The Journal of Antibiotics (2013) 66, 633–640

www.nature.com/ja

ORIGINAL ARTICLE

Streptomyces siamensis sp. nov., and *Streptomyces similanensis* sp. nov., isolated from Thai soils

Paranee Sripreechasak[1], Atsuko Matsumoto[2], Khanit Suwanborirux[3], Yuki Inahashi[2], Kazuro Shiomi[2,4], Somboon Tanasupawat[1] and Yoko Takahashi[2,4]

Three actinomycete strains, KC-038^T, KC-031 and KC-106^T, were isolated from soil samples collected in the southern Thailand. The morphological and chemotaxonomic properties of strains KC-038^T, KC-031 and KC-106^T were consistent with the characteristics of members of the genus *Streptomyces*, that is, the formation of aerial mycelia bearing spiral spore chains; the presence of LL-diaminopimelic acid in the cell wall, MK-9 (H_6), MK-9 (H_4) and MK-9 (H_8) as the predominant menaquinones; and $C_{16:0}$, iso-$C_{16:0}$ and anteiso-$C_{15:0}$ as the major cellular fatty acids. 16S rRNA gene sequence analyses indicated that strains KC-038^T and KC-031 were highly similar (99.9%), and they were closely related to *S. olivochromogenes* NBRC 3178^T (98.1%) and *S. psammoticus* NBRC 13971^T (98.1%). Strain KC-106^T was closely related to *S. seoulensis* NBRC 16668^T (98.9%), *S. recifensis* NBRC 12813^T (98.9%), *S. chartreusis* NBRC 12753^T (98.7%) and *S. griseoluteus* NBRC 13375^T (98.4%). The values of DNA–DNA relatedness between the isolates and the type strains of the related species were below 70%. On the basis of the polyphasic evidence, the isolates should be classified as two novel species, namely *Streptomyces siamensis* sp. nov. (type strain, KC-038^T = NBRC 108799^T = PCU 328^T = TISTR 2107^T) and *Streptomyces similanensis* sp. nov. (type strain, KC-106^T = NBRC 108798^T = PCU 329^T = TISTR 2104^T).
The Journal of Antibiotics (2013) **66,** 633–640; doi:10.1038/ja.2013.60; published online 12 June 2013

Keywords: new species; polyphasic taxonomy; *Streptomycetaceae*; *Streptomyces siamensis*; *Streptomyces similanensis*

INTRODUCTION

The genus *Streptomyces* belonging to the family *Streptomycetaceae* was proposed by Waksman and Henrici[1] to accommodate aerobic, Gram-positive and spore-forming actinomycetes. The *Streptomyces* strains represent a group of actinomycetes that are widely distributed in nature. At present, the genus comprises more than 550 recognized species with validly published names, and recently some novel species, including *S. cocklensis*,[2] *S. gramineus*,[3] *S. nanhaiensis*,[4] *S. panacagri*,[5] *S. pharmamarensis*,[6] *S. qinglanensis*[7] and *S. staurosporininus*,[8] have been described. Strains of the genus *Streptomyces* are superior to other actinomycete strains in their ability to produce various bioactive metabolites, especially antibiotics. Well-known antibiotics derived from *Streptomyces* strains include tetracycline, streptomycin, chloramphenicol, neomycin, nystatin, amphotericin, kanamycin and cycloheximide. *Streptomyces* strains are still a rich source of commercially significant compounds, such as antibiotics, enzymes, enzyme inhibitors and other pharmacologically active agents.[9] Therefore, new species in the genus *Streptomyces* remains a focus of efficient research for the discovery of new bioactive compounds. In this paper, we report the taxonomic status of *Streptomyces* strains KC-038^T, KC-031 and KC-106^T, which were isolated from soils in the south of Thailand.

MATERIALS AND METHODS

Strains KC-038^T and KC-031 were isolated from soil samples collected from the Krung Ching Waterfall, Khao Luang National Park, Nakhon Si Thammarat Province, Thailand, and strain KC-106^T was isolated from the Similan Island National Park (8°39′09″N 97°38′27″E), Phanga Province, Thailand. The soil samples were serially diluted with distilled water, heated at 55 °C for 5 min and plated onto potato starch-glycerol agar[10] and starch casein nitrate agar[11] containing nystatin (25 mg l^{-1}) and tetracycline (10 mg l^{-1}). The resulting pure isolates were maintained on SYM agar (starch 1.0%, NZ amine 0.3%, yeast extract 0.1%, meat extract 0.1%, $CaCO_3$ 0.3%, agar 1.2%, pH 7.0). Genomic DNA of each isolate was obtained by sonication of a suspension of cells[12] grown in YD broth (yeast extract 1.0%, dextrose 1.0%, pH 7.0). The 16S rRNA gene was amplified using the primers described by Takahashi *et al.*[13] The PCR products were sequenced on a DNA sequencer (model 3130 Genetic Analyzer; Applied Biosystems, Foster City, CA, USA) using a BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems), according to the manufacturer's instructions. The closest phylogenetic neighbors were identified by BLAST searches using the EzTaxon-e server.[14] The clustalw2

[1]Department of Biochemistry and Microbiology, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand; [2]Kitasato Institute for Life Sciences, Kitasato University, Minato-ku, Tokyo, Japan; [3]Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand and [4]Graduate School of Infection Control Sciences, Kitasato University, Minato-ku, Tokyo, Japan
Correspondence: Dr S Tanasupawat, Department of Biochemistry and Microbiology, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok 10330, Thailand.
E-mail: somboon.t@chula.ac.th
or Dr Y Takahashi, Kitasato Institute for Life Sciences, Kitasato University, 5-9-1 Shirokane, Minato-ku, Tokyo 108 8641, Japan.
E-mail: ytakaha@lisci.kitasato-u.ac.jp
Received 4 January 2013; revised 2 May 2013; accepted 9 May 2013; published online 12 June 2013

program was used for multiple alignments with selected sequences for calculating evolutionary distances[15] with SeaView version 4.2.[16] Phylogenetic trees were constructed using the neighbour-joining,[17] maximum-likelihood[18] and maximum-parsimony[19] methods. Data were resampled with 1000 bootstrap replications.[20] Values for sequence similarity among the closely related strains were determined using the EzTaxon-e server.[14]

Table 1 Cellular fatty acid compositions (%) of strains KC-038^T, KC-031 and closely related type strains

