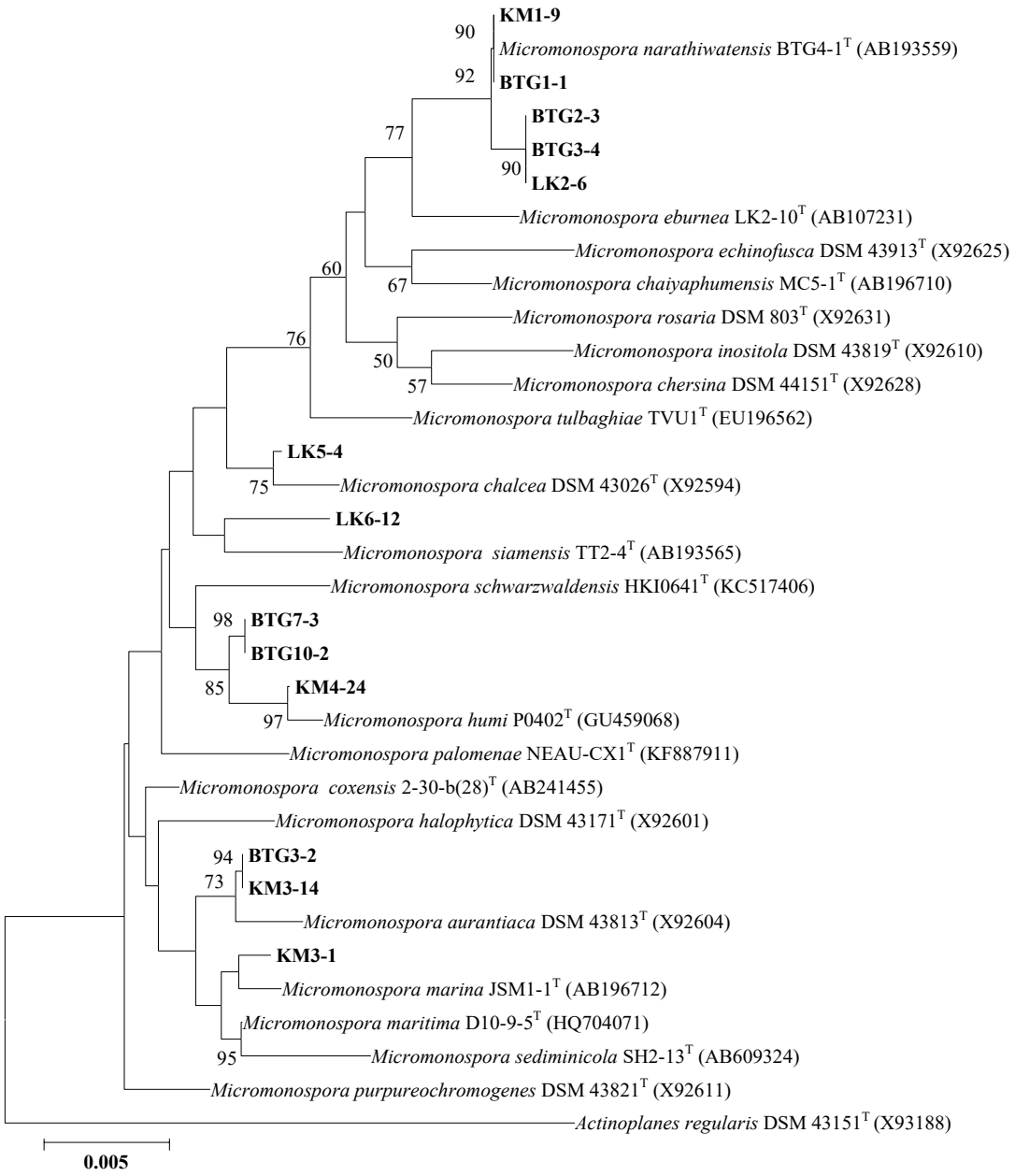

Fig. 1: Neighbour-joining tree based on distance analysis representing relationship between the partial 16S rRNA gene sequences of 13 *Micromonospora* isolates and the nearest relatives obtained from EzTaxon.

