The Journal of Antibiotics (2015) 68, 322–327

www.nature.com/ja

ORIGINAL ARTICLE

Streptomyces lactacystinicus sp. nov. and *Streptomyces cyslabdanicus* sp. nov., producing lactacystin and cyslabdan, respectively

Akira Také[1], Atsuko Matsumoto[1,2], Satoshi Ōmura[2] and Yōko Takahashi[1,2]

Actinomycete strains OM-6519^T and K04–0144^T produce the bioactive compounds lactacystin and cyslabdan, respectively. Here, the taxonomic positions of these two strains were determined. The morphological and chemical features of strains OM-6519^T and K04–0144^T indicated that they belonged to the genus *Streptomyces*. Strain OM-6519^T showed the highest 16S rRNA gene sequence similarities with *Streptomyces xanthocidicus* NBRC 13469^T (99.7%), *Streptomyces chrysomallus* subsp. *fumigatus* NBRC 15394^T (99.6%) and *Streptomyces aburaviensis* NRRL B-2218^T (99.5%). However, the DNA–DNA relatedness values between strain OM-6519^T and the three related strains were below 70%. Strain K04–0144^T showed the highest 16S rRNA gene sequence similarities with *Streptomyces corchorusii* NBRC 13032^T (99.4%), *Streptomyces olivaceoviridis* NBRC 15394^T (99.4%) and *Streptomyces canarius* NRRL B-2218^T (99.3%). However, the DNA–DNA relatedness values between strain K04–0144^T and the three related strains were also below 70%. Based on morphological, cultural and physiological characteristics and DNA–DNA relatedness data, strains OM-6519^T and K04–0144^T should be classified as new species of the genus *Streptomyces*, for which the names *Streptomyces lactacystinicus* sp. nov. and *Streptomyces cyslabdanicus* sp. nov. are proposed. The type strain of *S. lactacystinicus* is OM-6519^T (= NBRC 110082^T, DSM 43136^T). The type strain of *S. cyslabdanicus* is K04–0144^T (= NBRC 110081^T, DSM 42135^T).
The Journal of Antibiotics (2015) **68,** 322–327; doi:10.1038/ja.2014.162; published online 10 December 2014

INTRODUCTION

Approximately 12 000 bioactive compounds produced by *Actinobacteria* have been identified, of which three-quarters are produced by *Streptomyces* species, which constitute the majority of *Actinobacteria* in soil.[1,2] For this reason, a large number of *Streptomyces* strains have been isolated from soil samples in the search for new bioactive compounds. To date, nearly 600 species of *Streptomyces* have been identified.[3] Among these isolates, numerous bioactive compounds have been characterized. We have also found avermectin[4] produced by *Streptomyces avermectinius* (*S. avermitilis*) MA-4680^T, herbimycin[5] produced by *Streptomyces hygroscopicus* subsp. *hygroscopicus* AM-3672 and a lot of other compounds. In addition, two *Streptomyces* strains, OM-6519^T and K04–0144^T, which produce lactacystin and cyslabdan, respectively, have also been identified;[6,7] however, their taxonomic positions are unclear.

Streptomyces strain OM-6519^T was originally isolated from soil and produces lactacystin, a bioactive compound that induces neuritogenesis in neuroblastoma cells.[6,8] Lactacystin also inhibits proteasome activity by interacting with the β subunit of the 20S proteasome.[9] The functional mechanism[9,10] biosynthetic pathway[11] and total synthesis[12] of lactacystin have been determined, and the compound is frequently used as a biochemical reagent.

Strain K04–0144^T was also isolated from soil and produces several bioactive compounds, including cyslabdan,[7] nosokomycins, which are a group of novel antibiotics that are active against methicillin-resistant *Staphylococcus aureus*,[13] amphotericin B and nocardamine. Cyslabdan is a new compound that consists of a unique labdane-type diterpene and *N*-acetylcysteine residue, and is of particular interest because it potentiates the activity of β-lactams against methicillin-resistant *Staphylococcus aureus*.[14,15] Although some plants and fungi produce compounds that contain labdane-type diterpenes, such as pacovatinins[16] and botryosphaerins,[17] strain K04–0144^T is the first member of the genus *Streptomyces* to reportedly produce labdane-type diterpene.

In this paper, we report the taxonomic characterization of strains OM-6519^T and K04–0144^T.