Fatty acid	*KC-038^T*	*KC-031*	*S. olivochromogenes NBRC 3178^T*	*S. psammoticus NBRC 13971^T*
Saturated straight chain				
$C_{14:0}$	4.0	3.0	0.7	3.2
$C_{16:0}$	23.5	19.7	7.6	23.9
$C_{17:0}$	1.2	1.0	0.5	1.0
$C_{18:0}$	—	—	—	0.5
$C_{17:0}$cyclo	0.5	0.7	2.9	3.7
Unsaturated straight chain				
$C_{17:1}$ *ω8c*	0.6	0.6	—	—
Saturated branched chain				
iso-$C_{14:0}$	8.6	8.5	8.0	2.0
iso-$C_{15:0}$	7.7	8.9	13.0	10.8
iso-$C_{16:0}$	18.4	22.3	22.1	10.5
iso-$C_{17:0}$	1.7	2.7	4.9	2.9
iso-$C_{18:0}$	—	—	0.9	0.6
anteiso-$C_{13:0}$	0.5	—	—	—
anteiso-$C_{15:0}$	17.7	16.6	25.2	22.6
anteiso-$C_{17:0}$	4.2	4.6	6.7	9.8
Unsaturated branched chain				
iso-$C_{16:1}$ H	0.6	0.9	1.0	—
iso-$C_{17:1}$ *ω9c*	0.6	1.0	1.9	0.5
anteiso-$C_{17:1}$ *ω9c*	0.5	0.6	1.2	0.7
Summed feature[a] 3	6.0	5.5	1.2	4.4

—, the amount of fatty acid less than 0.5% was omitted.
[a]Summed feature 3 comprises $C_{16:1}$ ω7c and/or $C_{16:1}$ ω6c.

Table 2 Cellular fatty acid compositions (%) of strain KC-106^T and closely related type strains

Fatty acid	*KC-106^T*	*S. seoulensis NBRC 16668^T*	*S. recifensis NBRC 12813^T*	*S. griseoluteus NBRC 13375^T*	*S. chartreusis NBRC 12753^T*
Saturated straight chain					
$C_{14:0}$	0.6	0.8	—	0.8	2.0
$C_{16:0}$	5.4	4.4	5.7	8.5	9.7
$C_{18:0}$	1.1	2.1	0.6	—	—
$C_{17:0}$ cyclo	2.0	2.4	3.3	1.8	0.4
Unsaturated straight chain					
$C_{18:1}$ *ω9c*	—	2.2	—	—	—
Saturated branched chain					
iso-$C_{14:0}$	5.8	10.2	6.7	4.2	7.3
iso-$C_{15:0}$	8.6	8.9	10.6	9.3	11.6
iso-$C_{16:0}$	23.2	20.1	19.3	17.9	19.8
iso-$C_{17:0}$	3.3	1.1	3.6	2.8	2.4
iso-$C_{18:0}$	1.5	1.0	1.5	1.1	—
anteiso-$C_{13:0}$	ND	—	—	0.6	1.3
anteiso-$C_{15:0}$	25.0	26.2	29.3	29.9	2.8
anteiso-$C_{17:0}$	10.3	4.2	8.0	13.6	5.8
Unsaturated branched chain					
iso-$C_{16:1}$ H	4.4	5.9	2.2	1.3	1.6
iso-$C_{17:1}$ *ω9c*	1.6	1.7	2.2	1.7	1.4
anteiso-$C_{17:1}$ *ω9c*	4.1	4.3	3.2	2.6	1.2
Summed feature[a] 3	0.9	2.0	1.4	2.2	6.9

Abbreviation: ND, not detected.
—, the amount of fatty acid less than 0.5% was omitted.
[a]Summed feature 3 comprises $C_{16:1}$ ω7c and/or $C_{16:1}$ ω6c.

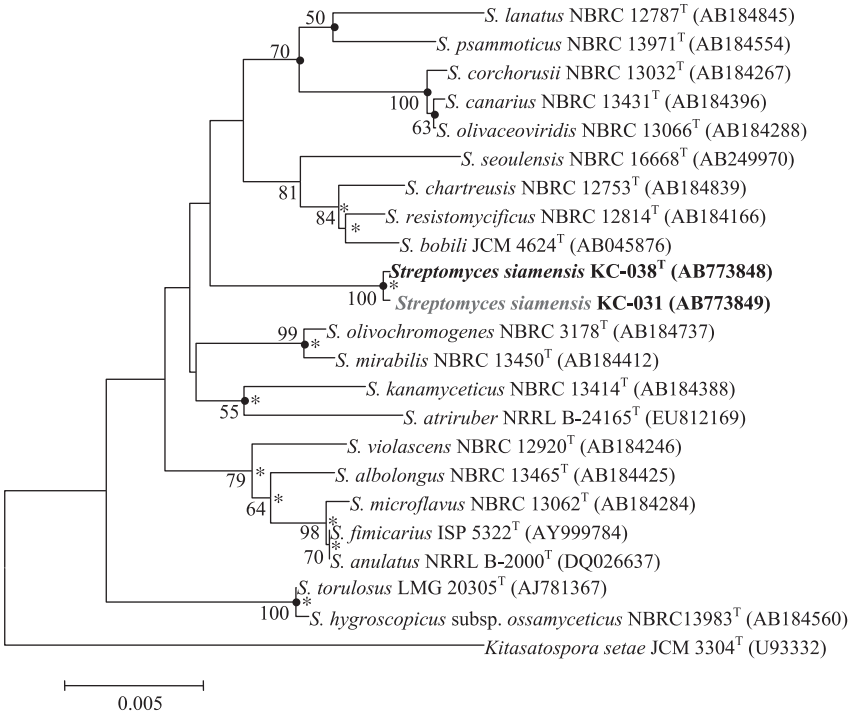

Figure 1 Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the relationship between strains KC-038^T, KC-031 and closely related type strains of the genus *Streptomyces*. Only bootstrap values above 50% (percentages of 1000 replications) are indicated. (●), branches were also recovered in the maximum-parsimony tree; (*), branches were also recovered in the maximum-likelihood tree; Bar, 0.005 nucleotide substitutions per site.

