Description of two novel species of the genus *Kitasatospora* Ōmura *et al.* 1982, *Kitasatospora cineracea* sp. nov. and *Kitasatospora niigatensis* sp. nov.

Research Center for
Biological Function, The
Kitasato Institute, 5-9-1,
Shirokane, Minato-ku,
Tokyo 108-8642, Japan

Koichi Tajima, Yōko Takahashi, Akio Seino, Yuzuru Iwai
and Satoshi Ōmura

Author for correspondence: Satoshi Ōmura. Tel: +81 3 3444 6161. Fax: +81 3 3444 8360.
e-mail: omura-s@kitasato.or.jp

Five actinomycete strains, SK-3255^T, SK-3406^T, SK-3412, SK-3421 and OM-5023, were isolated using a novobiocin-containing agar medium from soil samples. These strains produced long spore chains on aerial mycelium and contained LL- and meso-diaminopimelic acids (DAPs) in the cell wall. On the basis of morphological and chemotaxonomic characteristics and phylogenetic analysis, these five strains were classified into the genus *Kitasatospora*. DNA–DNA hybridization and comparison of physiological characteristics revealed that strains SK-3255^T and SK-3406^T differed from known species. Strains SK-3406^T, SK-3412 and SK-3421 were regarded as the same species. Strain OM-5023 was identified as *Kitasatospora griseola*. Therefore, two novel species are proposed, *Kitasatospora cineracea* sp. nov. and *Kitasatospora niigatensis* sp. nov., with the type strains *K. cineracea* SK-3255^T (= IFO 16452^T = JCM 10915^T = NRRL B-23134^T) and *K. niigatensis* SK-3406^T (= IFO 16453^T = JCM 10916^T = NRRL B-24135^T).

Keywords: *Kitasatospora cineracea* sp. nov., *Kitasatospora niigatensis* sp. nov., soil bacteria, entire 16S rDNA

INTRODUCTION

The actinomycete genus *Kitasatospora* was proposed by Ōmura *et al.* (1982). *Kitasatospora* strains contain both LL-diaminopimelic acid (DAP) and meso-DAP as diamino acids in the cell wall. Aerial spores on solid culture and submerged spores in liquid culture contain LL-DAP, while mycelium in both cultures mainly contains meso-DAP (Ōmura *et al.*, 1981, 1982; Takahashi *et al.*, 1984a). These properties are unique characteristics of *Kitasatospora* strains. Members of the genus *Kitasatospora* were transferred to the genus *Streptomyces* on the basis of rRNA-targeted oligonucleotide probes and phenotypic properties (Wellington *et al.*, 1992). However, the genus *Kitasatospora* was later revived by phylogenetic study

based on 16S rDNA sequence data (Zhang *et al.*, 1997).

The physiological regulation and mode of spore formation of *Kitasatospora* strains were investigated by Takahashi *et al.* (1991, 1996). These data showed that *Kitasatospora* strains were different from *Streptomyces* strains in their mode of submerged spore formation. While the submerged spores of *Streptomyces griseus* were produced abundantly by random fragmentation of the mycelium, those of *Kitasatospora* strains were newly produced at the tips of the mycelium and the chains were released from the mycelium to yield the submerged spores.

To date, nine species of the genus *Kitasatospora* have been recognized in accordance with the Bacteriological Code: *Kitasatospora setae* Ōmura *et al.* 1983, *Kitasatospora phosalacinea* Takahashi *et al.* 1985, *Kitasatospora griseola* Takahashi *et al.* 1985, *Kitasatospora mediocidica* Labeda 1988, *Kitasatospora cystarginea* Kusakabe and Isono 1992, *Kitasatospora cochleata* (Nakagaito *et al.* 1993) Zhang *et al.* 1997,

Abbreviations: DAP, diaminopimelic acid; ISP, International Streptomyces Project.

The DDBJ accession numbers for the 16S rDNA sequences of strains SK-3255^T, SK-3406^T, SK-3412, SK-3421 and OM-5023 are respectively AB022875–AB022879.

Table 1. Cultural characteristics of *Kitasatospora* strains newly isolated from soils

Colour names and codes (in parentheses) are taken from the *Color Harmony Manual* (Taylor *et al.*, 1958). Strains SK-3255^T, SK-3406^T, SK-3412 and SK-3421 did not produce soluble pigments on any of the media listed.