MATERIALS AND METHODS

Strains OM-6519^T and K04–0144^T were previously isolated from soil samples collected near lake at Inba, Chiba, Japan and under a tree on Ishigaki Island, Okinawa, Japan, respectively.[6,7] To observe cultural and morphological characteristics, the strains were cultured for 2 weeks at 27 °C on yeast extract-malt agar (ISP 2; Difco, Detroit, MI, USA), oatmeal agar (ISP 3; Nihon Pharmaceutical Co., Ltd., Tokyo, Japan), inorganic salts-starch agar (ISP 4; Difco), glycerol-asparagine agar (ISP 5; Nihon Pharmaceutical Co., Ltd.),

[1]Graduate School of Infection Control Sciences, Kitasato University, Tokyo, Japan and [2]Kitasato Institute for Life Sciences, Kitasato University, Tokyo, Japan
Correspondence: Dr A Matsumoto, Kitasato Institute for Life Sciences, Kitasato University, 5-9-1 Shirokane, Minato-ku, Tokyo 108–8641, Japan.
E-mail: amatsu@lisci.kitasato-u.ac.jp
Received 23 June 2014; revised 27 October 2014; accepted 6 November 2014; published online 10 December 2014

peptone-yeast extract-iron agar (ISP 6; Nihon Pharmaceutical Co., Ltd.) and tyrosine agar (ISP 7; Nihon Pharmaceutical Co., Ltd.).[18] The color of aerial and vegetative mycelia and soluble pigments were determined according to the Color Harmony Manual.[19] Morphological characteristics were observed by light microscopy and scanning electron microscopy (JEOL-JSM 5600; JEOL Ltd., Tokyo, Japan). The temperature range, pH range and NaCl tolerance for growth were determined using ISP 2. ISP 4 was used to detect starch hydrolysis, and potassium nitrate broth (ISP 8) (beef extract (Difco) 0.3%, peptone (Difco) 0.5% and KNO_3 0.1%) was used to examine nitrate reduction.[20] Glucose–peptone–gelatin medium (glucose 2.0%, peptone 0.5%, gelatin (Difco) 20%, pH 7.0) was used to detect gelatin hydrolysis at 20 °C, and 10% skim milk (Difco) was used to assess the coagulation and peptonization of milk at 37 °C.[20] Utilization of carbohydrates as the sole carbon source was tested using a basal medium (ISP 9; Difco), according to the method of Pridham and Gottlieb.[21] Nitrogen source utilization was examined using the basal medium recommended by Williams *et al.*[22] Biomass for molecular systematics and chemotaxonomic studies was obtained after cultivation on a rotary shaker in yeast extract-glucose broth (yeast extract (Difco) 1.0% and glucose 1.0%, pH 7.0) for 3 days at 27 °C. Isomers of diaminopimelic acid were determined by TLC using whole-cell hydrolysis.[23] Whole-cell sugar composition was analyzed according to the method of Becker *et al.*[23] Cell phospholipids were extracted and identified by the method of Minnikin *et al.*[24] Isoprenoid quinones were extracted from cells as described by Collins *et al.*[25] and were analyzed by LC/MS (JMS-T 100LP; JEOL Ltd.) using a CAPCELL PAK C18 UG120 column (Shiseido Co. Ltd., Tokyo, Japan) with methanol/2-propanol (7:3). Methyl esters of cellular fatty acids were prepared by direct transmethylation with methanolic hydrochloride, and were analyzed on a GLC system (HP 6890; Hewlett Packard, Palo Alto, CA, USA). Identification and quantification of the fatty acid methyl esters, as well as numerical analysis of fatty acid profiles, were performed according to the instructions of the Microbial Identification System (MIDI, Newark, DE, USA) using the ACTIN 6 database.[26] Genomic DNA from both isolates was prepared by sonication of cell suspensions, and 16S rRNA gene sequences were analyzed as described previously.[27] The phylogenetically closest neighbors of strains OM-6519^T and K04–0144^T were identified by BLAST search using the EzTaxon-e server (http://eztaxon-e.ezbiocloud.net).[3] Evolutionary distances were estimated using SeaView version 4.2.[28,29] Multiple alignments with selected sequences were performed using the ClustalW2 program. A phylogenetic tree was constructed based on the neighbor-joining method,[30] maximum-likelihood method[31] and maximum-parsimony method.[32] Data were re-sampled with 1000 bootstrap replications.[33] The values of sequence similarities with the closest strains were determined using the EzTaxon server.[3] For determination of the G+C content and performing DNA–DNA hybridizations, genomic DNA was prepared in accordance with the procedure of Saito and Miura.[34] DNA G+C content was determined by HPLC, according to the method of Tamaoka and Komagata,[35] and DNA–DNA hybridization was performed using the photobiotin-labeling method of Ezaki *et al.*[36]