Strains KC-038^T, KC-031 and KC-106^T were cultivated at 27 °C for 2 weeks on ISP (International *Streptomyces* Project) 2, 3, 4, 5, 6 and 7 media,[21] YS agar (yeast extract 2.0%, starch 1.0%, agar 1.5%, pH 7.0) and nutrient agar. The Color Harmony Manual[22] was used to determine the color of aerial and substrate mycelia and soluble pigment. The features of the substrate and aerial mycelia and spores were observed by light microscopy (Nikon; model Labophoto-2, Tokyo, Japan) and scanning electron microscopy (model JSM-5600, JEOL, Tokyo, Japan) after cultivation on agar media at 27 °C for 3 weeks. For scanning electron microscopy investigation, the cultures were fixed with 4% osmium tetraoxide vapor *in situ* for 16 h at room temperature, and then dried at room temperature.[23] Physiological characteristics, NaCl tolerance, and the temperature and pH ranges required for growth were determined on ISP 2 medium. Utilization of various carbohydrates as the sole carbon source was tested using ISP 9 medium.[24] Starch hydrolysis was examined using ISP 4 medium, while nitrate medium (beef extract 0.3%, peptone 0.5%, KNO_3 0.1%, pH 7.0) was used to assess nitrate reduction, and glucose–peptone–gelatin medium (glucose 2.0%, peptone 0.5%, gelatin 20%, pH 7.0) was used to examine gelatin liquefaction. Skim milk (10%) was used to assess coagulation and peptonization of milk, and skim milk agar was used to examine casein hydrolysis. Enzyme activities were determined using the API ZYM system (bioMérieux, Lyon, France), according to the manufacturer's instructions. Biomass for the genotypic and chemotaxonomic studies was obtained after cultivation in YD broth on a rotary shaker at 27 °C for 3 days. Diaminopimelic acid isomers in whole cells were determined by TLC using whole-cell hydrolysates.[25] Whole-cell sugar composition was analyzed according to the methods of Becker *et al.*[25] Isoprenoid quinones were extracted according to the method of Collins *et al.*[26], and were analyzed by LC/MS (JMS-T 100LP, JEOL) using a CAPCELL PAK C18 UG120 column (Shiseido, Tokyo, Japan) with methanol/2-propanol (7:3). The *N*-acyl types of muramic acid were determined by using the method of Uchida and Aida.[27] Phospholipids were extracted and identified by using the method of Minnikin *et al.*[28] The presence of mycolic acids was examined by TLC following the protocol of Tomiyasu.[29] Cellular fatty acid composition was determined by gas liquid chromatography, according to the Microbial Identification System (MIDI) Sherlock version 6.0 using the RTSBA6 MIDI database as described by Sasser.[30] For DNA base composition analysis, chromosomal DNA was prepared following the procedure of Saito and Miura,[31] and the DNA G+C content was determined by HPLC according to the method of Tamaoka and Komagata.[32] DNA–DNA hybridization was performed using the photobiotin-labeling method of Ezaki *et al.*[33]

RESULTS AND DISCUSSION

Chemotaxonomic characteristics

Strains KC-038^T, KC-031 and KC-106^T exhibited typical characteristics of the genus *Streptomyces*. LL-diaminopimelic acid was detected in whole-cell hydrolysates. The menaquinones detected were as follows: MK-9 (H_6) (62%), MK-9 (H_4) (23%) and MK-9 (H_8) (15%) for KC-038^T, and MK-9 (H_8) (70%), MK-9 (H_6) (21%) and MK-9 (H_4) (10%) for KC-106^T. The *N*-acyl type of muramic acid was acetyl. Strains KC-038^T and KC-106^T contained diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositol and unknown phospholipids as phospholipid composition. Strains

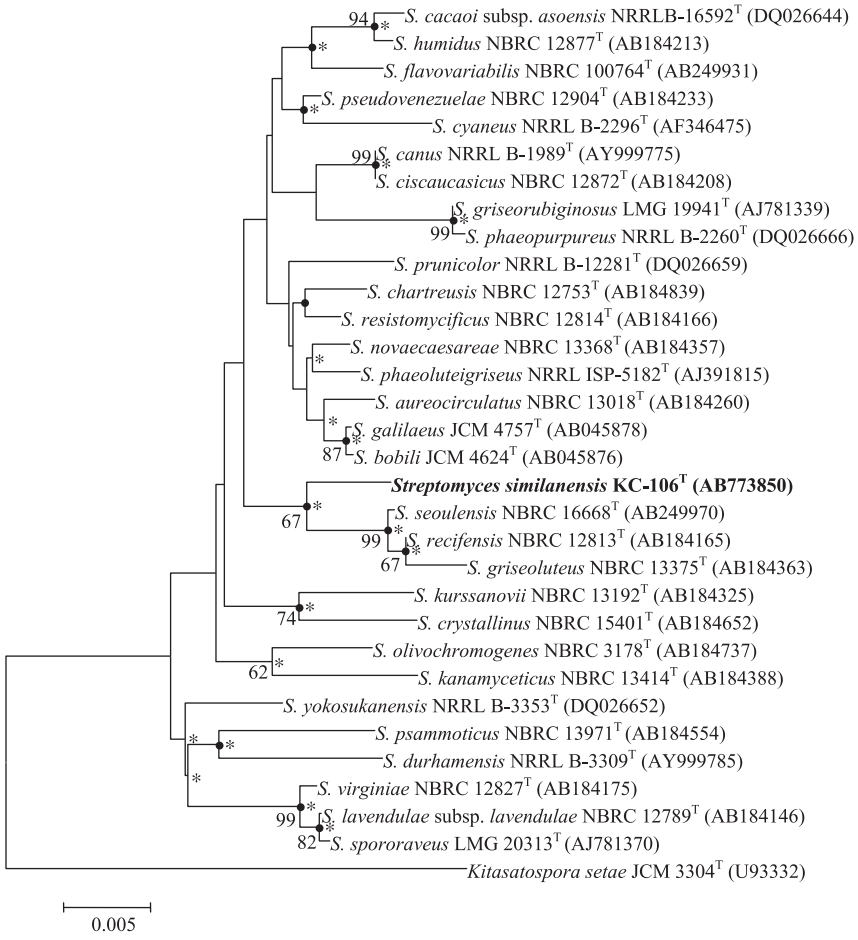

Figure 2 Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the relationship between strain KC-106^T and closely related type strains of the genus *Streptomyces*. Only bootstrap values above 50% (percentages of 1000 replications) are indicated. (●), branches were also recovered in the maximum-parsimony tree; (*), branches were also recovered in the maximum-likelihood tree; Bar, 0.005 nucleotide substitutions per site.

