Journal of Applied Pharmaceutical Science Vol. 4 (10), pp. 024-031, October, 2014
Available online at http://www.japsonline.com
DOI: 10.7324/JAPS.2014.401005
ISSN 2231-3354

Characterization and antimicrobial activity of *Streptomyces* strains from soils in southern Thailand

Paranee Sripreechasak[1], Khanit Suwanborirux[2] and Somboon Tanasupawat[1]

[1]Department of Biochemistry and Microbiology, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok 10330, Thailand.
[2]Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok 10330, Thailand.

ARTICLE INFO

Article history:
Received on: 02/08/2014
Revised on: 16/08/2014
Accepted on: 09/09/2014
Available online: 30/10/2014

Key words:
Actinomycetes, *Streptomyces*, antimicrobial activity, soil

ABSTRACT

Streptomyces strains are superior to other actinomycete strains in their ability to produce large numbers and varieties of antibiotics. The aim of this research was to study on the identification and antimicrobial activity of *Streptomyces* strains which were isolated from thirteen soil samples collected around Angthong Islands National Park, Surat Thani province, the southern part of Thailand. Twenty-six strains were isolated by using the dilution plating method on starch casein nitrate agar plate and potato starch-glycerol agar plate. On the basis of phenotypic characteristics and the 16S rRNA gene sequence analysis, they were belonged to the genus *Streptomyces* and were identified as *S. tendae* (5 isolates), *S. malachitospinus* (2 isolates), *S. marokkonensis* (2 isolates), *S. parvulus* (2 isolates), *S. fragilis* (1 isolate), *S. diastaticus* (1 isolate), *S. drozdowiczii* (1 isolate), *S. olivochromogenes* (1 isolate), *S. aureus* (2 isolates), *S. iranensis* (1 isolate), *S. rapamycinicus* (1 isolate), *S. yatensis* (1 isolate), *S. samsunensis* (4 isolates) and *S. spiralis* (2 isolates). On the antimicrobial activity screening, 15 isolates exhibited activity against *Bacillus subtilis* ATCC 6633, 13 isolates against *Kocuria rhizophila* ATCC 9341, 6 isolates against *Mucor racemosus* IFO 4581 and *Candida albicans* KF1, one isolate against *Escherichia coli* NIHJ KB213 and 5 isolates against *Xanthomonas campestris* pv. *oryzae* KB88.

INTRODUCTION

Streptomycetes are environmental filamentous Gram-positive bacteria of great commercial value. They have been well known as the producers of various useful bioactive metabolites particularly the antibiotics (Goodfellow *et al.*, 1988). The genus *Streptomyces* belonging to the family *Steptomycetaceae* was an aerobic, Gram-positive, spore-forming actinomycetes (Kämpfer, 2012). Strains of the genus *Streptomyces* have been well known as the producers of various useful bioactive metabolites particularly the antibiotics including erythromycin, tetracycline, streptomycin, chloramphenicol, neomycin, nystatin, amphotericin, kanamycin and cycloheximide (Glasby, 1993; Berdy, 2005). *Streptomyces* strain represents a group of micro-organisms widely distributed in nature. *Streptomyces* strains remains a focus of systematic research because they are still a rich source of commercially significant compounds such as antibiotics, enzymes, enzyme inhibitors and pharmacologically active agents (Goodfellow *et al.*, 1988). In this study, we focused to isolate and identify of *Streptomyces* strains and screen of antimicrobial activity of *Streptomyces isolates* from soils in the southern part of Thailand.

** Corresponding Author*
Somboon Tanasupawat, Department of Biochemistry and Microbiology, Faculty of Pharmaceutical Sciences, Chulalongkorn University, 254 Phayathai Road, Wangmai, Bangkok 10330, Thailand.
E-mail: Somboon.T@chula.ac.th, tanalab@yahoo.com

MATERIALS AND METHODS

Characterization of the isolates

Isolation and phenotypic characterization of the isolates

Actinomycete strains were isolated from thirteen soil samples collected around Angthong Islands National Park, Surat Thani province, the southern part of Thailand. The dried soil samples were suspended in distilled water and heated at 55 °C for 5 minutes. Serial dilutions of the suspension were prepared by the 10-fold dilution method. 10^{-2} and 10^{-3} dilutions of the suspension, aliquots of 0.1 ml were spreaded on surface of potato starch-glycerol agar (potato starch 1.0 %, glycerol 1.0 %, K_2HPO_4 0.2 %, $(NH_4)_2SO_4$ 0.2 %, $MgSO_4.7H_2O$ 0.1 %, $NaCl_2$ 0.1 %, $CaCO_3$ 0.2 %

and agar 1.2%, pH 7.0) (Tajima *et al*., 2001) and starch casein nitrate agar (starch 1.0%, sodium caseinate 0.03%, KNO_3 0.2% and agar 1.5%, pH 7.0) (Seong, 2001) supplemented with 25 mg l^{-1} of nystatin for antifungal. Each agar plate was supplemented with 10 mg l^{-1} of tetracycline and/or 50 mg l^{-1} of novobiocin.

The different colonies were picked up and streaked for further purification on International *Streptomyces* Project (ISP) media, ISP 2 (Shirling and Gottlieb, 1966). The pure isolates were observed for their cultural characteristics after cultivation on ISP 2 and ISP 3 agar at 28 °C for 2 weeks. The colors of substrate, aerial mycelium and soluble pigments were determined using the NBS/IBCC color chart (Mundie, 1995). Morphological observation was done by using a light microscope and scanning electron microscope (JSM-5410LV, Japan) on the culture grown on ISP 2, ISP 3, ISP 4 or YS (Yeast extract-starch) agar plates at 30 ^{o}C for 14 days. The phenotypic characteristics of isolates were determined as described by Shirling and Gottlieb (1966) and Arai (1975).