RESULTS AND DISCUSSION

Taxonomic study of strain OM-6519^T

Strain OM-6519^T grew well on all of the tested media and formed brown colonies. Substrate mycelia fragmented under submerged growth conditions, but did not fragment when cultured on agar media. Gray aerial mycelia were abundantly produced on ISP 2 and 3. A yellow soluble pigment was also produced (Supplementary Table S1). The mature spore chains were straight and had more than 20 spores per chain. The spores were cylindrical with a rugose surface and size of 1.1–1.3 × 0.6–0.7 μm (Figure 1). The strain grew at 15–37 °C, pH 5–10 and 0–4% (w/v) NaCl. Optimum growth was observed at 18–30 °C and pH 6–10. Melanoid pigment was produced on ISP 7. Coagulation and peptonization of milk, nitrate reduction and hydrolysis of starch were positive, but gelatin liquefaction and cellulose hydrolysis were negative. The strain utilized D-glucose, D-fructose and sucrose as sole carbon sources, but did not utilize L-arabinose, D-xylose, raffinose, melibiose, D-mannitol, L-rhamnose or *myo*-inositol. L-Aspartic acid, L-threonine, glycine, L-phenylalanine, L-arginine and L-ornithine were utilized as sole nitrogen sources, but the strain did not utilize L-methionine or D-valine. Strain OM-6519^T contained LL-diaminopimelic acid as the diamino acid in whole-cell hydrolysates and the whole-cell sugar pattern consisted of glucose, ribose and rhamnose. Phosphatidylethanolamine was detected, but phosphatidylglycerol, diphosphatidylglycerol, phosphatidylcholine and phosphatidylinositol were not found. The major menaquinones were MK-9(H_6) (71%) and MK-9(H_8) (29%), and the predominant fatty acids were $C_{16:0}$ (29.6%), anteiso-$C_{15:0}$ (17.7%) and iso-$C_{15:0}$ (13.3%) (Supplementary Table S2). The G+C content of the genomic DNA was 73 mol%. These phenotypic and chemotaxonomic characteristics indicated that strain OM-6519^T belonged to the genus *Streptomyces*.

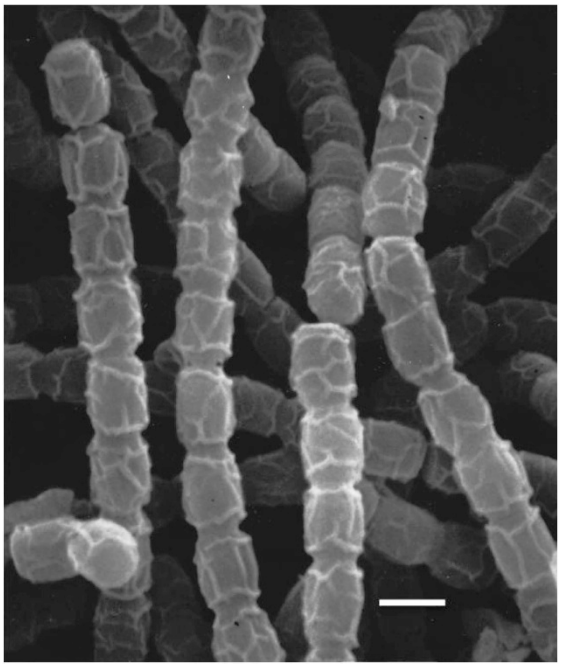

Figure 1 Scanning electron micrograph of strain OM-6519^T grown on ISP 4 for 2 weeks at 27 °C.

The nearly complete 16S rRNA gene sequence (1482 bp) of strain OM-6519^T was determined and deposited under the DDBJ accession number AB915215. Phylogenetic analysis with the type strains of 20 species showing the highest similarities to strain OM-6519^T indicated that the strain clustered with *Streptomyces xanthocidicus* NBRC 13469^T, *S. chrysomallus* subsp. *fumigatus* NBRC 15394^T and *S. aburaviensis* NRRL B-2218^T (Figure 2). The similarity values of the 16S rRNA gene sequences between the three strains and strain OM-6519^T were 99.7, 99.6 and 99.5%, respectively. Strain OM-6519^T was differed from the related strains with respect to spore surface morphology, as its closest phylogenetic neighbors had spores with a smooth surface, as opposed to rugose. Moreover, these strains also differed in several physiological characteristics, as only strain OM-6519^T utilized L-phenylalanine and L-ornithine as nitrogen sources, and did not utilize D-xylose as a sole source of carbon (Table 1). The levels of DNA–DNA relatedness between strain OM-6519^T and *S. xanthocidicus* NBRC 13469^T, *S. chrysomallus* subsp. *fumigatus* NBRC 15394^T and *S. aburaviensis* NRRL B-2218^T were 57 ± 7%, 41 ± 10% and 44 ± 6%, respectively. These values were clearly below the 70% threshold value for assigning strains to the same species.[37]

