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Identification and antimicrobial activity of *Streptomyces* and *Actinoplanes* strains from lichens

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ABSTRACT

Twelve actinomycete strains were isolated from lichens collected in Thailand. On the basis of their phenotypic and chemotaxonomic characteristics including 16S rRNA gene sequence analysis, they were divided into 2 genera, *Streptomyces* (Group 1, 9 isolates) and *Actinoplanes* (Group 2, 3 isolates). Isolates LDG1-03 and LDG1-15; LDG1-05 and LDG2-02; LDG1-08; LDG1-16; LDG2-09; LLG1-01; and LLG1-03 were closely related to *S. malaysiensis* NBRC 16446^T (98.95-99.85%); *S. lomondensis* NBRC^T (99.77-99.78%); *S. graminearus* NBRC 15420^T (98.72 %); *S. parvulus* NBRC 13193^T (100%); *S. cinereoruber* subsp. *cinereoruber* NBRC 12756^T(99.11%); *S. seoulensis* NBRC 16668^T (100%); and *S. cinerochromogenes* NBRC 13822 ^{T} (99.17%) based on 16S rRNA gene sequence analysis, respectively. The isolates LDG1-06, LDG1-22A and LDG1-22B are closely related to *A. deccanensis* IFO 13994^T and *A. nipponensis* FH 2241 ^{T} (97.97-98.93 %), respectively. The isolates in Group 1 contained LL-diaminopimelic acid in cell wall and had MK-9(H_6), MK-9(H_8) and MK-9(H_4) as the major menaquinones. The isolates in Group 2 contained *meso*-diaminopimelic acid and had MK-9(H_6) and MK-9(H_4) as the major menaquinones. Four *Streptomyces* isolates exhibited antimicrobial activity against *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633, *Kocuria rhizophila* ATCC 9341 and *Candida albicans* ATCC 27853 while *Actinoplanes* isolate LDG1-06 exhibited against *C. albicans* ATCC 27853.

INTRODUCTION

Lichens are the symbiotic partnership between fungi and green algae or cyanobacteria with a wide variety of morphologies that are different from their parent (Jovan,2008; González *et al.*,2005; Boonprakob,2014). They live and grow on extremophilic condition such as extremely hot or cold and high ultraviolet radiation (Boonprakob,2014). Lichens as pioneers of new terrestrial habitat that cause to change physical of the habitat and develop the habitat by producing more organic compounds to soil. This changing is let some organisms (animals and plants) to effect the habitat and change it to ecosystem. (González *et al.*, 2005; Boonprakob, 2014). Several lichens can be found on stones (lithic), soil, or as epiphytes on plants (González *et al.*, 2005). Some species of lichens are sensitive to climate change, so research use them as biological indicator for the detection of air pollution and natural resource management (Jovan, 2008). Actinomycetes are the most widely group of bacteria that well known as antibiotic producer and other bioactive compounds which primarily isolated from soils (Inderiati *et al.*, 2008; Kumar *et al.*, 2010).

Interestingly, some actinomycetes are distributed in lichens and can be divided into 7 families; *Micromonosporaceae* (*Actinoplanes*, *Micromonospora*), *Streptomycetaceae* (*Streptomyces*), *Pseudonocardiaceae* (*Actinomycetospora*, *Amycolatopsis*, *Pseudonocardia*, *Saccharopolyspora*), *Nocardiaceae* (*Rhodococcus*), *Streptosporangiaceae* (*Planobispora*, *Streptosporangium*), *Thermomonosporaceae* (*Actinomadura*) and *Geodermatophilaceae* (*Geodermatophilus*) (González *et al.*, 2005; Yamamura *et al.*, 2011). *Amycolatopsis* strain is one of actinomycetes that found in lichens. *A. mediterranei* strains produce rifamycin as an important antibiotic that has antimicrobial activity against *Mycobacterium tuberculosis* and the infection of HIV in patients (González *et al.*, 2005; Mejía *et al.*, 1998).

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In this investigation, actinomycete strains isolated from lichens in Thailand were screened for the antimicrobial activity and identified based on the phenotypic and chemotaxonomic characteristics including 16S rRNA gene sequence analysis.

MATERIALS AND METHODS

Isolation and identification of isolates

Actinomycete strains were isolated from lichens collected from Mahasarakham province, Thailand (16°19′22″N 103°17′48″E) (Table 1) using an air dry and heat treatment method (at 100℃ for 1 h, Nonomura and Ohara,1971). Serial dilutions of the samples were prepared by the 10-fold dilution method, 10-1, 10-2 and 10-3 dilutions of the suspension. The 0.1 ml of aliquot was spreaded on surface of AV agar paltes (Nonomura and Ohara, 1969) and HV agar plates (Hayakawa and Nonomura, 1987) supplemented with nalidixic acid (25 mg/l) cycloheximide (50 mg/l).