16S rRNA gene sequencing and phylogenetic analysis

Genomic DNA of each isolates was prepared by sonication of the cell suspension (Matsumoto *et al.,* 2006) cultured in YD broth (1.0% yeast extract and 1.0% dextrose). The 16S rRNA gene was amplified using the primers described by Takahashi *et al*. (2002). The reaction was carried out in a 50 µl reaction volume consisting of 5.0 µl of DNA solution, 29.6 of sterile Milli Q water, 5.0 ml of 10x Taq buffer, 2 ml of dNTP, 4.0 ml of each primer in primer pairs (11F & 925R), 0.4 µL of Ex Taq DNA polymerase. DNA thermal cycler was use for 16S rRNA gene amplification using the temperature profile of initial denaturation at 95 °C for 1 min, followed by 30 cycles of denaturation at 95 °C for 1 min, primer annealing at 50 °C for 1 min, extension at 72 °C for 1.5 min, and a final extension at 72 °C for 2 min. The PCR products were checked by agarose gel electrophoresis. The PCR products were sequenced on a DNA sequencer (Applied Biosystems 3130 Genetic Analyzer) using a BigDye Terminator v3.1 cycle Sequencing kit (Applied Biosystems), according to the manufacturer's instructions.

The obtained sequence was compared with all sequences from GenBank using the BLAST program. The clustalw2 program was used for multiple alignments with selected sequences for calculating evolution distances (Kimura, 1980) by Sea View version 4.2 (Gouy *et al*., 2010). The phylogenetic tree was constructed using the neighbor-joining method (Saitou and Nei, 1987). Data were resampled with 100 bootstrap replications. The values for sequence similarities among the most closely related strains were determined using the EzTaxon-e server (Kim et al., 2012).

Antimicrobial activities of the isolates

Each isolate was cultured in 10 ml of 301 seed medium (2.4% starch, 0.1% glucose, 0.3% peptone, 0.3% meat extract, 0.5% yeast extract, 0.4% $CaCO_3$, pH 7.0) and cultivated on shaker (200 rpm) at 28 °C for 3-7 days. One percentage of seed culture was transferred into 10 ml of 51 medium (0.5 % glucose, 0.5 % corn steep powder, 1.0 % oatmeal, 1.0 % pharmamedia, 0.5 % K_2HPO_4, 0.5 % $MgSO_4.7H_2O$, 1 ml/l trace metal solution, tap water) and 53 medium (2.0 % soluble starch, 2.0 % glycerol, 2.0 % nutrient broth, 1.0 % defatted wheat germ, 0.3 % $CaCO_3$, tap water) and cultivated on shaker (200 rpm) at 28 °C for 6 days. The 6-day-cultured broth was extracted with 10 ml of ethanol by shaking (200 rpm) for 30 min, mixed well and centrifuged (3,000 rpm) for 5 min.

Supernatant (50% ethanol extract) was examined antimicrobial assay against microorganisms, *Kocuria rhizophila* ATCC 9341, *Bacillus subtilis* ATCC 6633, *Escherichia coli* NIHJ KB213, *Xanthomonas campestris* pv. *oryzae* KB88, *Candida albicans* KF1 and *Mucor racemosus* IFO 4581 by a paper disc diffusion assay (Lorain, 1991).

RESULTS AND DISCUSSION

Characterization of the isolates

Twenty-six strains were isolated from thirteen soil samples collected around Angthong Islands National Park, Surat Thani province, the southern part of Thailand. On the basis of morphological, cultural, physiological and biochemical characteristics including the 16S rRNA gene sequence analysis, they were belonged to the genus *Streptomyces* (Kämpfer, 2012). The phylogenetic tree (Figure 1) based on 16S rRNA gene sequences similarity showed that they were located within the lineage of the genus *Streptomyces*. The isolates showed good growth and formed extensively branched substrate mycelia and spores on ISP 2 and ISP 3 agar plates. Their morphological on ISP 2, ISP 3, ISP 4 or YS (Yeast extract-starch) agar plates are shown in Figure 2.

The cultural characteristics such as upper colony, aerial mass color, reversed colonial appearance and soluble pigment are described in Tables 1 and 2. All isolates grew at pH 5-11 and they hydrolysed starch, but did not hydrolyse gelatin. Most of them reduced nitrate to nitrite and some showed peptonization/ coagulation. Some grew on 4-10% NaCl. The utilization of carbon sources was variable (Table 3). The details of their phenotypic characteristics are listed in Tables 1, 2 and 3.

Group 1 contained 5 isolates (Table 4). The calculated pair-wise sequence similarities of isolates KC-054, KC-066, KC-074, KC-075 and KC-076 were 100% to *S. tendae* ATCC 19812^{T} (Figure 1). They were identified as *S. tendae* (Syed *et al*., 2007).

Group 2 contained 2 isolates, KC-060 and KC-088 (Table 4). The calculated pair-wise sequence similarities of isolates were 99.8 and 99.9%, respectively to *S. malachitospinus* NBRC 101004^{T}. They were identified as *S. malachitospinus* (Kalakoutskii *et al.,* 1990).

Group 3 contained 2 isolates, KC-073 and KC-155 (Table 4). The calculated pair-wise sequence similarity of them was 99.4% to *S. marokkonensis* Ap1^{T}. These strains were identified as *S. marokkonensis* (Bouizgarne *et al*., 2009).

Group 4 contained 2 isolates, KC-055 and KC-090 (Table 4). The calculated pair-wise sequence similarities of KC-055 and KC-090 were 100% to *S. parvulus* NBRC 13193^T. They were identified as *S. parvulus* NBRC 13193^T (Reddy *et al*., 2011).

Group 5 contained 1 isolate, KC-072 (Table 4). The calculated pair-wise sequence similarity of KC-072 was 99.3% to *S. fragilis* NRRL 2424^T. This strain was identified as *S. fragilis* (Santhanam *et al.*, 2013).