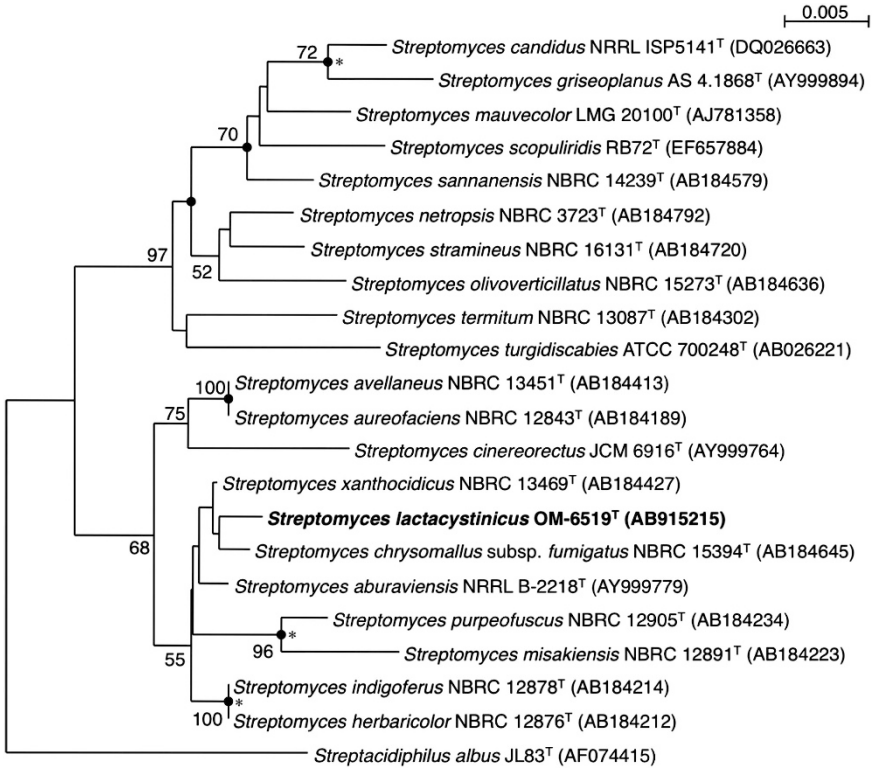

Figure 2 Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences from strain OM-6519^T and the type strains of related species in the genus *Streptomyces*. Only bootstrap values above 50% (percentages of 1000 replications) are indicated. Branches marked with a solid circle were also recovered in the maximum-likelihood tree; asterisks show branches recovered in the maximum-parsimony tree. Bar, 0.005 nucleotide substitutions per site.

Table 1 Morphological and cultural features and physiologically differential characteristics of strain OM-6519^T and type strains of related species

	OM-6519^T	*S. xanthocidicus NBRC 13469^T*	*S. chrysomallus subsp. fumigatus NBRC 15394^T*	*S. aburaviensis NRRL B-2218^T*
Spore chain	Straight	Straight	Straight	Straight
Spore surface	Rugose	Smooth	Smooth	Smooth
Colony color on ISP 3	Light brown	Pale yellowish brown	Yellowish brown	Yellowish brown
Colony color on ISP 7	Yellowish brown	Dark yellowish brown	Dark Brown	Dark yellowish brown
Aerial mass color on ISP 3	Shadow gray	White	Silver gray	Shadow gray
Aerial mass color on ISP 7	Whitish purple	Pinkish white	Ivory tint	Shadow gray
Nitrate reduction	+	–	–	+
Growth on 5% NaCl	–	+	+	–
Carbon utilization				
L-Arabinose	–	+	+	–
D-Xylose	–	+	+	+
Raffinose	–	+	–	+
Melibiose	–	–	–	+
D-Fructose	w	+	w	w
Sucrose	w	+	w	–
Nitrogen utilization				
L-Phenylalanine	+	–	–	–
L-Arginine	+	+	+	–
L-Ornithine	+	–	–	–

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

Based on the above results, we propose that strain OM-6519^T represents a novel species in the genus *Streptomyces*, for which the name *Streptomyces lactacystinicus* sp. nov. is proposed.