The agar plates were incubated at 30 ◦C for 14-21 days and a single colony was transferred and purified on yeast extract–malt extract agar (International *Streptomyces* Project , ISP2 medium) as described by Shirling and Gottlieb (1996). The purified isolates were cultivated at 30 °C for 14 days on ISP2 agar plates and their cultural characteristics were observed. The colour of upper colony, reverse colony and soluble pigments were determined using the NBS/IBCC colour chart (Mundie, 1995). Morphological observation was done by using a light microscope and scanning electron microscope (JSM-5410LV, Japan) of the cultures grown on ISP 2 or ISP 4 agar plates at 30 ^{o}C for 7, 14 or 21 days.

The phenotypic characteristics were determined as described by Shirling and Gottlieb (1966) and Arai (1975). Cell wall diaminopimelic acid (DAP) isomers was determined as described by Kutzner (1981). Menaquinone system was analysed as previous report (Komagata and Suzuki, 1987).

16S rRNA gene sequence and phylogenetic analyses

DNA of the strains was isolated from cells grown in ISP2 medium and purified as described by Saito and Miura (1963). The complete 16S rRNA gene was amplified by PCR using primers, 8-27f and 1492r. The amplified 16S rRNA gene was used as templates for sequencing with Big Dye Terminator sequencing Kit (Perkin Elmer) and analyzed by AB1377 automated DNA sequencer (Perkin Elmer).

The sequencing reaction for each sample was performed in DNA Thermal Cycler (Gene Amp PCR System 2400; Perkin Elmer) by using primers, 8-27f (5'-AGAGTTTGATC (A/C)TGGCTCAG-3'), 530f (5'-GTGCCAGC(A/C)GCCGCGG-3') and 1114f (5'GCAACGAGCGCAACCC-3'). Homology search was performed using the standard BLAST sequence similarity searching from the web server, http://www.ezbiocloud.net/eztaxon/identify against previously reported sequence at the GenBank/EMBL/DDBJ database. The sequence was multiply aligned with selected sequences obtained from Genbank/EMBL/DDBJ by using the CLUSTAL_X (Thompson *et al*., 1997). The alignment was manually verified and adjusted prior to the construction of phylogenetic tree. The phylogenetic tree was constructed by using neighbour-joining (Saitou and Nei, 1987) in the MEGA program version 6 (Tamura *et al*., 2013).

The confidence values of branches of the phylogenetic tree were determined using the bootstrap analyses based on 1000 resamplings (Felsenstien, 1985). The 16S rRNA gene sequence of *Micromonospora siamensis* TT2-4^{T} is used as an outgroup. The values for sequence similarity among the closely related strains were determined using the EzTaxon-e server (Kim *et al*., 2012).

Antimicrobial activities of 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 (180 rpm) at 30 ℃ for 3-7 days.

One percent of seed culture was transferred into 10 ml of A16 medium (0.2%glucose, 1.5% pharmamedia, 0.3% $CaCO_3$, 1% Diaion HP-20, pH 7.0), 54 medium (2% soluble starch, 0.5% glycerol, 1% defatted wheat germ, 0.3 % meat extract,0.3% yeast extract, 0.3% $CaCO_3$, tap water) and ISP2 medium (Glucose 0.4%, yeast extract 0.4%, malt extract 1%) and cultivated on shaker (200 rpm) at 30 °C for 6 days. The 6 days old culture broth was added with 10 ml of 50% ethanol and extracted by shaking (200 rpm) for 1 h, mixed well and centrifuged (3,000 rpm) for 5 min.

The supernatant was collected and examined for its antimicrobial activity against microorganisms such as *Stapylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633, *Kocuria rhizophila* ATCC 9341, *Eschrichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Cadida albicans* ATCC 10231 by disc diffusion assay (20 µl/ disc) (Lorain, 1991).

RESULTS AND DISCUSSION

Isolation and identification of isolates

The actinomycete strains LLG1-01 and LLG1-03 were isolated from lichen, *Chrysothrix* sp. and LDG1-03, LDG1-05, LDG1-08, LDG1-15, LDG1-16, LDG2-02, LDG2-09, LDG1-06 and LDG1-22A and LDG1-22B were from *Flavopamelia capelata* collected in Mahasarakham province, Thailand (Table 1). On the basis of their morphological, cultural, physiological, biochemical and chemotaxonomic characteristics including the16S rRNA gene sequence analysis, 12 isolates were identified as *Streptomyces* (9 isolates, Group 1) and were identified as *Actinoplanes* (3 isolates, Group 2) (Figure 1) as described here.

Group 1 contained 9 isolates (Table 1). The morphology of isolates LLG1-01, LLG1-03, LDG1-08 grew on ISP2 medium agar at 30 ^{o}C for 7 days while isolates LDG1-03, LDG1-05, LDG1-15, LDG2-02, LDG2-09 grew on ISP2 medium agar for 14 days were showed as mature spore chains in Figure 2. Their

colonies colour on ISP2 medium were described in Table 1. Most isolates grew at pH 5-11 and hydrolysed starch, but did not grow at 40- 45 ^{o}C.