Group 6 contained 1 isolate, KC-070 (Table 4). The calculated pair-wise sequence similarity of KC-070 was 99.7% to *S. diastaticus* subsp. *ardesiacus* NRRL B-1773^T. This strain was identified as *S. diastaticus* subsp. *ardesiacus* (Arumugam *et al.*, 2011).

Group 7 contained 1 isolate, KC-085 (Table 4). The calculated pair-wise sequence similarity of KC-085 was 100% to *S. drozdowiczii* NBRC 101007^T (Figure 1). This strain was identified as *S. drozdowiczii* (Semedo *et al*., 2004).

Group 8 contained 1 isolate, KC-156 (Table 4). The calculated pair-wise sequence similarity of the isolate was 98.4% to *S. olivochromogenes* NBRC 3178^T (98.4). It was identified as *S. olivochromogenes* (Sripreechasak *et al*., 2013a).

Group 9 contained 2 isolates, KC-141 and KC-142 (Table 4). The calculated pair-wise sequence similarities of them was 99.5 % and 99.6% to *S. aureus* NBRC 100912^T. These strains were identified as *S. aureus* (Manfio *et al*., 2003).

Group 10 contained 1 isolate, KC-087 (Table 4). The calculated pair-wise sequence similarity of KC-087 was 99.8% to *S. iranensis* HM 35^T. This strain was identified as *S. iranensis* (Hamedi *et al*., 2010).

Group 11 contained 1 isolate, KC-058 (Table 4). The calculated pair-wise sequence similarity of KC-058 was 99.3% to *S. rapamycinicus* NRRL B-5491^T. This strain was identified as *S. rapamycinicus* (Kumar and Goodfellow, 2008).

Group 12 contained 1 isolate, KC-079 (Table 4). The calculated pair-wise sequence similarity of KC-079 was 99.4% to *S. yatensis* NBRC 101000^T. This strain was identified as *S. yatensis* (Saintpierre *et al*., 2003).

Group 13 contained 4 isolates, KC-080, KC-093, KC-094 and KC-151 (Table 4). The calculated pair-wise sequence similarities of KC-080, KC-093, KC-094 and KC-151 were 99.9-100% to *S. samsunensis* M1463^T. These strains were identified as *S. samsunensis* (Sazak *et al*., 2011).

Group 14 contained 2 isolates, KC-062 and KC-063 (Table 4). The calculated pair-wise sequence similarities of KC-062 and KC-063 were 99.0-99.4% to *S. spiralis* NBRC 14215^T. These strains were identified as *S. spiralis* (Kämpfer, 2012).

Antimicrobial activities of the isolates

On the antimicrobial activitiy screening, based on the cultivation in 51 medium and 53 medium (Table 4), Group 1 isolates, KC-066 and KC-075 identified as *S. tendae* and Group 3 isolates, KC-073 and KC-155 identified as *S. marokkonensis* showed antibacterial activity against *B. subtilis* and *K. rhizophila* strain when fermented in both media. Group 2 isolate, KC-060 identified as *S. malachitospinus* and Group 14 isolates, KC-062 and KC-063 identified as *S. spiralis* could inhibit *B. subtilis*, *K. rhizophila* or *X. campestris* strain when fermented in 53 medium. Group 4 isolates, KC-055 and KC-090 exhibited against *B. subtilis*, *K. rhizophila* and *X. campestris* strain when fermented in both media.

The isolates in Group 5, 6 and 7 showed no antimicrobial activity on the tested strains. Group 8 isolate, KC-156 identified as *S. olivochromogenes* showed antibacterial activity against *B. subtilis* and *K. rhizophila* strain when fermented in both media and it inhibited *E. coli* when fermented in 53 medium. Group 9 isolate, KC-141 identified as *S. aureus* showed antibacterial activity against *B. subtilis* and *K. rhizophila* strain when fermented in 51 medium.

Group 10 isolate, KC-087 identified as *S. iranensis* showed antibacterial activity against *B. subtilis*, *K. rhizophila*, *C. albicans* and *M. racemosus* strain when fermented in 53 medium. Group 11, Group 12 and Group 13 isolates showed antibacterial activity against *B. subtilis*, *K. rhizophila*, *C. albicans* and *M. racemosus* or *X. campestris* strain when fermented in both media.
This study, *S. tendae*, *S. malachitospinus*, *S. marokkonensis*, *S. parvulus*, *S. fragilis*, *S. diastaticus*, *S. drozdowiczii*, *S. olivochromogenes*, *S. aureus*, *S. iranensis*, *S. rapamycinicus*, *S. yatensis*, *S. samsunensis*, and *S. spiralis* were distributed in soil samples collected around Angthong Islands National Park, Surat Thani province, the southern Thailand.

The *S. tendae* strains have been reported to produce nikkomycin (Roos *et al.*, 1992); cyclohexenylglycine, an isoleucine antagonist with antibacterial activity; the naphthoquinone compound juglomycin, which had antitumor activity and chlorothricin; a glycosylated macrolide antibiotic that acted as an antagonist of acetyl-coenzyme A in bacteria and antifungal, chitin-binding protein (Bormann *et al*., 1999). *S. parvulus* strain produced polypeptide antibiotic and showed antibacterial activity (Shetty *et al*., 2014).

The strain of *S. fragilis* produced azaserine and 6-diazo-5-oxo-L-norleucine (DON) (Pittillo and Hunt, 1967) while the strains of *S. diastaticus* have been reported to produce two polyene macrolide antibiotics, rimocidin and CE-108 (Seco *et al*., 2005) and antifungus, oligomycins A and C (Yang *et al.*, 2010). *S. olivochromogenes* produced 4-hydroxy-3-methoxycinnamic (ferulic) acid esterase (Faulds and Williamson, 1991) whereas the strain *S. rapamycinicus* strain NRRL 5491 was reported to produce the important drug rapamycin (Baranasic *et al.*, 2013).