Taxonomic study of strain K04–0144^T

Strain K04–0144^T grew well on ISP 2, 4, 6 and 7, and formed brown colonies. Substrate mycelia did not show fragmentation. Gray aerial mycelia were abundantly produced on ISP 2, 3, 4 and 7. A yellow soluble pigment was produced (Supplementary Table S3). The mature spore chains were spiral and had more than 20 spores per chain. Spores were cylindrical with a smooth surface and size of 1.0–1.3 × 0.7–0.8 μm (Figure 3). Strain K04–0144^T grew at 12–42 °C, pH 4–10 and 0–7% (w/v) NaCl. Optimum growth was observed at 16–36 °C and pH 5–10. The strain did not produce melanoid pigment. Coagulation and peptonization of milk, nitrate reduction, gelatin liquefaction and starch hydrolysis were positive, but cellulose hydrolysis was negative. The strain utilized D-glucose, D-xylose, raffinose, melibiose, D-mannitol, D-fructose and L-rhamnose as sole carbon sources, but did not utilize L-arabinose, *myo*-inositol or sucrose. L-Aspartic acid, L-threonine, glycine, L-phenylalanine, L-arginine, L-methionine and L-ornithine were utilized as sole nitrogen sources, but D-valine was not utilized. Strain K04–0144^T contained LL-diaminopimelic acid as a diamino acid in whole-cell hydrolysates, the whole-cell sugar pattern consisted of glucose and mannose. Phosphatidylethanolamine and diphosphatidylglycerol were detected, but phosphatidylglycerol, phosphatidylcholine and phosphatidylinositol were not found. The major menaquinones were MK-9 (H_8) (62%) and MK-9 (H_6) (35%) and the predominant fatty acids were iso-$C_{16:0}$ (22.5%), anteiso-$C_{15:0}$ (22.5%) and iso-$C_{15:0}$ (13.7%) (Supplementary Table S2). The G+C content of the genomic DNA was 73 mol%. These phenotypic and chemotaxonomic characteristics indicated that strain K04–0144^T belonged to the genus *Streptomyces*.

The nearly complete 16S rRNA gene sequence (1484 bp) of strain K04–0144^T was determined and was deposited under the DDBJ accession number AB915216. Phylogenetic analysis based on the sequences of the type strains of the 20 species showing the highest similarities to strain K04–0144^T showed that strain K04–0144^T clustered with eight strains (Figure 4). *Streptomyces corchorusii* NBRC 13032^T showed the highest similarity with strain K04–0144^T (99.4%), followed by *S. olivaceoviridis* NBRC 15394^T (99.4%) and *S. canarius* NRRL B-2218^T (99.3%). However, strain K04–0144^T differed physiologically from these three species with respect to nitrate reduction, gelatin liquefaction, utilization of L-arabinose, *myo*-inositol and sucrose as sole carbon sources, and utilization of L-threonine and L-methionine as sole nitrogen sources (Table 2). The levels of DNA–DNA relatedness between strain K04–0144^T and *S. corchorusii* NBRC 13032^T, *S. olivaceoviridis* NBRC 15394^T and *S. canarius* NRRL B-2218^T were 32 ± 15%, 35 ± 13% and 38 ± 18%, respectively. These values are clearly below the 70% threshold value for assigning strains to the same species.[37]

Based on these findings, we propose that strain K04–0144^T represents a novel species in the genus *Streptomyces*, for which the name *Streptomyces cyslabdanicus* sp. nov. is proposed.

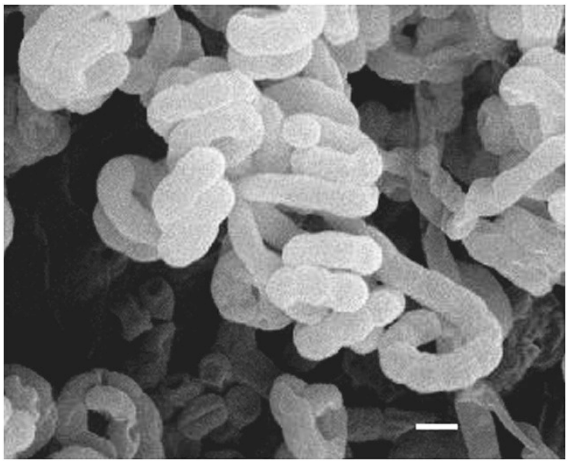

Figure 3 Scanning electron micrograph of strain K04–0144^T grown on ISP 2 for 2 weeks at 27 °C.

Description of *Streptomyces lactacystinicus* sp. nov

Streptomyces lactacystinicus (lac.ta.cys.ti'ni.cus. N.L. n. *lactacystinum*, lactacystin; L. masc. suff. *-icus*, suffix used with the sense of pertaining to; N.L. masc. adj. *lactacystinicus*, pertaining to lactacystin, a proteasome inhibitor produced by the organism).