Most of them showed peptonization reaction. Some isolates reduced nitrate to nitrite and hydrolysed gelatin. Some grew on 0-8 % NaCl. They did not utilized raffinose and the variable utilization of carbon sources was showed in Table 2. The tested isolates in this Group contained MK-9(H_6) (33.42-60.07 %), MK-9(H_8) (9.47-48.73 %), MK-9(H_4) (1.31-33.26 %) and MK-9(H_2) (7.08-49.66) as menaquinones components and had LL-diaminopimelic acid as cell wall peptidoglycan type (Tables 3).

Phylogenetic analysis of isolates LDG1-03, LDG1-05, LDG1-08, LDG1-15, LDG1-16, LDG2-02, LDG2-09, LLG1-01 and LLG1-03 revealed that they were belonged to the genus *Streptomyces* (Figure 4).

Isolates LDG1-03 and LDG1-15 were closely related to each other and showed 98.95% and 99.85% similarility to *S. malaysiensis* NBRC 16446^{T} (AI-Tai *et al*.,1999). Isolates LDG1-05 and LDG2-02 were closely related to each other and showed 99.78% and 99.77% similarility to *S. lomondensis* NBRC^{T} (Johnson and Dietz 1969). Isolate LDG1-08 was closely related to *S. graminearus* NBRC 15420^{T} (Preobrazhenskaya, 1986) (98.72 %). Isolate LDG1-16 was closely related to *S. parvulus* NBRC 13193^{T} (100%) (Reddy *et al*., 2011). Isolate LDG2-09 was closely related to *S. cinereoruber* NBRC 12756^{T} (Corbaz *et al.,* 1957) (99.11%).

Isolate LLG1-01 was closely related to *S. seoulensis* NBRC 16668^{T} (Chun *et al*., 1997) (100%). Isolate LLG1-03 was closely related to *S. cinerochromogenes* NBRC 13822 ^{T} (Miyairi *et al.,* 1966) (99.17%). The percentage of 16S rRNA gene sequence similarity of *Streptomyces* isolates compared to type strains were showed in Table 3.

Group 2 contained 3 isolates (Table 1). The morphology of isolates LDG1-06 and LDG1-22B grew on ISP2 medium agar and isolate LDG1-22A grew on ISP4 medium agar at 30 ^{o}C for 21 days were showed as young mature sporangium (Figure 3). The isolates grew at pH 5-12 but did not grow at 40- 45 ^{o}C. They grew on 0-1% NaCl and hydrolysed starch but did not reduced nitrate to nitrite.

They showed weakly on peptonization/ and showed negative coagulation. Only isolate LDG1-06 did hydrolyse gelatin. They did not utilized raffinose and the variable utilization of carbon sources was showed in Table 2. They contained MK-9(H_6) (10.33-31.90 %) and MK-9(H_4) (60.67-87.49%) were major menaquinones while MK-9(H_2) (5.57%) and MK-10(H_4) (1.86%) were minor menaquinones components. They contained *meso*-diaminopimelic acid as cell wall peptidoglycan type (Table 3). Phylogenetic analysis of strains LDG1-06, LDG1-22A and LDG1-22B revealed that they were belonged to the genus *Actinoplanes* (Figure 4). The percentage of 16S rRNA gene sequence similarity of *Actinoplanes* strains to another strains were showed in Table 3. Strain LDG1-06 was closely related to *Actinoplanes deccanensis* IFO 13994^{T} (Parenti *et al.,* 1975) (97.97%). The strains LDG1-22A and LDG1-22B were closely related to each other and showed 98.92 %and 98.93 % similarility with *Actinoplanes nipponensis* FH 2241^{T} (Wink *et.al.,* 2013).

Antimicrobial activity of isolates

Isolates LDG1-03 and LDG1-15 exhibited antimicrobial activity against *S. aureus* ATCC 25923, *B.subtilis* ATCC 6633, *K. rhizophila* ATCC 9341and *C.albicans*ATCC 10231. StrainLDG1-16 exhibited *B. subtilis* ATCC 6633 and *K. rhizophila* ATCC 9341. StrainLLG1-03 exhibited *B. subtilis* ATCC 6633. All 5 strains did not showed activitiy against *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853. Strain LDG1-06 exhibited *C.albicans* ATCC 10231. Strain LDG1-05, LDG1-08, LDG2-02, LDG2-09, LLG1-01, LDG1-22A and LDG1-22B did not show antimicrobial activity.

This study, 7 isolates were identified as *S. malaysiensis*, *S. lomondensis*, *S. graminearus*, *S parvulus*, *S. cinereoruber*, *S. seoulensis* and *S. cinerochromogenes* while isolates LDG1-03 and LDG1-08 were closely related to *S. malaysiensis* NBRC 16446^{T} (98.95%) and *S. graminearus* NBRC 15420^{T} (98.72 %), respectively. In addition isolate LDG1-06 was closely related to *A. deccanensis* IFO 13994^{T} (97.97%). LDG1-22A and LDG1-22B were closely related to *A. nipponensis* FH 2241 ^{T} 98.92 %and 98.93 %, respectively. These isolates were required for further study on DNA-DNA hybridization to propose them as the novel species.