Group 2 contained one isolate, LK5-4. Colonies were deep orange in ISP2 medium, turning to black after sporulation. They utilized D-glucose, D-melibiose, D-mannitol, Draffinose, L-rhamnose, glycerol, lactose, D-galactose, cellobiose, D-fructose, and D-xylose, but not salicin, L-arabinose, and D-ribose. Nitrate reduction was positive. This strain grew on 6% NaCl concentration. The predominant cellular fatty acids were iso-$C_{16:0}$, anteiso-$C_{17:0}$, iso-$C_{15:0}$ and 10-Methyl $C_{17:0}$, and a small amount of

iso-$C_{17:0}$ and anteiso-$C_{15:0}$. MK-10(H_4), and MK-10(H_6) were major menaquinones. The DNA G+C content of strain was 72.3 mol%. The BLAST analysis of 16S rRNA gene sequence of strain LK5-4 indicated that the strain was closely related to *Micromonospora chalcea* (99.71% similarity) as shown in Table 1. Phylogenetic analysis of strain LK5-4 revealed that it was identified as *M. chalcea* (Kawamoto, 1989) (Figure 1).

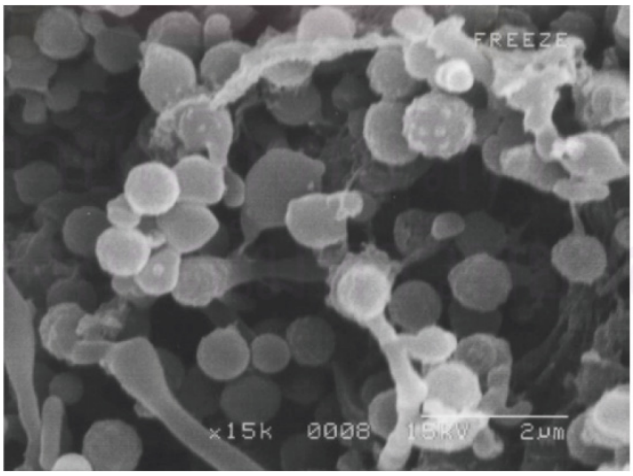

Fig. 2: The scanning electron micrograph of strain BTG 2-3 on ISP2 medium (21 days).

Table 3: Cellular fatty acid profiles of *Miromonospora* strains.

Fatty acid	Group					
	1	2	3	4	5	6
Saturated fatty acids						
$C_{15:0}$	2.8	-	1.9	3.3-9.7	-	-
$C_{16:0}$	1.7	-	2.4	1.4-1.9	0.7	0.9
$C_{17:0}$	7.7	0.8	4	9.2-9.8	2.8	1.7
$C_{18:0}$	1	1.6	1.8	0.5-0.7	1.2	1.6
Unsaturated fatty acids						
$C_{17:1}$ω8c	6.9	2.7	9.5	11.4-14	8.7	6.4
$C_{18:1}$ω7c	-	-	0.7	-	-	-
$C_{18:1}$ω9c	0.7	4.5	4.3	0.7	4.7	4.9
Branched fatty acids						
iso-$C_{14:0}$	1	1.6	0.9	2.5-7.7	0.5	0.5
iso-$C_{15:0}$	42.4	5.6	18.6	11.6-20.4	18.8	25.4
anteiso-$C_{15:0}$	10.3	4.3	6	9.3-16.1	10.3	6.4
iso-$C_{16:0}$	11.5	45.4	18.2	17.9-23.1	13.3	14.7
iso-$C_{17:0}$	5.3	2.5	5.2	0.6-2.3	8	10.4
anteiso-$C_{17:0}$	4.6	9.6	7.7	1.7-4.4	14.2	10.8
10-Methylated fatty acids						
10-Methyl $C_{17:0}$	1.1	7.4	1.4	2.1-2.8	1.2	1.4
10-Methyl $C_{18:0}$ TBSA	-	1.5	-	-	1.3	0.5
Summed feature 3[a]	0.9	0.5	1.6	0.7-0.8	0.9	1.2
Summed feature 6[b]	-	-	-	-	0.5	-

Group1: LK2-6; 2, LK5-4; 3, LK6-12; 4, KM4-24 and BTG10-2; 5, BTG3-2; 6, KM3-1. -, not detected. [a]Summed feature 3 contained one or more of the following fatty acids: 2-OH-i-$C_{15:0}$ and/or $C_{16:1}$ω7c. [b]Summed feature 6 contained one or more of the following fatty acid: $C_{19:1}$ω11c/ $C_{19:1}$ω9c.