In southern Thailand, actinobacteria isolated from soils collected in Krung Ching Waterfall National Park, Nakhon Si Thammarat were reported to be *Streptomyces exfoliates*, *S. vinaceusdrappus*; *S. tendae*, *S. aureus*, *S. atriruber*, *S. olivochromogenes*, *S. malaysiensis*, *S. purpeofuscus*, *S. sparsogenes*, *S. aldersoniae*, *S. rapamycinicus*, and *S. youssoufiensis*; *Nocardia niigatensis*; *Amycolatopsis rifamycinica*; *Kitasatospora saccharophila*; *Rhodococcus triatomae*; and *Gordonia alkanivorans* (Sripreechasak *et al.*, 2013b).

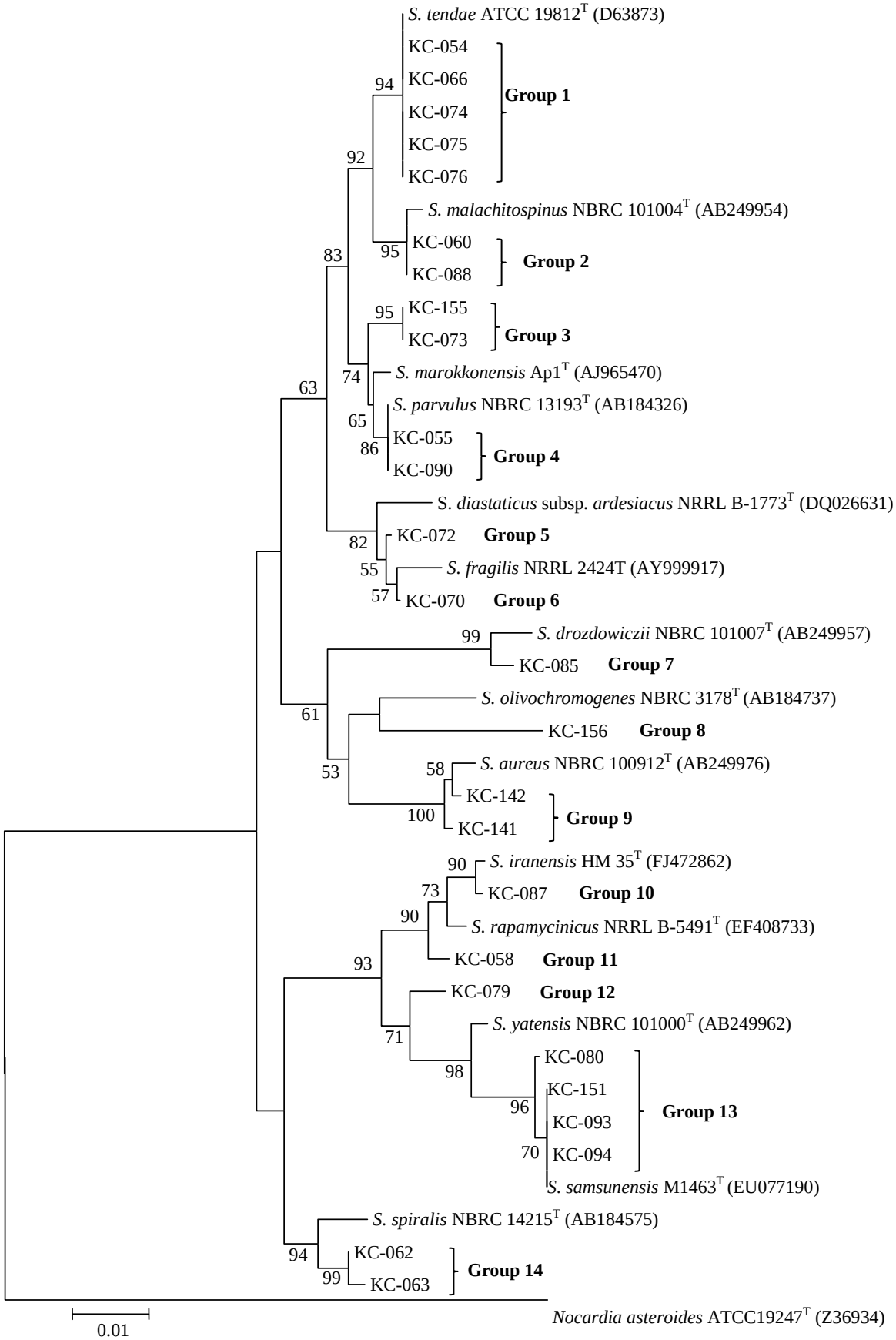
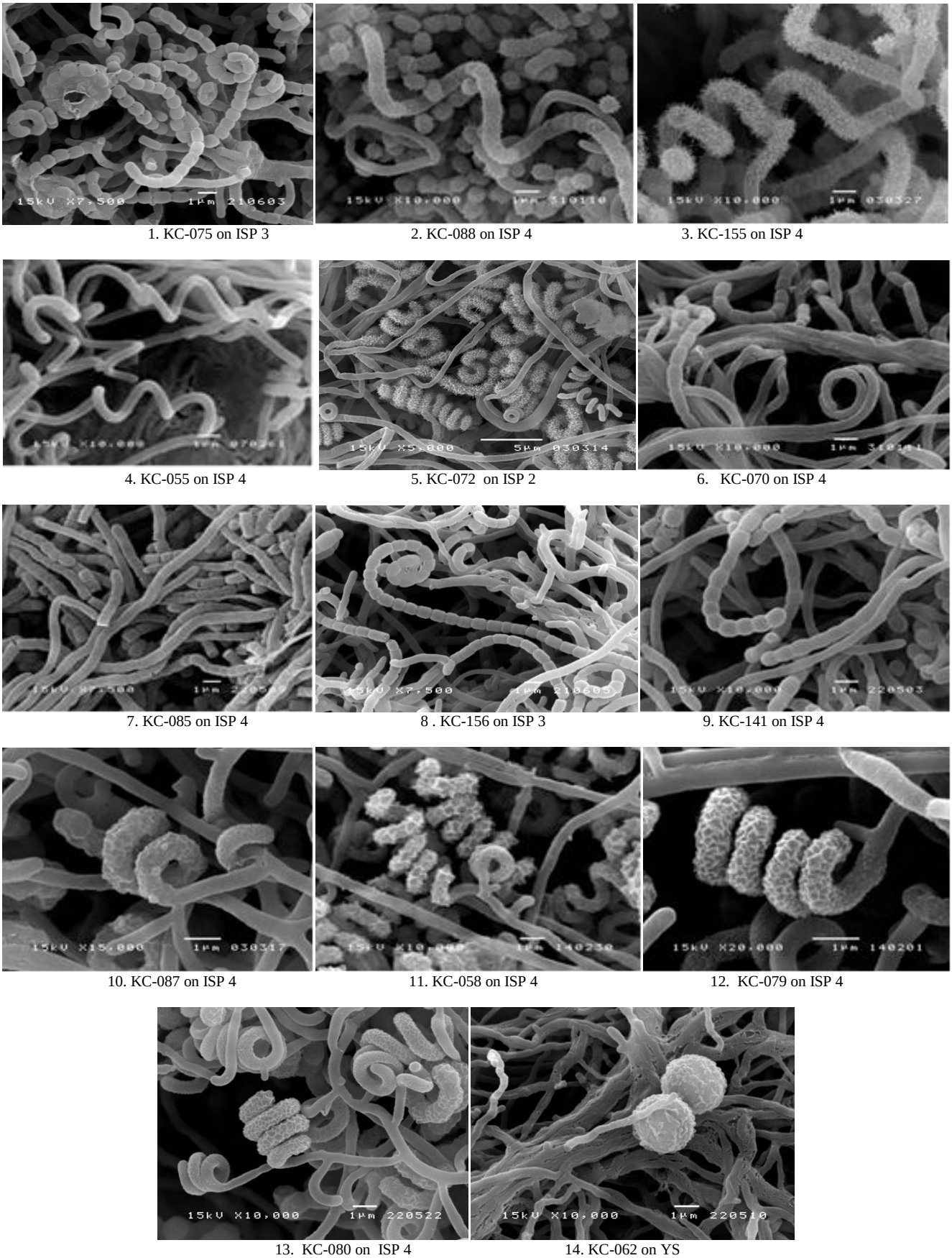