The strains of *S. lomondensis* could produce lomofungin (Johnson and Dietz, 1969) while *S. malaysiensis* showed antibacterial and antifungal (AI-Tai *et.al.,* 1999), *S. graminearus* produced gougerotin, a peptidyl nucleoside antibiotic that possessed antitumor, antiviral, antibacterial, antimycoplasma, anthelmintic, and acaricidal activities (Jiang *et.al.,* 2013; Niu *et.al.,* 2013) *S. parvulus* strains produced actinomycin D (Williams and Katz, 1977; Ochi and Katz, 1978). *A. deccanensis* produced lipiarmycin, a metabolite of is highly active against Gram-positive bacteria, including strains resistant to the medically important antibiotics and protects mice experimentally infected with *Streptococcus haemolyticus*. Liparmycin inhibits growth of susceptible bacteria by interfering with RNA synthesis. (Coronelli *et.al.,*1975).

In southern Thailand, *S. siamensis*, *S. similanensis* (Sripreechasak *et al.,* 2013a), *S. 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*; *A. rifamycinica*; and *Kitasatospora saccharophila* (Sripreechasak *et al.,* 2013b) were distributed in soils and only *S. malaysiensis* was found as the same species.

Table 1: Sources, isolation , isolate number, cultural characteristics of isolates.

Lichen	Treatment /medium	Isolate no.	Colony colour		Soluble pigment
			Upper colony	Reverse colony	
***Chrysothrix* sp.**	Air dry /AV	LLG1-01	Bluish gray (191)	Olive gray (113)	-
		LLG1-03	Light bluish gray (190) to white	Light olive brown (94) to deep yellow (85)	-
Flavopamelia capelata	Air dry /AV	LDG1-03	White to reddish black (24)	Moderate yellow(87) to dark grayish yellowish brown (81)	-
		LDG1-05	Moderate yellowish brown (77) to white	Strong yellowish brown (74)	-
		LDG1-06	Strong orange yellow (68)	Brownish orange (54)	-
		LDG1-08	Light bluish gray (190) to white	Light olive brown (94) to dark yellow (88)	-
	Heat dry /AV	LDG1-15	Brownish black (65)	Dark yellow (88) to olive gray (113)	Brilliant yellow green (116)
		LDG1-16	Strong yellowish brown (74) to white	Strong yellowish brown (74)	Brilliant yellow (83)
		LDG1-22A	Brilliant orange yellow (70)	Vivid orange yellow (67)	-
		LDG1-22B	Brilliant orange yellow (70)	Brilliant orange yellow (70)	Brilliant yellow (83)
	Air dry/HV	LDG2-02	Light greenish gray (154) to white	Strong brown (55)	-
		LDG2-09	Pale blue (185)	Deep yellow (85) to deep yellowish brown (75)	-

Table 2: Phenotypic characteristics of isolates.

Characteristics	LDG1-03	LDG 1-15	LDG 1-05	LDG 2-02	LDG 1-08	LDG 1-16	LDG 2-09	LLG 1-01	LLG 1-03	LDG 1-06	LDG 1-22A	LDG 1-22B
Max. NaCl (%,w/v)	7	8	7	8	6	8	6	6	8	1	0	0
pH range for growth	5-12	5-12	5-12	5-12	4.5-11	7-12	4.5-12	4.5-12	4.5-12	5-12	5-12	5-12
Gelatin liquefaction	+	+	-	-	-	-	+	+	w	+	-	-
Nitrate reduction	+	+	-	+	-	-	+	-	-	-	-	-
Peptonization	+	+	w	+	+	+	+	+	+	-	w	w
Coagulation	-	-	-	+	+	+	-	-	-	-	-	-
Utilization of :												
Amygdalin	-	+	-	-	-	+	+	-	-	-	-	-
meso-Erythritol	-	-	-	-	-	-	+	-	-	-	-	-
D-Fructose	-	-	-	-	-	-	+	+	-	-	-	-
D-Galactose	-	-	-	+	+	-	+	-	-	-	-	-
D-Glucose	+	-	+	-	-	+	-	-	-	-	-	-
myo-Inositol	-	-	-	+	-	-	+	+	-	-	-	-
D-Lactose	-	-	+	-	-	-	+	-	-	-	+	+
D-Mannitol	+	+	-	-	-	+	-	+	+	-	-	-
D-Melibiose	+	-	-	-	-	-	+	-	-	-	-	-
D-Moltose	-	-	-	-	-	-	+	-	-	-	+	+
D-Xylose	-	-	-	-	-	-	+	-	-	-	-	-
L-Arabinose	-	-	-	-	-	-	-	-	-	+	+	-
L-Rhamnose	-	-	-	-	-	-	+	+	-	-	+	+
Xylitol	-	-	-	-	-	-	-	-	+	-	+	+

Table 3: Menaquinone components and cell wall peptidoglycan type of isolates.