Table 3 Cultural characteristics of strains KC-038^T, KC-031 and closely related type strains

Medium	*KC-038^T*	*KC-031*	*S. olivochromogenes NBRC 3178^T*	*S. psammoticus NBRC 13971^T*
ISP medium 2				
Growth	Good	Good	Good	Good
	Mustard (2le)	Bamboo (2gc) to mustard (2le)	Bamboo (2gc)	Bamboo (2gc)
Reverse	Golden olive (2lg) to beaver (3li)	Camel (3ie) to bamboo (2gc)	Bamboo (2gc)	Bamboo (2gc)
Aerial mycelium	Abundant, white (a) to ashes (5fe)	Moderate, white (a)	Poor, white (a)	Moderate, white (a)
Soluble pigment	None	None	None	None
ISP medium 3				
Growth	Good	Good	Good	Good
	Light antique gold ($1_{1/2}$ic) to antique gold ($1_{1/2}$ne)	Light mustard tan (2ie)	Ivory (2db) to mustard (2le)	Yellow maple (3ng)
Reverse	Light antique gold ($1_{1/2}$ic) to mustard tan (2lg)	Mustard (2le) to mustard tan (2lg)	Ivory (2db) to mustard (2le)	Camel or tan (3ie)
Aerial mycelium	Abundant, slate tan (2ig)	Abundant, beige (3ge) to slate tan (2ig)	Moderate, white (a)	Abundant, white (a) to sand (3cb)
Soluble pigment	None	None	None	None
ISP medium 4				
Growth	Good	Good	Good	Good
	Antique gold ($1_{1/2}$ne)	Light mustard tan (2ie) to mustard tan (2lg)	Mustard (2le)	Mustard tan (2ie)
Reverse	Light mustard tan (2ie) to mustard tan (2ng)	Mustard brown (2pi)	Bamboo (2gc)	Bamboo (2gc)
Aerial mycelium	Abundant, natural (3dc) to silver gray (3fe)	Abundant, silver gray (3fe)	None	Moderate, white (a)
Soluble pigment	None	None	None	None
ISP medium 5				
Growth	Good	Good	Good	Good
	Biscuit (2ec) to antique gold ($1_{1/2}$ne)	Sand (3cb)	White (a)	Pearl (3ba)
Reverse	Biscuit (2ec) to light mustard tan (2ie)	Biscuit (2ec)	White (a)	Pearl (3ba)
Aerial mycelium	Abundant, beige (3gc)	Abundant, sand (3cb)	Poor, white (a)	Abundant, white (a)
Soluble pigment	None	None	None	None
ISP medium 6				
Growth	Good	Good	Good	Good
	Chocolate (4nl)	Light mustard tan (2ie)	Bamboo (2gc)	Honey gold (2ic)
Reverse	Chocolate (4nl)	Light mustard tan (2ie)	Bamboo (2gc)	Honey gold (2ic)
Aerial mycelium	None	None	None	None
Soluble pigment	Chocolate brown (4pn)	Mustard gold (2pg)	None	Amber (3pc)
ISP medium 7				
Growth	Good	Good	Good	Good
	Biscuit (2ec) to dusty yellow (2gc)	Convert tan (2ge)	White (a)	Pearl (3ba)
Reverse	Natural (3dc) to antique gold ($1_{1/2}$ne)	Natural (3dc)	White (a)	Pearl (3ba)
Aerial mycelium	Abundant, beige (3ge)	Abundant, natural (3dc)	Moderate, white (a)	Abundant, white (a)
Soluble pigment	None	None	None	None
YS agar				
Growth	Good	Good	Good	Good
	Biscuit (2ec) to chartreuse yellow (2ie)	Bamboo (2gc)	Cream ($1_{1/2}$ca)	Mustard tan (2ie)
Reverse	Bamboo (2gc) to light mustard tan (2ie)	Bamboo (2gc)	Cream ($1_{1/2}$ca)	Bamboo (2gc) to mustard tan (2ie)
Aerial mycelium	Abundant, white to slate tan (2ig)	Moderate, slate tan (2ig)	None	Abundant, white (a)
Soluble pigment	None	None	None	None
Nutrient agar				
Growth	Good	Good	Good	Good
	Cream ($1_{1/2}$ca)	Cream ($1_{1/2}$ca)	Cream ($1_{1/2}$ca) to bamboo (2gc)	Cream ($1_{1/2}$ca)
Reverse	Cream ($1_{1/2}$ca) to light ivory (2ca)	Cream ($1_{1/2}$ca)	Cream ($1_{1/2}$ca) to bamboo (2gc)	Cream ($1_{1/2}$ca)
Aerial mycelium	Poor, white (a)	None	None	Poor, white (a)
Soluble pigment	None	None	None	None

Numbers and letters in parentheses referred to the color based on the Color Harmony Manual.[22]

KC-038^T and KC-031 contained $C_{16:0}$ (23.5, 19.7%), iso-$C_{16:0}$ (18.4, 22.3%) and anteiso-$C_{15:0}$ (17.7, 16.6%) (Table 1), whereas strain KC-106^T contained anteiso-$C_{15:0}$ (25.0%), iso-$C_{16:0}$ (23.2%) and anteiso-$C_{17:0}$(10.3%) as major cellular fatty acids (Table 2). The cellular fatty acid profiles of strains KC-038^T, KC-031 and KC-106^{T}were almost the same as those of the type strains, but the amount of some fatty acids was different, as shown in Tables 1 and 2. The DNA G + C content was 72 mol% for strains KC-038^T and KC-031, and 73 mol% for strain KC-106^T.

Phylogenetic analysis

The 16S rRNA gene sequence similarity value between strains KC-038^T and KC-031 was 99.9%, and they showed the highest sequence similarities to *S. olivochromogenes* NBRC 3178^T (98.1%) and

S. psammoticus NBRC 13971^T (98.1%) and clustered with them (Figure 1).

The 16S rRNA gene sequence of strain KC-106^T was most similar to those of *S. seoulensis* NBRC 16668^T (98.9%), *S. recifensis* NBRC 12813^T (98.9%), *S. chartreusis* NBRC 12753^T (98.7%) and *S. griseoluteus* NBRC 13375^T (98.4%). The phylogenetic tree showed that strain KC-106^T forms a cluster with three of the above species, with the exception of *S. chartreusis* (Figure 2).

Phenotypic characteristics

The cultural characteristics of strains KC-038^T and KC-031, along with those of the type strains of the closest related species, *S. olivochromogenes* NBRC 3178^T[34] and *S. psammoticus* NBRC 13971^T,[35] are shown in Table 3. Strains KC-038^T and KC-031 grew well and formed extensively branched substrate mycelia on the various agar media tested. Aerial mycelia of white to gray color were produced on ISP 2–5 and 7 media, YS agar and nutrient agar, while the related type strains produced white aerial mycelia. The aerial mycelia consisted of long spiral chains with a smooth surface and the spores were rod shaped (Figures 3 and 4). Soluble pigment was produced on ISP 6 medium. The phenotypic and differential characteristics of strains KC-038^T and KC-031 are listed in the species description below and in Table 4. Strains KC-038^T and KC-031 were highly similar to each other but were differentiated from the closest related type strains with respect to carbon utilization.