Fig. 1: Neighbor-joining tree based on 16S rRNA gene sequences showing relationship between 26 isolates and closely related type strains of the genus *Streptomyces*. Only bootstrap values above 50% (percentages of 1000 replications) are indicated. Bar, 0.01 nucleotide substitutions per site.



1. KC-075 on ISP 3 2. KC-088 on ISP 4 3. KC-155 on ISP 4

4. KC-055 on ISP 4 5. KC-072 on ISP 2 6. KC-070 on ISP 4

7. KC-085 on ISP 4 8 . KC-156 on ISP 3 9. KC-141 on ISP 4

10. KC-087 on ISP 4 11. KC-058 on ISP 4 12. KC-079 on ISP 4

13. KC-080 on ISP 4 14. KC-062 on YS

Fig. 2: Scanning electron micrograph of isolates in Group 1 to Group 14, grown on ISP 2, ISP3, ISP4 or YS agar plates at 30 ˚C for 2 weeks.

Table 1: Cultural characteristics of *Streptomyces* isolates in Group 1 to Group 14 on ISP 2 agar .

Group	Growth	Upper Colony	Aerial mass color	Reverse colony	Soluble pigment
1	Good	Pale greenish yellow – light greenish yellow	Abundant, white – bluish gray	Pale greenish yellow – moderate olive	None
2	Good	Pale yellow – grayish greenish yellow	None	Pale yellow – grayish greenish yellow	None
3	Good	Pale yellow	None	Pale yellow – pale yellowish green	None
4	Good	Pale yellow – grayish greenish yellow	None	Pale yellow – grayish greenish yellow	None
5	Good	Grayish greenish yellow	Abundant, white – brownish black	Light grayish olive – grayish olive	None
6	Good	Brownish pink – light reddish brown	Abundant, white – medium gray	Dark grayish yellow – grayish reddish brown	None
7	Good	Colorless	Abundant, bluish gray	Light grayish olive – brownish black	Dark grayish yellow
8	Good	Strong brown	Abundant, light gray	Light brown – dark brown	Light brown
9	Good	Pale yellow	None	Pale yellow	None
10	Good	Pale yellow	Abundant, white – bluish gray	Dark yellow	Grayish yellow
11	Good	Dark grayish yellow	Abundant, greenish white – greenish black	Light grayish olive – grayish olive	None
12	Good	Colorless – pale yellow	Abundant, greenish white – grayish olive	Light yellow – dark olive brown	None
13	Good	Colorless – light olive gray	Abundant, greenish white – medium gray	Light yellow – light olive gray	None – deep yellow
14	Good	Strong greenish yellow	Abundant, white	Strong greenish yellow	None

Table 2: Cultural characteristics of *Streptomyces* isolates in Group 1 to Group 14 on ISP 3 agar.