Characteristics	LDG1-15	LDG1-05	LDG1-08	LDG1-16	LLG1-01	LLG1-03	LDG1-06	LDG1-22A	LDG1-22B
Menaquinones (%)									
MK-9(H_6)	49.95	59.5	60.07	42.06	33.42	59.12	10.33	31.90	22.07
MK-9(H_8)	48.73	40.5	27.15	17.60	9.47	40.88	2.18	-	-
MK-9(H_4)	1.31	-	12.78	33.26	7.45	-	87.49	60.67	77.93
MK-9(H_2)	-	-	-	7.08	49.66	-	-	5.57	-
MK-10(H_4)	-	-	-	-	-	-	-	1.86	-
Diaminopimelic acid	LL-DAP	LL-DAP	LL-DAP	LL-DAP	LL-DAP	LL-DAP	*meso*-DAP	*meso*-DAP	*meso*-DAP

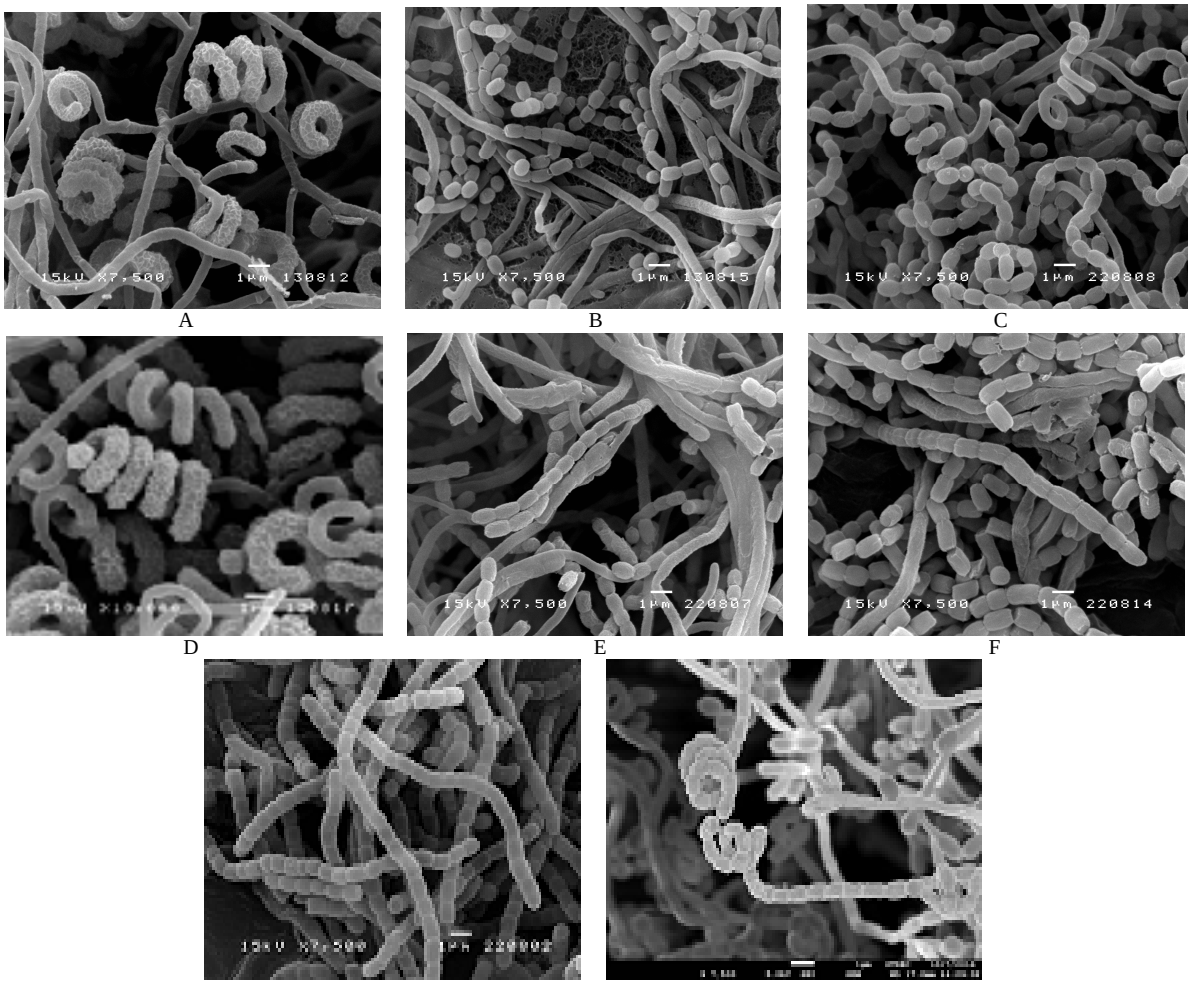
Table 4: Antimicrobial activity of isolates.