The cultural characteristics of strain KC-106^T and the type strains of the closest related species, *S. seoulensis* NBRC 16668^T,[36] *S. recifensis* NBRC 12813^T,[37] *S. chartreusis* NBRC 12753^T,[38] and *S. griseoluteus* NBRC 13375^T,[39] are shown in Table 5. The strain grew well and formed extensively branched substrate and aerial mycelia on all agar media tested. Aerial mycelia of white to brownish gray color were produced. The aerial mycelia consisted of long and spiral spore chains with a hairy surface and oval-shaped spores (Figure 5), which is clearly different from the smooth spores produced by the related strains. The phenotypic and differential characteristics of strain KC-106^T are listed in the species description below and in Table 6. Strain KC-106^T was also differentiated from the closest related type strains with respect to carbon utilization.

DNA–DNA hybridization

The DNA–DNA relatedness value between strains KC-038^T and KC-031 was 100%; therefore, these two strains were classified as the same species. The DNA–DNA relatedness values between strain KC-038^T and the closest related type strains, *S. olivochromogenes* NBRC 3178^T and *S. psammoticus* NBRC 13971^T, were in the range of 4–36% (Table 7). The DNA–DNA relatedness values between strain KC-106^T and the closest type strains, *S. seoulensis* NBRC 16668^T, *S. recifensis* NBRC 12813^T, *S. chartreusis* NBRC 12753^T and *S. griseoluteus* NBRC 13375^T, were in the range of 7–46% (Table 8). These values were below the 70% cutoff point recommended by Wayne *et al.*[40] for assigning strains to the same species, and these results thus confirm that strains KC-038^T and KC-106^T are distinct from their closely related phylogenetic neighbors. Therefore, strains KC-038^T and KC-106^T are clearly the two novel species within the genus *Streptomyces*.

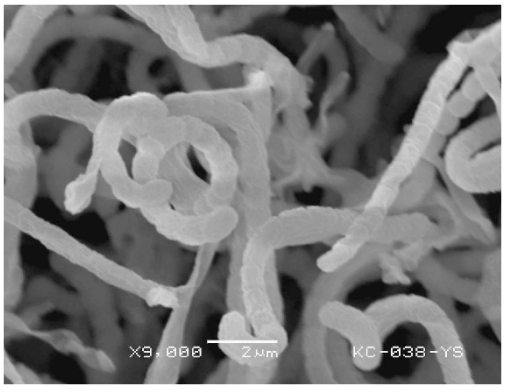

Figure 3 Scanning electron micrograph of strain KC-038^T grown on YS agar for 3 weeks at 27 °C.

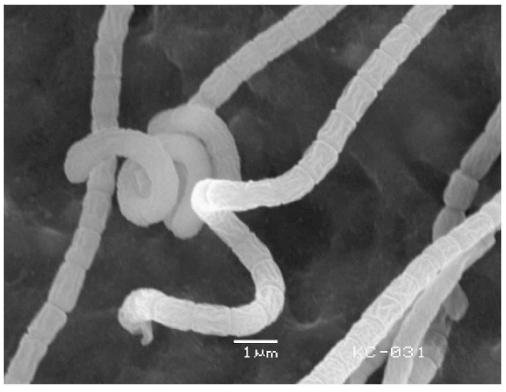

Figure 4 Scanning electron micrograph of strain KC-031 grown on ISP 4 medium for 3 weeks at 27 °C.

Table 4 Differential characteristics of strains KC-038^T, KC-031 and closely related type strains

Characteristics	*KC-038^T*	*KC-031*	*S. olivochromogenes NBRC 3178^T*	*S. psammoticus NBRC 13971^T*
Spore chain	Spiral	Spiral	Spiral	Ractiflexibiles
Spore surface	Smooth	Smooth	Smooth	Smooth
Utilization of				
L-Arabinose	+	+	+	−
D-Xylose	+	+	+	−
Raffinose	+	+	−	−
Melibiose	+	+	+	−
D-Mannitol	+	+	+	−
L-Rhamnose	+	+	+	−
myo-Inositol	+	+	+	−
Sucrose	−	−	+	+
Gelatin liquefaction	−	−	w	−
NaCl tolerance (%)	6	6	6	3
Enzyme activity of				
Esterase lipase C8	−	w	w	w
Cystine arylamidase	w	w	w	−
Trypsin	w	+	−	−
α-Galactosidase	−	−	w	−
β-Galactosidase	w	+	+	+
α-Glucosidase	−	w	+	+
β-Glucosidase	+	+	−	−
N-Acetyl-β-glucosaminidase	+	+	w	−
α-Mannosidase	w	w	−	+

Abbreviations: +, positive; w, weakly positive; −, negative.

Table 5 Cultural characteristics of strain KC-106^T and closely related type strains