Medium	SK-3255 ^T			SK-3406 ^T , SK-3412 and SK-3421			OM-5023		
	Growth	Aerial mycelium	Growth	Growth	Aerial mycelium	Growth	Aerial mycelium	Growth	Soluble pigment
Yeast extract/malt extract agar (ISP 2)	Good, camel (3ie)	Abundant, pussy willow-grey (5de)	Good, ashes (5fe)	Good, camel (3ie)	Abundant, orchid tint (10ba)	Good, camel (3ie)	Moderate, light grey (c)	None	None
Oatmeal agar (ISP 3)	Good, pearl (3ba)	Moderate, ashes (5fe)	Good, sand (2ec)	Moderate, light wheat (2ea)	Abundant, ashes (71/2ia)	Moderate, light wheat (2ea)	Very poor, grey (g)	Light wheat (2ea)	Light wheat (2ea)
Inorganic salts/starch agar (ISP 4)	Good, bamboo (2ec)	Moderate, silver-grey (3fe)	Good, covert tan (2 ge)	Good, bamboo (2 ge)	Poor, sand (3cb)	Good, bamboo (2 ge)	Abundant, shadow-grey (5ih)	None	None
Glycerol/asparagine agar (ISP 5)	Good, light mustard-tan (2ie)	Abundant, oyster-white (b)	Good, covert tan (2 ge)	Good, camel (3ie)	Abundant, ashes (5fe)	Good, camel (3ie)	Abundant, grey (g)	Pastel yellow (11/2fb)	Pastel yellow (11/2fb)
Peptone/yeast extract iron agar (ISP 6)	Good, oatmeal (2ec)	Very poor	Moderate, ivory (2db)	Good, ivory (2db)	Moderate, sand (3cb)	Good, ivory (2db)	None	None	None
Tyrosine agar (ISP 7)	Good, light mustard-tan (2ie)	Abundant, silver-grey (3fi)	Moderate, pussy willow-grey (5de)	Good, light-brown (3lg)	Abundant, ashes (5fe)	Good, light-brown (3lg)	Abundant, shadow-grey (5ih)	Pastel yellow (11/2fb)	Pastel yellow (11/2fb)
Sucrose/nitrate agar	Good, natural (3dc)	Moderate, oyster-white (b)	Moderate, natural (3dc)	Moderate, ivory tint (2cb)	Moderate, sand (3cb)	Moderate, ivory tint (2cb)	Very poor, sand (3cb)	None	None

Kitasatospora paracochleata (Nakagaito *et al.* 1993) Zhang *et al.* 1997, *Kitasatospora azatica* (Nakagaito *et al.* 1993) Zhang *et al.* 1997 and *Kitasatospora cheerisanensis* Chung *et al.* 1999 (Omura *et al.*, 1982, 1985; Takahashi *et al.*, 1984b; Labeda, 1988; Kusakabe & Isono, 1988; Nakagaito *et al.*, 1992a; Chung *et al.*, 1999). Strains of the genus have been found to produce more than 10 new compounds, with a variety of biological activities and structures (Takahashi *et al.*, 1999). Since *Kitasatospora* strains seem to be useful as a microbial source in screening for new bioactive compounds, we have tried selective isolation of *Kitasatospora* strains from soil samples. Based on the observation that *Kitasatospora* strains were resistant to novobiocin (Iwai & Takahashi, 1992), we carried out the isolation of actinomycetes from soil samples using a novobiocin-containing agar medium. We isolated 174 novobiocin-resistant actinomycetes from 13 different soil samples. Five of them, strains SK-3255^T, SK-3406^T, SK-3412, SK-3421 and OM-5023, were identified as belonging to the genus *Kitasatospora*. The taxonomic characterization of these five actinomycete strains is presented here.

METHODS

Selective isolation of novobiocin-resistant strains. Actinomycete strains SK-3255^T and OM-5023 were isolated from soil samples collected in Nishigahara, Kita-ku, Tokyo, Japan. Strains SK-3406^T, SK-3412 and SK-3421 were isolated from soil samples collected in Niigata City, Japan. These strains were isolated by using a novobiocin-containing agar medium consisting of 1 % potato starch, 1 % glycerol, 0.2 % K₂HPO₄, 0.2 % (NH₄)₂SO₄, 0.1 % MgSO₄·7H₂O, 0.1 % NaCl, 0.2 % CaCO₃, 50 µg novobiocin ml⁻¹ and 1.2 % agar (pH 7.0).