Isolate no.	Nearest relatives (% identity)	Inhibitory against indicator strains (mm) [1]		
		A16 medium	54 medium	ISP 2 medium
Group 1				
LDG1-03	*Streptomyces malaysiensis* NBRC 16446^T(98.95%)	*K* (11), *S* (9), *B* (9)	*K* (34), *S* (25), *B* (25), *Ca* (16)	*K* (30), *S* (25) *B* (21)
LDG1-15	*Streptomyces malaysiensis* NBRC 16446^T(99.85%)	-	*K* (20),*S(15)*,*B* (12) , *Ca* (15)	*K* (20),*S(15)*,*B* (11)
LDG1-05	*Streptomyces lomondensis* NBRC^T (99.78%)	-	-	-
LDG2-02	*Streptomyces lomondensis* NBRC 15426^T (99.77%)	-	-	-
LDG1-08	*Streptomyces graminearus* NBRC 15420^T (98.72 %)	-	-	-
LDG1-16	*Streptomyces parvulus* NBRC 13193^T (100%)	-	*K* (14),B(10)	
LDG2-09	*Streptomyces cinereoruber* subsp. *cinereoruber* NBRC 12756^T(99.11%)	-	-	-
LLG1-01	*Streptomyces seoulensis*NBRC 16668^T (100%)	-	-	-
LLG1-03	*Streptomyces cinerochromogenes* NBRC 13822 ^{T} (99.17%)	-	*B* (06)	-
Group 2				
LDG1-06	*Actinoplanes deccanensis* IFO 13994^T (97.97%)		*C*a (23)	
LDG1-22A	*Actinoplanes nipponensis* FH 2241 ^{T} (98.92 %)	-	-	-
LDG1-22B	*Actinoplanes nipponensis* FH 2241 ^{T} (98.93 %)	-	-	-

B, Bacillus subtilis ATCC 6633; *E, Eschrichia coli* ATCC 25922; *K, Kocuria rhizophila* ATCC 9341; *S, Stapylococcus aureus* ATCC 25923; *P, Pseudomonas aeruginosa* ATCC 27853 and *Ca, Cadida albicans* ATCC 10231; -, no activity

A **B**

Fig. 1: Lichens on tree barks; *Chrysothrix* sp. (A) and *Flavopamelia capelata* (B).

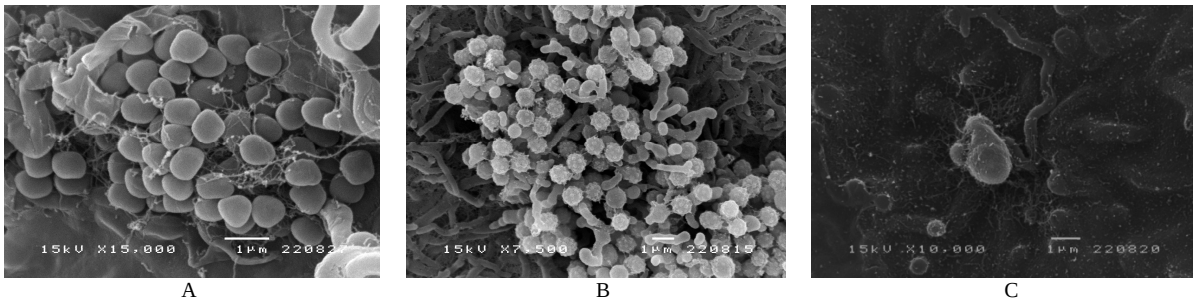


A B C

D E F

G H

Fig. 2: Scanning electron micrograph of isolates in Group 1; LDG1-03 (A), LDG1-05 (B) , LDG1-08 (C), LDG1-15 (D),LDG2-02 (E), LDG2-09 (F), LLG1-01 (G) and LLG1-03(H).



A B C

Fig. 3: Scanning electron micrograph of isolates in Group 2; LDG1-06 (a), LDG1-22A (b), LDG1-22B(c).