Group 3 contained one isolate, LK6-12. Colonies were deep orange in ISP2, turning to dark grayish brown after sporulation. The spore surface appeared rough (Figure 3). Pale yellow soluble pigment is produced on ISP2 medium. The strain LK6-12 utilized D-glucose, D-raffinose, salicin, lactose, glycerol, D-mannitol, L-rhamnose, cellobiose and D-xylose but not D-melibiose, D-galactose, L-arabinose, D-ribose and D-fructose. Nitrate reduction was negative. This strain grew on 4% NaCl concentration. The predominant cellular fatty acids were iso-$C_{15:0}$, iso-$C_{16:0}$, iso-$C_{17:0}$, $C_{17:1}$ω8c, anteiso-$C_{15:0}$ and anteiso-$C_{17:0}$. Major menaquinones were MK-10(H_4), MK-10(H_6) and MK-9(H_4). The DNA G+C content was 72.6 mol%. The BLAST analysis of 16S rRNA gene sequence of strain LK6-12 indicated that the strain was closely related to *Micromonospora siamensis* (99.16% similarity) (Thawai *et al.*, 2005b) and showed low similarity that will be the novel species of the genus *Micromonospora* (Figure 1).

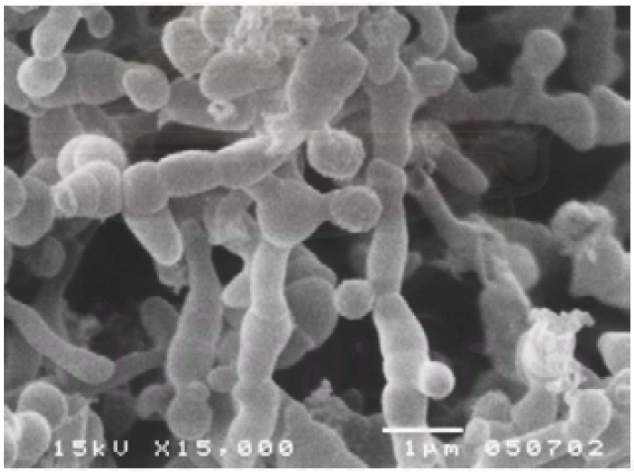

Fig. 3: The scanning electron micrograph of strain LK6-12 on YMA medium (21 days).

Group 4 comprised of three isolates, KM4-24, BTG7-3 and BTG10-2. Colonies were vivid orange on ISP2 medium. The spores surface appeared smooth (Figure 4). Pale yellow soluble pigment was produced on ISP2 medium. They utilized D-glucose, D-melibiose, D-raffinose, lactose, glycerol, L-rhamnose, cellobiose and D-xylose. Nitrate reduction was negative. This strain grew on 4% NaCl concentration. The major cellular fatty acids were $C_{17:0}$, $C_{17:1}$ω8c, iso-$C_{15:0}$, anteiso-$C_{15:0}$ and iso-$C_{16:0}$. MK-10(H_4) and MK-10(H_6) were the predominant menaquinones. The DNA G+C content of strain BTG10-2 was 72.5 mol%. The strain BTG7-3 and BTG10-2 produced secondary metabolites that could inhibit growth of *Bacillus subtilis* ATCC 6633 and *Kocuria rhizophila* ATCC 9341. The BLAST analysis of 16S rRNA gene sequence of strains KM4-24, BTG7-3 and BTG10-2 indicated that the strains were closely related to *M. humi* (99.72-100% similarity) as shown in Table 1. Phylogenetic analysis of strains KM4-24, BTG7-3 and BTG10-2 revealed that they were belonged to *M. humi* (Songsumanus *et al.*, 2011) (Figure 1).

Group 5 consisted of two isolates, BTG3-2 and KM3-14. Colonies are light yellowish brown in ISP2, turning to dark yellowish brown after sporulation. They utilized D-melibiose, D-glucose, D-raffinose, D-galactose, cellobiose, D-fructose,

and D-xylose, but not D-mannitol, L-rhamnose, glycerol, lactose, salicin, L-arabinose, and D-ribose. Nitrate reduction is positive. Growth occured in the presence of NaCl up to 4%. The predominant cellular fatty acids contained $C_{17:1}\omega8c$, iso-$C_{15:0}$, anteiso-$C_{15:0}$, iso-$C_{16:0}$, iso-$C_{17:0}$, and anteiso-$C_{17:0}$. The predominant menaquinones of all isolates are MK-10(H_4), and MK-9(H_4). The DNA G+C content of strain KM3-14 was 72.4 mol%. The BLAST analysis of 16S rRNA gene sequence of strains BTG3-2 and KM3-14 indicated that the strains were closely related to *M. aurantiaca* (100% similarity) as shown in Table 1. Phylogenetic analysis of strains BTG3-2 and KM3-14 revealed that they were belonged to *M. aurantiaca* (Sveshnikova *et al.*, 1969) (Figure 1).