Medium	*KC-106^T*	*S. seoulensis NBRC 16668^T*	*S. recifensis NBRC 12813^T*	*S. griseoluteus NBRC 13375^T*	*S. chartreusis NBRC 12753^T*
ISP medium 2					
Growth	Good	Good	Good	Good	Good
	Dusty yellow ($1_{1/2}$gc)	Colorless	Colorless	Mustard tan (2ie)	Light ivory (2ca)
Reverse	Bamboo (2gc) to mustard brown (2ni)	Bamboo (2gc)	Bamboo (2gc) to slate tan (2ig)	Bamboo (2gc) to mustard brown (2ni)	Bamboo (2gc) to light olive gray ($1_{1/2}$ge)
Aerial mycelium	Abundant, white (a) to silver gray (3fe)	Abundant, white (a) to ashes (5fe)	Abundant, white (a) to lead gray (5ih)	Abundant, white (a) to ashes (5fe)	Abundant, white (a) to aqua gray (19fe)
Soluble pigment	None	None	None	None	None
ISP medium 3					
Growth	Good	Good	Good	Good	Good
	Light mustard tan (2ie) to mustard tan (2lg)	Convert tan (2ge)	Colorless	Biscuit (2ec)	Light ivory (2ca) to bamboo (2gc)
Reverse	Beige (3ge)	Ivory (2db) to convert tan (2ge)	Pearl (3ba) to natural (2dc)	Ivory (2db) to convert brown (2li)	Light ivory (2ca) to light mustard tan (2ie)
Aerial mycelium	Abundant, fawn (4ig)	Abundant, ashes (5fe)	Abundant, white (a) to silver gray (3fe)	Abundant, white (a) to lead gray (5ih)	Abundant, white (a) to down blue (15dc)
Soluble pigment	None	None	None	None	None
ISP medium 4					
Growth	Good	Good	Good	Good	Good
	Light mustard tan (2ie)	Convert gray (2fe)	Light ivory (2ca) to ivory (2db)	Bisque (3ec) to camel (3ie)	Ivory (2db)
Reverse	Light ivory (2ca) to mustard tan (2lg)	Ivory (2db) to slate tan (2ig)	Ivory (2db)	Light fawn (4ge)	Ivory (2db)
Aerial mycelium	Abundant, white (a) to silver gray (3fe)	Abundant, white (a) to ashes (5fe)	Abundant, white (a)	Abundant, white (a)	Abundant, white (a) to gray(e)
Soluble pigment	None	None	None	None	None
ISP medium 5					
Growth	Good	Good	Good	Good	Good
	Pearl (3ba) to mustard (2le)	Pearl (3ba)	Convert gray (2fa)	Pearl (3ba)	White (a) to ivory (2db)
Reverse	Pearl (3ba) to light fawn (4ge)	Sand (3cb) to silver gray (3fe)	Natural (2dc)	Pearl (3ba) to ashes (5fe)	White (a) to light ivory (2ca)
Aerial mycelium	Abundant, white (a) to silver gray (3fe)	Abundant, white (a)	Abundant, Natural (3dc) to silver gray (3fe)	Abundant, white (a) to lead gray (3ih)	Moderate, white (a)
Soluble pigment	None	None	None	None	None
ISP medium 6					
Growth	Good	Good	Good	Good	Good
	Light ivory (2ca)	Cream ($1_{1/2}$ca)	Colorless	Cream ($1_{1/2}$ca)	Light ivory (2ca)
Reverse	Light wheat (2ea)	Cream ($1_{1/2}$ca) to light wheat (2ea)	Light ivory (2ca)	Light wheat (2ea)	Light ivory (2ca)
Aerial mycelium	Abundant, white (a)	Abundant, white (a)	Abundant, white (a)	Abundant, white (a)	None
Soluble pigment	None	None	None	None	None
ISP medium 7					
Growth	Good	Good	Good	Good	Good
	Cream ($1_{1/2}$ca) to mustard (2le)	Pearl (3ba)	Sand (3cb)	Pearl (3ba)	Golden brown (3pi) to clover brown (3pl)
Reverse	Ivory (2dc) to mustard (2le)	Pearl (3ba) to natural (3dc)	Natural (3dc)	Pearl (3ba) to slate tan (2ig)	Beige (3gc) to beaver (4li)
Aerial mycelium	Abundant, white (a) to silver gray (3fe)	Abundant, natural (3dc) to silver gray (3fe)	Abundant, natural (3dc)	Abundant, white (a) to silver gray (3fe)	Abundant, natural (3dc) to dawn blue (15dc)
Soluble pigment	None	None	None	None	None
YS agar					
Growth	Good	Good	Good	Good	Good
	Light mustard tan (2ie)	Convert tan (2ge)	Colorless	Ivory (2db)	Pearl (3ba) to light ivory (2ca)
Reverse	Mustard tan (2lg) to mustard brown (2ni)	Beige (3ge) to dark brown (3nl)	Ivory (2db) to dark brown (3nl)	Bisque (3ec) to dark brown (3nl)	Biscuit (2ec)
Aerial mycelium	Abundant, white (a) to silver gray (3fe)	Abundant, white (a) to lead gray (5ih)	Abundant, white(a) to ashes (5fe)	Abundant, white (a) to lead gray (5ih)	Abundant, white (a) to aqua gray (19fe)
Soluble pigment	None	None	None	None	None
Nutrient agar					
Growth	Good	Good	Good	Good	Good
	Ivory (2db) to biscuit (2ec)	Pearl (2cb)	Pearl (2cb)	Pearl (3ba)	Cream ($1_{1/2}$ca)
Reverse	Bamboo (2gc) to light mustard tan (2ie)	Light ivory (2ca)	Light ivory (2ca) to convert tan (2ge)	Pearl (3ba)	Cream ($1_{1/2}$ca)
Aerial mycelium	Abundant, white (a) to silver gray (3fe)	Abundant, white (a)	Abundant, white (a) to silver gray (3fe)	Abundant, white (a)	Poor, white (a)
Soluble pigment	None	None	None	None	None

Numbers and letters in parentheses referred to the color based on the Color Harmony Manual.[22]

Conclusion

Based on phylogenetic, phenotypic and chemotaxonomic characteristics, strains KC-038^T, KC-031 and KC-106^T are classified within the genus *Streptomyces*. The cultural, physiological and biochemical features, such as aerial mycelia color and utilization of various carbohydrates as the sole carbon source, indicate that strains KC-038^T, KC-031 and KC-106^T differ from the closest related species. The results of DNA–DNA relatedness also support their classification of these strains into two novel species. Therefore, the name *Streptomyces siamensis* sp. nov. is proposed for strains KC-038^T and KC-031, and the name *Streptomyces similanensis* sp. nov. is proposed for strain KC-106^T.

Description of *S. siamensis* sp. nov. (si.am.en'sis N.L. masc. adj. *siamensis*, belonging to Siam, the old name for Thailand, where the first strain was isolated)

Gram-positive, aerobic actinomycete, forming extensively branched substrate mycelia and aerial hyphae that differentiate into long and

spiral chains of smooth-surfaced, rod-shaped spores. The color of aerial mycelia varies from white to gray. Dark brown soluble pigment is produced in ISP6 medium. Growth occurs at 16–38 °C (optimum at 16–30 °C) and at pH 4–11 (optimum at pH 5–9). Hydrolyzes casein and starch but not gelatin. Nitrate is not reduced. Utilizes D-glucose, L-arabinose, D-xylose, raffinose, melibiose, D-mannitol, D-fructose, L-rhamnose and *myo*-inositol, but not sucrose. Positive for alkaline phosphatase, esterase (C4), leucine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrase, β-glucosidase and *N*-acetyl-β-glucosaminidase, and weakly positive for valine arylamidase, cystine arylamidase, trypsin, β-galactosidase and α-mannosidase. Negative for esterase lipase (C8), lipase (C14), osidase, α-galactosidase and α-glucosidase (API ZYM system). The menaquinones are MK-9 (H_6), MK-9 (H_4) and MK-9 (H_8). Major cellular fatty acids are $C_{16:0}$, iso-$C_{16:0}$ and anteiso-$C_{15:0}$. The DNA G + C content of the type strain is 72 mol%.

The type strain KC-038^T (= NBRC 108799^T = PCU 328^T = TISTR 2107^T) was isolated from the soil collected at Krung Ching Waterfall, Khao Luang National Park, Nakhon Si Thammarat Province, Thailand.