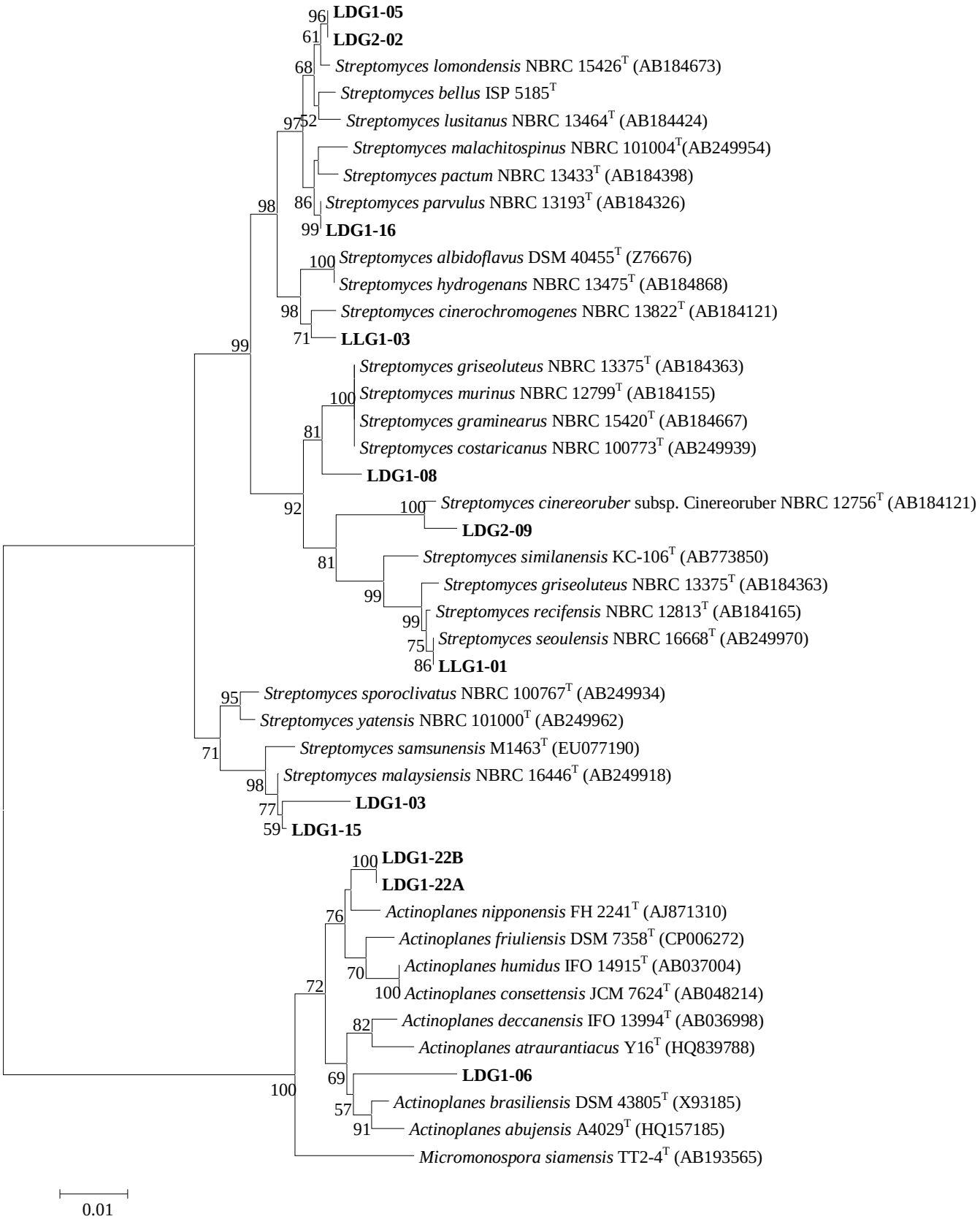


Fig. 4: Neighbour-joining tree showing actinomycete isolates based on 16S rRNA gene sequences. The numbers on the branches indicate the percentage from 1,000 replicate bootstrap samplings (>50% are indicated). Bar, 0.01 substitutions per nucleotide position.

CONCLUSION

The actinomycete strains LDG1-03, LDG1-05, LDG1-08, LDG1-15, LDG1-16, LDG2-02, LDG2-09, LLG1-01 and LLG1-03 were identified as *Streptomyces* and strains LDG1-06, LDG1-22A, LDG1-22B were identified as *Actinoplanes* based on their morphological, cultural, physiological and biochemical characteristics including the 16S rRNA gene sequence analysis. Four isolates in Group 1 exhibited antimicrobial activity against *S. aureus* ATCC 25923, *B. subtilis* ATCC 6633, *K. rhizophila* ATCC 9341 and *C. albicans* ATCC 27853 while *Actinoplanes* isolate LDG1-06 in Group 2 exhibited against *C. albicans* ATCC 27853.

REFERENCES

AI-Tai A, Kim B, Kim S B, Manfiol G P, Goodfellow M. *Streptomyces malaysiensis* sp. nov., a new streptomycete species with rugose, ornamented spores. Int J Syst Bacteriol,1999; 49: 1395-1402.

Arai T. 1975. Culture media for Actinomycetes, The Society for Actinomycetes, Japan, p.1-31.

Boonprakob K. Lichens: the better life to be ignored. SWU Sci J, 2014; 30, 1-10.

Chun J, Youn H D, Yim Y I, Lee H, Kim M Y, Hah Y C, Kang S O. *Streptomyces seoulensis* sp. nov. Int J Syst Evol Microbiol, 1997; 47(2): 492-498.

Corbaz R, Ettlinger L, Keller-Schierlein W, Zähner H. Zur Systematik der Actinomyceten. 1. Über Streptomyceten mit rhodomycinastigen Pigmenten. Arch Mikrobiol,1957; 25(4):325-332.

Coronelli C, White R J, Lancini G C, Parenti F. Lipiarmycin, a new antibiotic from *Actinoplanes*. II. Isolation, chemical, biological and biochemical characterization. J Antibiot, 1975; 28(4):253-259.

Felsenstien J. Confidence limits on phylogenies: an approach using the bootstrap. Evolution, 1985; 39:783-791.

González I, Ayuso-Sacido A, Anderson A, Genilloud O. Actinomycetes isolated from lichens: evaluation of their diversity and detection of biosynthetic gene sequences. FEMS Microbiol Ecol, 2005; 54:401-415.