A Gram-positive, aerobic actinomycete that forms straight spore chains. The spores are cylindrical with a rugose surface and size of 1.1–1.3 × 0.6–0.7 μm. Grows well on ISP 2, 3, 4, 5, 6 and 7, and forms brown colonies. Gray aerial mycelia are abundantly produced on ISP 2 and 3. A yellow soluble pigment is produced. Growth occurs at 15–37 °C and pH 5–10. No growth occurs at 5% (w/v) NaCl. Melanoid pigment is not produced. Milk is coagulated and peptonized. Nitrate is reduced to nitrite. Gelatin is not liquefied. Starch is hydrolyzed, but cellulose is not. D-glucose, D-fructose, sucrose, L-aspartic acid, L-threonine, glycine, L-phenylalanine, L-arginine and L-ornithine are utilized as sole carbon and nitrogen sources. L-Arabinose, D-xylose, raffinose, melibiose, D-mannitol, L-rhamnose, *myo*-inositol, L-methionine and D-valine are not utilized. Whole-cell hydrolysate contains LL-diaminopimelic acid as the diamino acid, in addition to glucose, ribose, and rhamnose. The polar lipids mainly consist of phosphatidylethanolamine. The major menaquinones are MK-9 (H_6) and MK-9 (H_8). The predominant fatty acids are $C_{16:0}$, anteiso-$C_{15:0}$, and iso-$C_{15:0}$. The G+C content of the genomic DNA of the type strain is 73 mol%. The type strain, OM-6519^T (= NBRC 110082^T, DSM 43136^T), was isolated from soil from Inba, Chiba, Japan, and produces lactacystin, a proteasome inhibitor.

Description of *Streptomyces cyslabdanicus* sp. nov

Streptomyces cyslabdanicus (cys.lab.da'ni.cus. N.L. n. *cyslabdanum*, cyslabdan; L. masc. suff. *-icus*, suffix used with the sense of pertaining to; N.L. masc. adj. *cyslabdanicus*, pertaining to cyslabdan, an antibiotic produced by the organism).

A Gram-positive, aerobic actinomycete that forms spiral spore chains. The spores are cylindrical with a smooth surface and size of 1.0–1.3 × 0.7–0.8 μm. Mycelia do not fragment. Grows well on ISP 2, 4, 6 and 7, and form brown colonies. Gray aerial mycelia are abundantly produced on ISP 2, 3, 4 and 7. A yellow soluble pigment is produced on agar media. Growth occurs at 12–42 °C, pH 4–10 and 0–7% (w/v) NaCl. Melanoid pigment is not produced. Milk is coagulated and peptonized. Nitrate is reduced to nitrite. Gelatin is liquefied. Starch is hydrolyzed, but cellulose is not. D-glucose, D-xylose, raffinose, melibiose, D-mannitol, D-fructose, D-rhamnose, L-aspartic acid, L-threonine, glycine, L-phenylalanine, L-arginine, L-ornithine and L-methionine are utilized as sole carbon and nitrogen sources. L-Arabinose, *myo*-inositol, sucrose and D-valine are not utilized. Whole-cell hydrolysate contains LL-diaminopimelic acid as the diamino acid, in addition to glucose and mannose. The main polar lipids