Group	Growth	Upper colony	Aerial mass color	Reverse colony	Soluble pigment
1	Good	Pale greenish yellow – grayish greenish yellow	Abundant, white – bluish gray	Pale greenish yellow – moderate yellow	None
2	Good	Pale yellowish green – grayish yellow	Abundant, white – light greenish gray	Pale yellowish green – light grayish yellowish brown	None – pale orange yellow
3	Good	Yellowish white –pale orange yellow	Moderate, greenish white	Yellowish white –pale orange yellow	None
4	Good	Pale yellowish green – strong greenish yellow	Abundant, greenish white – light greenish gray	Pale yellowish green – moderate olive	Pale yellowish green – light greenish yellow
5	Good	Grayish yellow – dark grayish yellow	Abundant, light greenish gray – greenish gray	Dark grayish yellow – dark olive brown	Grayish yellow
6	Good	Moderate reddish brown	Abundant, white – purplish gray	Very dark red – light grayish red	None
7	Good	Moderate olive brown	Abundant, bluish gray	Dark olive brown	Grayish yellow
8	Good	Grayish greenish yellow	Abundant, light gray	Dark grayish yellow	None
9	Good	Moderate brown	Abundant, light gray	Deep brown – dark brown	Strong brown
10	Good	Pale yellow	Abundant, white – medium gray	Moderate yellow	Grayish yellow
11	Good	Grayish greenish yellow	Abundant, greenish white – greenish black	Grayish olive green – dark grayish olive green	None
12	Good	Pale yellowish green – light grayish olive	Abundant, white – greenish black	Light grayish olive – olive gray	None
13	Good	Colorless – olive gray	Abundant, greenish white – light greenish gray	Light yellowish green – olive gray	None – dark yellow
14	Good	Moderate greenish yellow – deep yellowish pink	Abundant, white	Pale greenish yellow – deep pink	None

Table 3: Differential characteristics of *Streptomyces* isolates in Group 1 to Group 14.

Characteristics	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Nitrate reduction	+	+	– (+1)	–	–	+	+	+	+(–1)	+	–	–	+	–
Peptonization/Coagulation	–/–	–/–	+ (–1)	–/+	–/–	–/–	+/–	–/–	– (+)	w/–	–/–	+/–	+/–	–
NaCl tolerance (%)	7-9	8	4-5	8	10	10	8	8	7	7	4	4	8	9
Growth at pH	5–12	4–12	5–11	5–11	5–12	5–12	5–12	5–12	5–12	5–12	5–12	5–12	5–12	5–12
Utilization of:														
L-Arabinose	+	+	+	+	+	+	+	+	+ (–1)	w	–	–	+ (–1)	w
D-Fructose	+ (w1)	–	–	+	+	+	–	+	+ (w1)	+	–	w	+	+
D-Glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+
myo-Inositol	+	+	+ (w1)	+	+	w	–	+	+	+	+	+	– (+1)	+
D-Mannitol	+	+	+	+	+	+	–	+	+	+	+	+	+	+
Melezitose	–	–	–	–	–	–	–	–	–	–	–	–	–	–
D-Melibiose	–	+	– (w1)	–	+	+	+	–	– (+1)	+	+	+	+ (–1)	w
Raffinose	–	+	w	w	+	w	+	–	– (w1)	w	+	w	+ (–2)	w
L-Rhamnose	+	+	+	+	–	+	+	+	+	+	–	+	+ (–1)	w
D-Sorbitol	–	–	–	–	–	–	–	–	–	–	+	–	–	–
Sucrose	–	w	–	w	+	–	–	–	–	w	–	–	–	–
D-Xylose	+ (w1)	+	–	+	+	w	+	+	+ (–1)	+	–	–	+	w

+, positive; -, negative; w, weakly positive. Number in parentheses indicates the number of isolate shows positive, weakly positive or negative reaction.

Table 4: Antimicrobial activity of *Streptomyces* isolates in Group 1 to Group 14.

Group	Isolates	Nearest relatives (% identity)	Inhibition zone (mm)	
			51 production medium	53 production medium
1	KC-054	*S. tendae* ATCC 19812^T (100)	-	-
	KC-066	*S. tendae* ATCC 19812^T (100)	B(14), K(14)	B(9)
	KC-074	*S. tendae* ATCC 19812^T (100)	-	-
	KC-075	*S. tendae* ATCC 19812^T (100)	B(13), K(13)	B(10), K(10)
	KC-076	*S. tendae* ATCC 19812^T (100)	-	-
2	KC-060	*S. malachitospinus* NBRC 101004^T (99.8)	-	B(10), X(11)
	KC-088	*S. malachitospinus* NBRC 101004^T (99.9)	-	-
3	KC-073	*S. marokkonensis* Ap1^T (99.4)	B(13), K(9)	B(11), K(11)
	KC-155	*S. marokkonensis* Ap1^T (99.5)	B(15), K(14)	K(9)
4	KC-055	*S. parvulus* NBRC 13193^T (100)	B(17), K(22), X(13)	B(18), K(27), X(15)
	KC-090	*S. parvulus* NBRC 13193^T (100)	B(25), K(27), X(22)	B(20), K(18), X(11)
5	KC-072	*S. fragilis* NRRL 2424^T (99.3)	-	-
6	KC-070	*S. diastaticus* subsp. *ardesiacus* NRRL B-1773^T (99.7)	-	-
7	KC-085	*S. drozdowiczii* NBRC 101007^T (99.4)	-	-
8	KC-156	*S. olivochromogenes* NBRC 3178^T (98.4)	B(11), K(11)	B(11), K(13), E(12)
9	KC-141	*S. aureus* NBRC 100912^T (99.5)	B(13), K(13)	-
	KC-142	*S. aureus* NBRC 100912^T (99.6)	-	-
10	KC-087	*S. iranensis* HM 35^T (99.8)	-	B(12), K(15), Ca(12), Mu(14)
11	KC-058	*S. rapamycinicus* NRRL B-5491^T (99.3)	B(24), K(27), X(17), Ca(17), Mu(21)	B(20), K(22), X(14), Ca(16), Mu(22)
12	KC-079	*S. yatensis* NBRC 101000^T (99.4)	B(17), K(11), X(15), Ca(16), Mu(19)	B(12), K(15), Ca(12), Mu(14)
13	KC-080	*S. samsunensis* M1463^T (99.9)	B(14), K(12), Ca(9), Mu(10)	B(11), K(10), Ca(15), Mu(11)
	KC-093	*S. samsunensis* M1463^T (100)	B(15), K(12), Ca(13), Mu(19)	B(10), K(10), Ca(13), Mu(18)
	KC-094	*S. samsunensis* M1463^T (100)	B(18), K(15), Ca(15), Mu(18)	B(10), K(11), Ca(15), Mu(15)
	KC-151	*S. samsunensis* M1463^T (100)	B(10), Ca(11), Mu(11)	-
14	KC-062	*S. spiralis* NBRC 14215^T (99.4)	-	B(10), X(11)
	KC-063	*S. spiralis* NBRC 14215^T (99.0)	-	B(10), K(14)