Hayakawa M, Nonomura H. Humic acid vitamin agar, a new medium for the selective isolation of soil actinomycetes. J Ferment Technol, 1987; 65**:**501-509.

Inderiati S, Franco C M M. Isolation and identification of Endophytic actinomycetes and their antifungal activity. J Biotechnol Res Trop Reg, 2008; 1: 1-6.

Jiang L, Wei J, Li L , Niu G, Tan H. Combined gene cluster engineering and precursor feeding to improve gougerotin production in *Streptomyces graminearus*. Appl Microbiol Biotechnol, 2013; 97:10469-10477.

Johnson L E, Dietz A. Lomofungin, a New Antibiotic Produced by *Streptomyces lomondensis* sp. Appl Microbiol, 1969; 17(5): 755-759.

Jovan S. 2008. Lichen Bioindicationof Biodiversity, Air Quality,and Climate: Baseline Results From Monitoringin Washington, Oregon, and California. General Technical Report PNW-GTR-737.U.S.Department of Agriculture, Forest Service, Pacific Northwest Research Station, Portland, OR,115 pp.

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. Komagata K, Suzuki K. Lipid and cell-wall analysis in bacterial systematics. Methods Microbiol,1987; 19:161-207.

Kumar N, Ravi S K, Mishra S K, Sigh A K, Pachouri U C. Isolation and screening of soil Actinomycetes as source of antibiotics active against bacteria. Int J Microbiol Res, 2010; 2 (2): 12-16.

Kutzner HJ. 1981. The Prokaryotes: A handbook on habitats, isolation and identification of bacteria. vol. 2, Berlin: Springer-Verlag; pp. 2028- 2029.

Lorian V. 1991. Antibiotics in Laboratory Medicine, Baltimore: The Williams and Wilkins Company; pp. 1-51.

Miyairi N, Takashima M, Shimizu K, Sakai H . Studies on new antibiotics, cineromycins A and B. J Antibiot,1966; 19(2): 56-62.

Mejía A, Barrios-Gonzakez J, Viniegra-González G. Overproduction of rifamycin B by *Amycolatopsis mediterranei* and its relationship with the toxic effect of barbital on growth. J Antibiot,1998; 51(1): 58-63.

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.

Niu G, Li L, Wei J,Tan H. Cloning, heterologous expression, and characterization of the gene cluster required for gougerotin biosynthesis. Chem Biol, 2013; 20:34-44.

Nonomura H, Ohara Y**.** Distribution of actinomycetes in soil. VI. **A** culture method effective for both preferential isolation and enumeration of *Microbispora* and *Streptosporangium* strains in soil. J Ferment Techiiol, 1969; 47:463-469.

Nonomura H, Ohara Y. Distribution of actinomycetes in soil. VIII : Green- spore groupof *Microtetraspora*, its preferential isolation and taxonomic characteristics. J Ferment Technol, 1971; 49:1-7.

Ochi K, Katz E. The possible involvement of a plasmid(s) in actinomycin synthesis by *Streptomyces parvulus* and *Streptomyces antibioticus*. J Antibiot, 1978; 31(11):1143-1148.

Parenti F, Pagani H, Beretta G. Lipiarmycin, a new antibiotic from Actinoplanes. I. Description of the producer strain and fermentation studies. J Antibiot, 1975; 28(4): 247-52.

Preobrazhenskaya T P (1986) In: Gauze GF, Preobrazhenskaya, TP, Sveshnikova MA, Terekhova, LP, Maximova TS. A guide for the determination of actinomycetes. Genera Streptomyces, *Streptoverticillium*, and Chainia. Nauka, Moscow, USSR.

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.

Saito H, Miura K. Preparation of transforming deoxyribonucleic acid by phenol. Biochim Biophys Acta, 1963; 72:619-629.

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

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

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.

Tamura K, Stecher G, Peterson D, Filipski A and Kumar S. MEGA6: Molecular evolutionary genetics analysis version 6.0. Mol Biol Evol, 2013; 28(10):2731-2739.

Thompson J D , Gibson T J, Plewniak F, Jeanmougin F, Higgins D G. CLUSTAL_X windows interface: Flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res,1997; 25:4876-4882.

Williams W K, Katz E. Development of a chemically defined medium for the synthesis of actinomycin D by *Streptomyces parvulus*. Antimicrob Agents Chemother, 1977; 11 (2):281-290.

Wink J, Schumann P, Spöer C, Eisenbarth K, Glaeser S P , Martin K, Kämpfer P. Emended description of *Actinoplanes friuliensis* and description of *Actinoplanes nipponensis* sp. nov., antibiotic-producing species of the genus *Actinoplanes*. Int J Syst Evol Microbiol, 2013; 64:599-606.

Yamamura H, Ashizawa H, Nakagawa Y, Hamada M, Ishida Y, Otoguro M, Tamura T, Hayakawa M. *Actinomycetospora rishiriensis* sp. nov., isolated from a lichen. Int J Syst Evol Microbiol, 2011; 61: 2621 -2625.

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