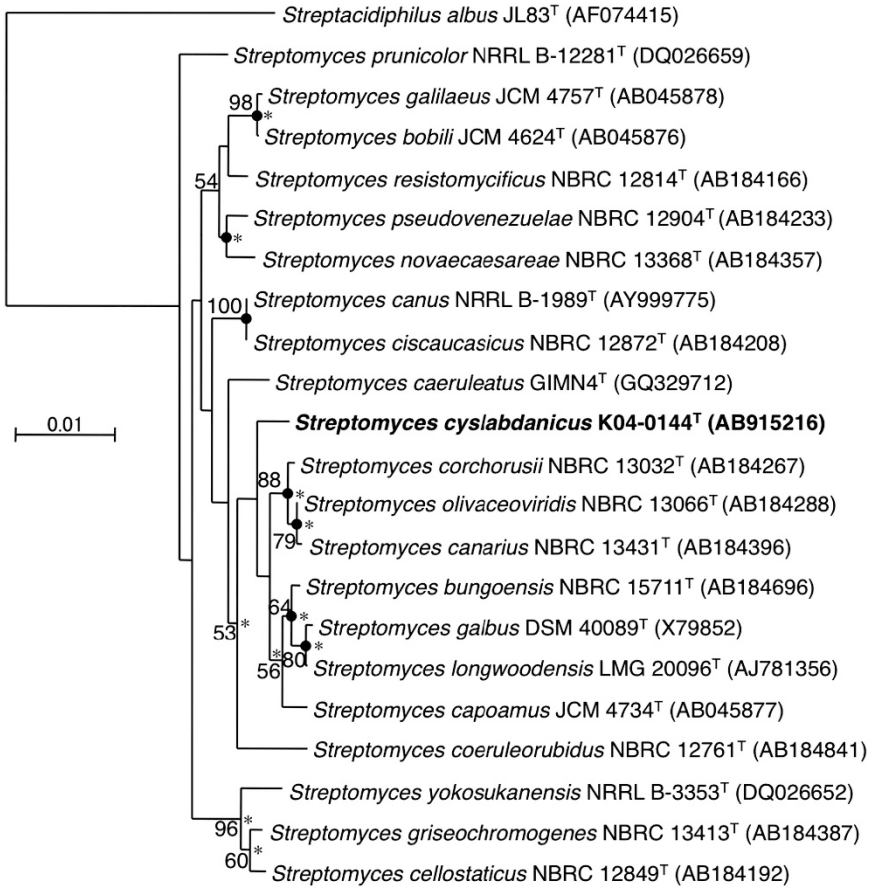

Figure 4 Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences from strain K04–0144^T and the type strains of related species in the genus *Streptomyces*. Only bootstrap values above 50% (percentages of 1000 replications) are indicated. Branches marked with a solid circle were also recovered in the maximum-likelihood tree asterisks show branches recovered in the maximum-parsimony tree. Bar, 0.01 nucleotide substitutions per site.

Table 2 Morphological and cultural features and physiologically differential characteristics of strain K04–0144^T and type strains of related species

	K04–0144^T	*S. corchorusii NBRC 13032^T*	*S. olivaceoviridis NBRC 13066^T*	*S. canarius NBRC 13431^T*
Spore chain	Spiral	Spiral	Spiral	Spiral
Spore surface	Smooth	Smooth	Smooth	Smooth
Colony color on ISP 3	Olive black	Yellow	Pale yellowish brown	Dark yellowish brown
Colony color on ISP 5	Dark brown	Yellow	Light olive gray	Grayish olive
Aerial mass color on ISP 3	Silver Gray	Dark olive gray	White	Celadon
Aerial mass color on ISP 5	Bluish gray	White	White	Greenish gray
Nitrate reduction	+	–	–	–
Coagulation of milk	+	+	+	–
Liquefaction of Gelatin	+	–	–	–
Growth at pH 4.0	+	+	–	–
Carbon utilization				
L-Arabinose	–	+	+	+
Raffinose	w	+	w	+
myo-Inositol	–	+	+	+
Sucrose	–	+	+	+
Nitrogen utilization				
L-Threonine	+	–	–	–
L-Phenylalanine	+	–	+	+
L-Methionine	+	–	–	–

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

are phosphatidylethanolamine and diphosphatidylglycerol. The major menaquinones are MK-9 (H_6) and MK-9 (H_8). The predominant fatty acids are iso-$C_{16:0}$, anteiso-$C_{15:0}$, and iso-$C_{15:0}$. The G+C content of the genomic DNA of the type strain is 73 mol%. The type strain, K04–0144^T (=NBRC 110081^T, DSM 42135T), was isolated from soil from Ishigaki Island, Okinawa, Japan, and produces cyslabdan, a bioactive compound that enhances the anti-MRSA activity of imipenem medication.

ACKNOWLEDGEMENTS

We would like to thank Professor Jean P. Euzéby (Society for Systematic and Veterinary Bacteriology) for his help with the nomenclature.