B, *Bacillus subtilis;* K, *Kocuria rhizophila* ; E, *Escherichia coli;* X, *Xanthomonas campestris* pv. *oryzae;* Ca, *Candida albicans;* Mu, *Mucor racemosus*

CONCLUSION

In conclusion, twenty-six strains of *Streptomyces* were isolated from thirteen soil samples collected around Angthong Islands National Park, Surat Thani province, the southern part of Thailand. They were identified as *S. tendae*, *S. malachitospinus*, *S. marokkonensis*, *S. parvulus*, *S. fragilis*, *S. diastaticus*, *S. drozdowiczii*, *S. olivochromogenes*, *S. aureus*, *S. iranensis*, *S. rapamycinicus*, *S. yatensis*, *S. samsunensis*, and *S. spiralis* based on their morphological, cultural, physiological and biochemical characteristics including the 16S rRNA gene sequence analysis. They exhibited antimicrobial activity against *B. subtilis ATCC 6633, K. rhizophila ATCC 9341, M. racemosus IFO 4581, C. albicans KF1, E. coli NIHJ KB213 and X. campestris pv. oryzae KB88.* Our strains in *S. malachitospinus*, *S. marokkonensis*, *S. drozdowiczii*,, *S. aureus*, *S. iranensis*, *S. yatensis*, *S. samsunensis*, and *S. spiralis* are required for further study on their bioactive compounds.

ACKNOWLEDGEMENTS

This study was supported by Chulalongkorn University Dutsadi Phiphat Scholarship (2008) for a Ph.D. Program to P. S. We thank Prof. Dr. Yoko Takahashi, Dr. Atsuko Matsumoto, Dr.Yuki Inahashi and the staffs of Kitasato Institute for Life Sciences, Kitasato University, Tokyo, Japan for supporting the screening and sequencing of the isolates.

REFERENCES

Arumugam M, Mitra A, Pramanik A, Saha M, Gachhui R, Mukherjee J. *Streptomyces sundarbansensis* sp. nov., an actinomycete that produces 2-allyloxyphenol, Int J Syst Evol Microbiol, 2011; 61: 2664-69.

Baranasic D, Gacesa R, Starcevic A, Zucko J, Blažič M, Horvat M, Gjuračić K, Fujs Š, Hranueli D, Kosec G, Cullum J, Petković H. Draft genome sequence of *Streptomyces rapamycinicus* strain NRRL 5491, the producer of the immunosuppressant rapamycin. Genome Announc, 2013; 1(4): e00581-13.

Berdy J. Bioactive microbial metabolites: Review article. J Antibiot, 2005; 58:1-26.

Bormann C, Baier D, Hörr I, Raps C, Berger J, Jung G, Schwarz H. Characterization of a Novel, Antifungal, chitin-binding protein from *Streptomyces tendae* Tü901 that interferes with growth polarity. J Bacteriol, 1999; 181(24) 7421-7429.

Bouizgarne B, Lanoot B, Loqman S, Sproer C, Klenk HP, Swings J, Ouhdouch Y. *Streptomyces marokkonensis* sp. nov., isolated from rhizosphere soil of Argania spinosa L. Int J Syst Evol Microbiol, 2009; 59:2857-2863.

Faulds CB, Williamson G. The purification and characterization of 4-hydroxy-3-methoxycinnamic (ferulic) acid esterase from *Streptomyces olivochromogenes*. J Gen Microbiol, 1991; 137(10): 2339-2345.

Glasby JS. 1993. *Encyclopedia of Antibiotics*. 3rd ed. John Wiley and Sons, Chichester, p. 1-507.

Gouy M, Gascuel S, Gascuel O. SeaView version 4.2: a multiplateform graphical user interface for sequence alignment and phylogenetic tree building. Mol Biol Evol, 2010; 27:221-224.

Goodfellow M, Williams ST, Mordarski M. 1988. Actinomycetes in Biotechnology, London: Academic Press Inc, p. 1-88.

Hamedi J, Mohammadipanah F, Klenk HP, Pőtter G, Schumann P, Sprőer C, Vonjan M, Kroppenstedt RM. *Streptomyces iranensis* sp. nov., isolated from soil. Int J Syst Evol Microbiol, 2010; 60:1504-1509.

Kämpfer P . 2012. Genus *Streptomyces*: The Actinobacteria In: Goodfellow M, Kämpfer P, Busse H-J, Trujillo, ME, Suzuki K, Ludwig W, Whitman, WB, eds. Bergey's Manual of Systematic Bacteriology, 2nd Edition, Part B, New York: Springer, p.1455-1767.

Kalakoutskii LV, Zenova GM, Soina VS, Likhacheva AA. Associations of actinomycetes with algae. Actinomycetes, 1990; 1: 27-42.

Kim OS, Cho YJ, Lee K, Yoon SH, Kim M, Na H, Park SC, Jeon YS, Lee JH, Yi H, Won S, Chun J. Introducing EzTaxon-e: a prokaryotic 16S rRNA gene sequence database with phylotypes that represent uncultured species. Int J Syst Evol Microbiol, 2012; 62: 716-721.

Kimura MA simple method for estimating evolutionary rates of base substitutions through comparative studies for nucleotide sequences. J Mol Evol, 1980; 16:111-120.

Kumar Y, Goodfellow M. Five new members of the *Streptomyces violaceusniger* 16S rRNA gene clade: *Streptomyces castelarensis* sp. nov., comb. nov., *Streptomyces himastatinicus* sp. nov., *Streptomyces mordarskii* sp. nov., *Streptomyces rapamycinicus* sp. nov. and *Streptomyces ruanii* sp. nov. Int J Syst Evol Microbiol, 2008; 58:1369-1378.