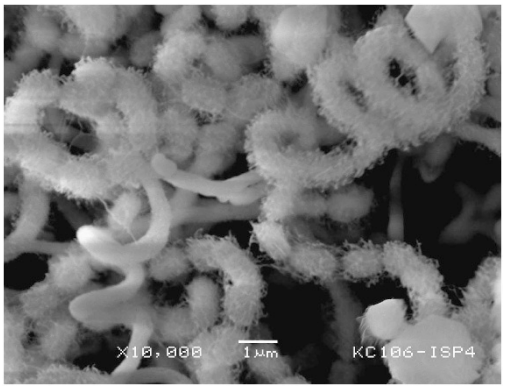

Figure 5 Scanning electron micrograph of strain KC-106 ^{T} grown on ISP 4 medium for 3 weeks at 27 °C.

Table 7 DNA–DNA relatedness between KC-038^T, KC-031 and closely related type strains

	DNA–DNA relatedness with labeled strains (%)[a]			
Strain	*KC-038^T*	*KC-031*	*S. olivochromogenes NBRC 3178^T*	*S. psammoticus NBRC 13971^T*
KC-038^T	100	100	36	13
KC-031	100	100	11	3
S. olivochromogenes NBRC 3178^T	17	21	100	4
S. psammoticus NBRC 13971^T	4	9	2	100

[a]Average of four independent determinations.

Table 6 Differential characteristics of strain KC-106^T and closely related type strains

Characteristics	*KC-106^T*	*S. seoulensis NBRC 16668^T*	*S. recifensis NBRC 12813^T*	*S. griseoluteus NBRC 13375^T*	*S. chartreusis NBRC 12753^T*
Spore chain	Spiral	Ractiflexibiles	Retinaculiaperti	Ractiflexibiles	Spiral
Spore surface	Hairy	Smooth	Smooth	Smooth	Spiny
Utilization of					
Raffinose	w	+	w	w	+
Melibiose	w	+	−	w	+
L-Rhamnose	−	+	−	−	+
myo-Inositol	−	−	−	−	+
Sucrose	w	+	+	w	+
Nitrate reduction	−	−	−	−	+
Gelatin liquefaction	−	+	+	w	+
NaCl tolerance (%)	7	8	6	5	6
Enzyme activity of					
Alkaline phosphatase	+	+	+	−	+
Esterase C4	+	+	+	w	+
Esterase lipase C8	−	+	w	−	w
Valine arylamidase	+	w	+	+	w
Cystine arylamidase	+	w	+	+	w
Trypsin	−	−	w	−	+
α-Chymotrypsin	−	−	−	−	+
Naphthol-AS-BI-phosphohydrase	+	+	+	w	+
β-Galactosidase	−	−	+	−	+
α-Glucosidase	+	w	+	−	−
β-Glucosidase	+	−	−	−	+
N-acetyl-β-glucosaminidase	+	+	+	w	+
α-Mannosidase	+	−	−	−	w

Abbreviations: +, positive; w, weakly positive; −, negative.

Table 8 DNA–DNA relatedness between KC-106^T and closely related type strains

Strain	DNA–DNA relatedness with labeled strains (%)[a]				
	KC-106^T	*S. seoulensis* NBRC 16668^T	*S. recifensis* NBRC 12813^T	*S. griseoluteus* NBRC 13375^T	*S. chartreusis* NBRC 12753^T
KC-106^T	100	30	28	19	18
S. seoulensis NBRC 16668^T	46	100	67	61	14
S. recifensis NBRC 12813^T	39	81	100	71	21
S. griseoluteus NBRC 13375^T	38	92	96	100	21
S. chartreusis NBRC 12753^T	7	24	20	24	100

[a]Average of four independent determinations.

Description of *S. similanensis* sp. nov. (si.mi.lan. en' sis N.L. masc. adj. *similanensis*, belonging to Similan, an island in the southern Thailand, where the first strain was isolated)

Gram-positive, aerobic actinomycete, forms extensively branched substrate mycelia and aerial hyphae that differentiate into long and spiral chains of hairy surfaced and oval spores. Aerial mycelia are white to brownish gray in color. Soluble pigment is not produced. Growth occurs at 12–40 °C (optimum at 18–30 °C) and at pH 4–11 (optimum at pH 4–9). Hydrolyzes casein and starch but not gelatin. Nitrate is not reduced. Utilizes D-glucose, L-arabinose, D-xylose, raffinose, melibiose, D-mannitol and D-fructose; weakly utilizes raffinose and melibiose, but not sucrose, L-rhamnose or *myo*-inositol. Positive for alkaline phosphatase, esterase (C4), leucine arylamidase, valine arylamidase, cystine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase and α-mannosidase. Negative for esterase lipase (C8), trypsin, α-chymotrypsin and β-galactosidase (API ZYM system). Menaquinones are MK-9 (H_8), MK-9 (H_6) and MK-9 (H_4). Major cellular fatty acids are anteiso-$C_{15:0}$, iso-$C_{16:0}$ and anteiso-$C_{17:0}$. The DNA G + C content is 73 mol%.

The type strain KC-106^T (= NBRC 108798^T = PCU 329^T = TISTR 2104^T) was isolated from soil collected at the Similan Island National Park, Phanga Province, Thailand.

ACKNOWLEDGEMENTS

This research was supported by a Chulalongkorn University Duthsadiphiphat Scholarship (2008), Chulalongkorn University, Bangkok, Thailand, and by the Kitasato Institute for Life Sciences, Kitasato University, Japan. We thank Professor JP Euzeby for the etymologies.