1 Bérdy, J. Thoughts and facts about antibiotics: where we are now and where we are heading. *J. Antibiot.* **65**, 385–395 (2012).
2 Xu, L.-H., Li, Q.-R. & Jiang, C.-L. Diversity of soil actinomycetes in Yunnan, China. *Appl. Environ. Microbiol.* **62**, 244–248 (1996).
3 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).
4 Burg, R. W. *et al.* Avermectins, new family of potent anthelmintic agents: producing organism and fermentation. *Antimicrob. Agents Chemother.* **15**, 361–367 (1979).
5 Ōmura, S. *et al.* Herbimycin, a new antibiotic produced by a strain of Streptomyces. *J. Antibiot.* **32**, 255–261 (1979).
6 Ōmura, S. *et al.* Lactacystin, a novel microbial metabolite, induces neuritogenesis of neuroblastoma cells. *J. Antibiot.* **44**, 113–116 (1991).
7 Fukumoto, A. *et al.* Cyslabdan, a new potentiator of imipenem activity against methicillin-resistant *Staphylococcus aureus*, produced by *Streptomyces* sp. K04-0144 I. Taxonomy, fermentation, isolation and structural elucidation. *J. Antibiot.* **61**, 1–6 (2008).
8 Ōmura, S. *et al.* Structure of lactacystin, a novel microbial metabolite which induces differentiation of neuroblastoma cells. *J. Antibiot.* **44**, 117–118 (1991).
9 Fenteany, G. *et al.* Inhibition of proteasome activities and subunit-specific amino-terminal threonine modification by lactacystin. *Science* **268**, 726–731 (1995).
10 Groll, M. *et al.* Structure of 20S proteasome from yeast at 2.4 Å resolution. *Nature* **386**, 463–471 (1997).
11 Takahashi, S. *et al.* Biosynthesis of lactacystin. *J. Antibiot.* **48**, 1015–1020 (1995).
12 Nagamitsu, T. *et al.* Total synthesis of (+)-lactacystin. *J. Am. Chem. Soc.* **118**, 3584–3590 (1996).
13 Uchida, R. *et al.* Nosokomycins, new antibiotics discovered in an in vivo-mimic infection model using silkworm larvae I: fermentation, isolation and biological properties. *J. Antibiot.* **63**, 151–155 (2010).
14 Fukumoto, A. *et al.* Cyslabdan, a new potentiator of imipenem activity against methicillin-resistant *Staphylococcus aureus*, produced by *Streptomyces* sp. K04-0144 II. Biological activities. *J. Antibiot.* **61**, 7–10 (2008).
15 Koyama, N. *et al.* The non-antibiotic small molecule cyslabdan enhances the potency of β-lactams against MRSA by inhibiting pentaglycine interpeptide bridge synthesis. *PLoS ONE* **7**, e48981 (2012).
16 Sekiguchi, M., Shigemori, H., Ohsaki, A. & Kobayashi, J. Pacovatinins A-C, new labdane diterpenoids from the seeds of Renealmia exaltata. *J. Nat. Prod.* **64**, 1102 (2001).
17 Yuan, L., Zhao, P.-J., Juan, M., Lu, C.-H. & Shen, Y.-M. Labdane and tetranorlabdane diterpenoids from Botryosphaeria sp. MHF, an endophytic fungus of Maytenus hookeri. *Helv. Chim. Acta* **92**, 1118–1125 (2009).
18 Shirling, E. B. & Gottlieb, D. Methods for characterization of Streptomyces species. *Int. J. Syst. Bacteriol.* **16**, 313–340 (1966).
19 Taylor, H. D., Knoche, L. & Grauville, W. C. *Color Harmony Manual*4th edn.Container Corporation of America: Chicago, (1958).
20 Gordon, R. E., Barnett, D. A., Handerhan, J. E. & Pang, C. H. N. Nocardia coeliaca, Nocardia autotrophica and the nocardin strain. *Int. J. Syst. Bacteriol.* **24**, 54–63 (1974).
21 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).
22 Williams, S. T. *et al.* Numerical classification of Streptomyces and related genera. *J. Gen. Microbiol.* **129**, 1743–1813 (1983).
23 Becker, B., Lechevalier, M. P. & Lechevalier, H. A. Chemical composition of cell-wall preparation from strains of various form-genera of aerobic actinomycetes. *Appl. Microbiol.* **13**, 236–243 (1965).
24 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).
25 Collins, M. D., Pirouz, T., Goodfellow, M. & Minnikin, D. E. Distribution of menaquinones in actinomycetes and corynebacteria. *J. Gen. Microbiol.* **100**, 221–230 (1977).
26 Sasser, M. *Identification of bacteria by gas chromatography of cellular fatty acids. MIDI Technical Note 101* (MIDI Inc: Newark, DE, 1990).
27 Inahashi, Y., Matsumoto, A., Danbara, H., Ōmura, S. & Takahashi, Y. Phytohabitans suffuscus gen. nov., sp. nov., a novel actinomycete of the family Micromonosporaceae isolated from a plant root. *Int. J. Syst. Evol. Microbiol.* **60**, 2652–2658 (2010).
28 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).
29 Gouy, M., Guindon, S. & Gascuel, O. SeaView version 4: A multiplatform graphical user interface for sequence alignment and phylogenetic tree building. *Mol. Biol. Evol.* **27**, 221–224 (2010).
30 Saitou, N. & Nei, M. The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* **4**, 406–425 (1987).
31 Felsenstein, J. Evolutionary trees from DNA sequences: A maximum likelihood approach. *J. Mol. Evol.* **17**, 368–379 (1981).
32 Fitch, W. M. Toward defining the course of evolution: minimum change for a species tree topology. *Syst. Biol.* **20**, 406–416 (1971).
33 Felsenstein, J. Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* **39**, 783–791 (1985).
34 Saito, H. & Miura, K. Preparation of transforming deoxyribonucleic acid by phenol treatment. *Biochim. Biophys. Acta.* **72**, 619–629 (1963).
35 Tamaoka, J. & Komagata, K. Determination of DNA base composition by reversed-phase high-performance liquid chromatography. *FEMS Microbiol. Lett.* **25**, 125–128 (1984).
36 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).
37 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).

Supplementary Information accompanies the paper on The Journal of Antibiotics website (http://www.nature.com/ja)