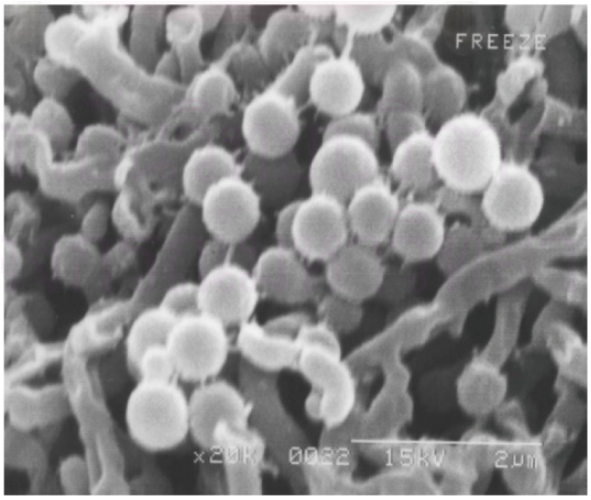

Fig. 4: The scanning electron micrograph of strain BTG10-2 on ISP2 medium (21 days).

Group 6 contained one isolates, including KM3-1. Colonies are deep orange and turning to black after sporulation. The strain KM3-1 utilized D-mannitol, D-raffinose, L-rhamnose, glycerol, lactose, D-galactose, D-fructose, cellobiose, D-melibiose, D-glucose, and D-xylose, but not salicin, L-arabinose, and D-ribose. Nitrate reduction is weak positive. Growth occured in the presence of NaCl up to 5%. The major cellular fatty acids were $C_{17:1}\omega8c$, iso-$C_{15:0}$, iso-$C_{16:0}$, iso-$C_{17:0}$, anteiso-$C_{15:0}$ and anteiso-$C_{17:0}$, and a small amount of iso-$C_{17:0}$, and anteiso-$C_{15:0}$ are also present. The predominant menaquinones were MK-10(H_4), and MK-9(H_4). The BLAST analysis of 16S rRNA gene sequence of strain KM3-1 indicated that the strain was closely related to *M. maritima* (99.79% similarity) as shown in Table 1. Phylogenetic analysis of strain KM3-1 revealed that it was belonged to *M. maritima* (Songsumanus *et al.*, 2013) (Figure 1).

Antimicrobial activity of isolates

All isolates exhibited no antimicrobial activity against *E. coli* ATCC 25922*, S. aureus* ATCC 25923, *P. aeruginosa* ATCC 27853 and *C. albicans* ATCC 10231, except strains BTG7-3 and BTG10-2 isolated from soils in Narathiwat province that showed activity against *B. subtilis* ATCC 6633 and *K. rhizophila* ATCC 9341 (Table 2).

In this study, the peat swamp forest soil contained pH ranged from 3.39 to 6.37 and the most soil samples are rather acidic pH (Table 1). *M. narathiwatensis*, *M. chalcea*, *M. humi*, *M. aurantiaca* and *M. maritima* strains were isolated and only *M. humi* strains exhibited antimicrobial activity against Gram-positive bacteria. The strains of *M. chalcea* are reported to produce antlermicins and tetrocarcins (aminoglycosides) and izumenolide (lactones) that exhibited antibacterial and anticancer activity (Kobinata *et al.*, 1980a; 1980b; Liu *et al.*, 1980; Tomita *et al.*, 1980a; 1980b). Therefore, the further study on the secondary metabolites and biological activity of *M. humi* strains are required. *Micromonospora* strains produced many kinds of antibiotics including aminoglycosides, ansamysins, cyclic depsipeptide, dibenzazepines, diketopiperazine, dipeptides, echinosporamicin, furan, indolocarbazole alkaloids, lactones, macrolides, naphthoquinones, polypeptide, pyrazines, quinone, quinocycline, tetrocarcin, and other new compounds. Antibacterial, antifungal, anticancer, antitumor, anti-inflammatory, antiparasitic activity, cytostatic effects and trehalase glycosidase inhibitor activity are presented as their biological activity (Boumehira *et al.*, 2016).

CONCLUSION

As part of the research on screening of actinomycete strains from soils in peat swamp forests collected in Narathiwat, Pattaloong and Yala provinces, thirteen isolates including, *M. narathiwatensis* strains were distributed in Narathiwat, Patthaloong, Yala provinces, the strain of *Micromonospora chalcea* was found in Yala, *Micromonospora humi*, *Micromonospora aurantiaca* strains were distributed in Narathiwat and Patthaloong provinces, while *Micromonospora maritima* strain was distributed in Patthaloong province. Two isolates in Group 4 expressed antimicrobial activity against *Bacillus subtilis* ATCC 6633 and *Kocuria rhizophila* ATCC 9341. This evidence showed that the new soil sources are useful for the investigation of new microorganisms and their secondary metabolites.

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CONFLICT OF INTERESTS

There are no conflicts of interest.

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