1 Waksman, S. A. & Henrici, A. T. The nomenclature and classification of actinomycetes. *J. Bacteriol*. **46,** 337–341 (1943).
2 Kim, B.-Y., Zucchi, T. D., Fiedler, H.-P. & Goodfellow, M. *Streptomyces cocklensis* sp. nov., a dioxamycin-producing actinomycete. *Int. J. Syst. Evol. Microbiol*. **62,** 279–283 (2012).
3 Lee, H.-J., Han, S.-I. & Whang, K.-S. *Streptomyces gramineus* sp. nov., an antibiotic-producing actinobacterium isolated from bamboo (Sasa borealis) rhizosphere soil. *Int. J. Syst. Evol. Microbiol*. **62,** 856–859 (2012).
4 Tian, X.-P. *et al*. *Streptomyces nanhaiensis* sp. nov., a marine streptomycete isolated from a deep-sea sediment. *Int. J. Syst. Evol. Microbiol*. **62,** 864–868 (2012).
5 Cui, Y. *et al*. *Streptomyces panacagri* sp. nov., isolated from soil of a ginseng field. *Int. J. Syst. Evol. Microbiol*. **62,** 780–785 (2012).
6 Carro, L., Zuniga, P., Calle, F. D. L. & Trujillo, M. E. *Streptomyces pharmamarensis* sp. nov.isolated from a marine sediment. *Int. J. Syst. Evol. Microbiol*. **62,** 1165–1170 (2012).
7 Hu, H. *et al*. *Streptomyces qinglanensis* sp. nov., isolated from mangrove sediment. *Int. J. Syst. Evol. Microbiol*. **62,** 596–600 (2012).
8 Kim, B.-Y., Zucchi, T. D., Fiedler, H.-P. & Goodfellow, M. *Streptomyces staurosporininus* sp. nov., a staurosporine-producing actinomycete. *Int. J. Syst. Evol. Microbiol*. **62,** 966–970 (2012).
9 Berdy, J. Bioactive microbial metabolites: review article. *J. Antibiot*. **58,** 1–26 (2005).
10 Tajima, K., Takahashi, Y., Seino, A., Iwai, Y. & Omura, S. Description of two novel species of the genus *Kitasatospora* Omura *et al*. 1982, *Kitasatospora cineracea* sp. nov. and *Kitasatospora niigatensis* sp. nov. *Int. J. Syst. Evol. Microbiol* **51,** 1765–1771 (2001).
11 Seong, C. N., Choi, J. H. & Baik, K.-S. An improved selective isolation of rare actinomycetes from forest soil. *J. Microbiol*. **39,** 17–23 (2001).
12 Matsumoto, A., Takahashi, Y., Iwai, Y. & Omura, S. Isolation of Gram-positive bacteria with high G + C from inside soil aggregates. *Actinomycetologica* **20,** 30–40 (2006).
13 Takahashi, Y. *et al*. *Streptomyces avermectinius* sp. nov., an avermectin-producing strain. *Int. J. Syst. Evol. Microbiol*. **52,** 2163–2168 (2002).
14 Kim, O. S. *et al*. Introducing EzTaxon-e: a prokaryotic 16S rRNA gene sequence database with phylotypes that represent uncultured species. *Int. J. Syst. Evol. Microbiol*. **62,** 716–721 (2012).
15 Kimura, M. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J. Mol. Evol*. **16,** 111–120 (1980).
16 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*. **27,** 221–224 (2010).
17 Saito, N. & Nei, M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol*. **4,** 406–425 (1987).
18 Felsenstein, J. Evolutionary trees from DNA sequences: a maximum likelihood approach. *J. Mol. Evol*. **17,** 368–379 (1981).
19 Kluge, A. G. & Farris, F. S. Quantitative phyletics and the evolution of anurans. *Syst. Zool*. **18,** 1–32 (1969).
20 Felsenstein, J. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution*. **39,** 783–791 (1985).
21 Shirling, E. B. & Gottlieb, D. Methods for characterization of *Streptomyces* species. *Int. J. Syst. Bacteriol*. **16,** 313–340 (1966).
22 Taylor, H. D., Knoche, L. & Grauville, W. C. *Color Harmony Manual*. 4th edn (Container Corporation of America, Chicago, IL, USA, 1958).
23 Inahashi, Y., Matsumoto, A., Danbara, H., Omura, S. & Takahashi, Y. *Phytohabitans suffuscus* gen. nov., sp. nov., an actinomycete of the family *Micromonosporaceae* isolated from plant roots. *Int. J. Syst. Evol. Microbiol*. **60,** 2652–2658 (2010).
24 Pridham, T. G. & Gottlieb, D. The utilization of carbon compounds by some Actinomycetales as an aid for species determination. *J. Bacteriol* **56,** 107–114 (1948).
25 Becker, B., Lechevalier, M. P. & Lechevalier, H. A. Chemical composition of cell-wall preparation from strains of various from-genera of aerobic actinomycetes. *Appl. Microbiol*. **13,** 236–243 (1965).
26 Collins, M. D., Pirouz, T., Goodfellow, M. & Minnikin, D. E. Distribution of menaquinones in actinomycetes and corynebacteria. *J. Gen. Microbiol*. **100,** 221–230 (1977).
27 Uchida, K. & Aida, K. Acyl type of bacterial cell wall: its simple identification by a colorimetric method. *J. Gen. Appl. Microbiol*. **23,** 249–260 (1977).
28 Minnikin, D. E., Patel, P. V., Alshamaony, L. & Goodfellow, M. Polar lipid composition in the classification of *Nocardia* and related bacteria. *Int. J. Syst. Bacteriol*. **27,** 104–117 (1977).
29 Tomiyasu, I. Mycolic acid composition and thermally adaptative changes in *Nocardia asteroids*. *J. Bacteriol*. **151,** 828–837 (1982).
30 Sasser, M. Identification of bacteria by gas chromatography of cellular fatty acids. *USFCC Newsl* **20,** 1–6 (1990).
31 Saito, H. & Miura, K. Preparation of transforming deoxyribonucleic acid by phenol treatment. *Biochim. Biophys. Acta*. **72,** 619–629 (1963).
32 Tamaoka, J. & Komagata, K. Determination of DNA base composition by reversed-phase high-performance liquid chromatography. *FEMS Microbiol. Lett*. **25,** 125–128 (1984).
33 Ezaki, T., Hashimoto, Y. & Yabuuchi, E. Fluorometric deoxyribonucleic acid-deoxyribonucleic acid hybridization in microdilution wells as an alternative to membrane filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains. *Int. J. Syst. Bacteriol*. **39,** 224–229 (1989).
34 Shirling, E. B. & Gottlieb, D. Cooperative description of type cultures of *Streptomyces*. IV. Species descriptions from the second, third and fourth studies. *Int. J. Syst. Bacteriol*. **19,** 391–512 (1969).
35 Virgilio, A. & Hengeller, C. Produzione di Tetraciclina con *Streptomyces psammoticus*. *Farm. Ediz. Scient*. **15,** 164–174 (1960).
36 Chun, J. *et al*. *Streptomyces seoulensis* sp. nov. *Int. J. Syst. Bacteriol*. **47,** 492–498 (1997).
37 Shirling, E. B. & Gottlieb, D. Cooperative description of type cultures of *Streptomyces*. II. Species descriptions from first study. *Int. J. Syst. Bacteriol*. **18,** 69–189 (1968).
38 Leach, B. E., Calhoun, K. M., Johnson, L. E., Teeters, C. M. & Jackson, W. G. Chartreusin, a new antibiotic produced by *Streptomyces chartreusis* a new species. *J. Am. Chem. Soc*. **75,** 4011–4012 (1953).
39 Umezawa, H., Hayano, S., Maeda, K., Ogata, Y. & Okami, Y. On a new antibiotic, griseolutein, produced by *Streptomyces*. *Jpn. Med. J*. **3,** 111–117 (1950).
40 Wayne, L. G. *et al*. International Committee on Systematic Bacteriology. Report of the *Ad Hoc* Committee on reconciliation of approaches to bacterial systematics. *Int. J.Syst. Bacteriol*. **37,** 463–464 (1987).