Lorain V. (1991). Antibiotics in Laboratory Medicine, Baltimore: The Williams & Wilkins Co., p. 1-51**.**

Manfio GP, Atalan E, Zakrzewska CJ, Mordarski M, Rodriguez C, Collins MD, Goodfellow M. Classification of novel soil streptomycetes as *Streptomyces aureus* sp. nov., *Streptomyces laceyi* sp. nov. and *Streptomyces sanglieri* sp. nov. Ant Van Leeuwenhoek, 2003; 85:245-55.

Matsumoto A, Takahashi Y, Iwai Y, Omura S. Isolation of Gram-positive bacteria with high G+C from inside soil aggregates. *Actinomycetologica*, 2006; 20:30-40.

Mundie DA. (1995). The NBS/ISCC Color System / David A. Mundie Pittsburgh, PA: Polymath Systems 535.6 dc-20 Available at: http://www.dodomagnifico.com/Colors/NBS.html. Accessed June 5, 2011.

Pittillo RF, Hunt DE. Azaserine and 6-diazo-*5*-oxo-L-norleucine (DON). J Antibiot, 1967; 1: 481-493.

Reddy TV, Mahmood S, Paris L, Reddy YH, Wellington EM, Idris MM. *Streptomyces hyderabadensis* sp. nov., an actinomycete isolated from soil. Int J Syst Evol Microbiol, 2011; 61:76-80.

Roos U, Mattern S, Schrempf H, Bormann C. Histidine aminotransferase activity in *Streptomyces tendae* and its correlation with nikkomycin production. FEMS Microbiol Lett, 1992; 76: 185-190.

Saintpierre D, Amir H, Pineau R, Sembiring L, Goodfellow M. *Streptomyces yatensis* sp. nov., a novel bioactive streptomycete isolated from a new-caledonian ultramafic soil. Antonie Van Leeuwenhoek, 2003; 83(1):21-26.

Saito N, Nei M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol, 1987; 4:406-425.

Seco EM, Cuesta T, Fotso S, Laatsch H, Malpartida F. Two polyene amides produced by genetically modified *Streptomyces diastaticus* var. 108, Chem Biol, 2005; 12 (5): 535-543.

Semedo LT, Gomes RC, Linhares AA, Duarte GF, Nascimento RP, Rosado AS, Margis PM, Margis R, Silva KR, Alviano CS, Manfio GP, Soares RM, Linhares LF, Coelho RR. *Streptomyces drozdowiczii* sp. nov., a novel cellulolytic streptomycete from soil in Brazil. Int J Syst Evol Microbiol, 2004; 54:1323-1328.

Santhanam R, Rong X, Huang Y, Andrews BA, Asenjo JA, Goodfellow M. *Streptomyces bullii* sp. nov., isolated from a hyper-arid Atacama Desert soil. Antonie Van Leeuwenhoek, 2013; 103(2):367-373.

Sazak A, Sahin N, Guven K, Isik K, Goodfellow M. *Streptomyces samsunensis* sp. nov., a member of the *Streptomyces violaceusniger* clade isolated from the rhizosphere of *Robinia pseudoacacia*. Int J Syst Evol Microbiol, 2011; 61:1309-1314.

Seong CN, Choi JH, Baik KS. An improved selective isolation of rare actinomycetes from forest soil. J Microbiol, 2001; 39:17-23.

Shetty PR, Buddana SK, Tatipamula VB, Naga YVV, Ahmad J. Production of polypeptide antibiotic from *Streptomyces parvulus* and its antibacterial activity**.** Braz J Microbiol, 2014; 45(1): 303-312.

Sripreechasak P, Matsumoto A, Suwanborirux K, Inahashi Y, Shiomi K, Tanasupawat S, Takahashi Y. *Streptomyces siamensis* sp. nov., and *Streptomyces similanensis* sp. nov., isolated from Thai soils. J Antibiot, 2013a, 66(11):633-640.

Sripreechasak P, Suwanborirux K, Inahashi Y, Matsumoto A, Tanasupawat S, Takahashi Y. Identification and antimicrobial activity of actinobacteria from soils in southern Thailand. Trop Biomed, 2013b; 30(1): 46-55.

Shirling EB, Gottlieb D. Methods for characterization of *Streptomyces* species. Int J Syst Bacteriol, 1966; 16:313-340.

Syed DG, Agasar D, Kim CJ, Li WJ, Lee JC, Park DJ, Xu LH, Tian XP, Jiang CL. *Streptomyces tritolerans* sp. nov., a novel actinomycete isolated from soil in Karnataka, India. Antonie Van Leeuwenhoek, 2007; 92(4):391-397.

Takahashi Y, Matsumoto A, Seino A, Ueno J, Iwai Y, Omura S. *Streptomyces avermectinius* sp. nov., an avermectin-producing strain. Int J Syst Evol Microbiol, 2002; 52:2163-2168.

Tajima K, Takahashi Y, Seino A, Iwai Y, Omura S. Description of two novel species of the genus *Kitasatospora*, *Kitasatospora cineracea* sp. nov. and *Kitasatospora niigatensis* sp. nov. Int J Syst Evol Microbiol, 2001; 51: 1765-1771.

Yang PW, Li MG, Zhao JY, Zhu MZ, Shang H, Li JR, Cui XL, Huang R, Wen ML. Oligomycins A and C, major secondary metabolites isolated from the newly isolated strain *Streptomyces diastaticus*. Folia Microbiol, 2010; 55(1):10-16.

How to cite this article:

Paranee Sripreechasak, Khanit Suwanborirux and Somboon Tanasupawat. Characterization and antimicrobial activity of *Streptomyces* strains from soils in southern Thailand. J App Pharm Sci, 2014; 4 (10): 024-031.