Micro-organisms. The following strains were used for comparative studies: *K. setae* KM-6054^T, *K. phosalacinea* KA-338^T, *K. griseola* KM-9660^T, *K. cochleata* IFO 14768^T, *K. cystarginea* IFO 14836^T, *K. paracochleata* IFO 14769^T and *Streptomyces kifunensis* IFO 15206^T.

Morphological characteristics. Cultures grown on inorganic salts/starch agar for 2 weeks at 28 °C were observed with a scanning electron microscope (model S-430; Hitachi). Samples for scanning electron microscopy were prepared by fixing the agar block in osmium tetroxide vapour for 12 h, dehydrating by critical-point drying and then sputter coating with palladium under vacuum. Cells grown in yeast extract/dextrose medium with submerged cultures were observed using a light microscope (model VANOX-S; Olympus).

Cultural and physiological characteristics. The International *Streptomyces* Project (ISP) media recommended by Shirling & Gottlieb (1966) were used to investigate cultural and physiological characteristics. Cultures were observed after incubation at 27 °C for 2 weeks. Colours and hues were determined according to the *Color Harmony Manual* (Taylor *et al.*, 1958). Utilization of carbon sources was tested by growth on carbon utilization medium (ISP 9) (Pridham & Gottlieb, 1948) supplemented with 1 % carbon sources at 27 °C for 2 weeks. Temperature ranges for growth were determined on inorganic salts/starch agar (ISP 4) using a temperature gradient incubator (Toyo Kagaku Sangyo).

Chemotaxonomic characterization. Isomers of DAP in whole-cell hydrolysates were determined by using TLC

Fig. 1. Scanning electron micrographs of the spore chains of strains SK-3255^T (a) and SK-3406^T (b) grown on inorganic salts/starch agar for 14 d at 27 °C. Bars, 1 µm.

Fig. 2. Light micrograph of strain SK-3255^T grown in yeast extract/dextrose medium for 2 d at 27 °C. The arrow shows a submerged spore. Bar, 10 µm.

according to the methods of Becker *et al.* (1965) and Hasegawa *et al.* (1983). The *N*-acyl types of muramic acid were determined using the colorimetric method of Uchida & Aida (1984). Whole-cell sugars were analysed by the method of Becker *et al.* (1965). Mycolic acids were detected by TLC according to Tomiyasu (1982). Menaquinones were extracted and purified according to Collins *et al.* (1977) and were analysed using HPLC equipped with a CAPCELL PAK C18 column (Shiseido) (Tamaoka *et al.*, 1983). Phospholipids were extracted and identified following the method of Minnikin *et al.* (1977).

DNA base composition and DNA–DNA hybridization. Chromosomal DNA was prepared following the method of Marmur (1961). The G + C content of DNA was determined by the HPLC method as described by Tamaoka & Komagata (1984). DNA–DNA hybridization was carried out according to the method of Ezaki *et al.* (1989).

Analysis of 16S rDNA sequence. The 16S rDNAs were amplified by PCR with a PCR thermal cycler (TaKaRa) using three pairs of universal primers: 11F (5' AGTTTG-ATCATGGCTCAG 3') and 925R (5' CGTCAATTCAT-TTGAGTT 3'), 1115R (5' AGGGTTGCGCTCGTTG 3') and 685F (5' GTAGCGGTGAAATGCGTA 3') and 1510R (5' GGTTACCTTGTTACGACT 3') and 685F (5' GTAG-CGGTGAAATGCGTA 3'). We also used two primer sets newly designed by the authors for the amplification and sequencing of the terminal regions of the 16S rRNA genes: 117R (5' CCCACGTGTTACTCACCC 3') and E-213F (5' AGCGTCCGTTTCCTTGAGA 3'), located outside the 16S rRNA gene, for the 5'-terminal region, and SKS90R (5' TCAACGTTCACCCATGAGC 3'), located within the 16S–23S rDNA internal transcribed spacer, and 1344F (5' CTAGTAATCGCAGATCAGCA 3'), for the 3'-terminal region. The PCR was performed with the following protocol: initial denaturation at 94 °C for 1 min followed by 30 cycles of denaturation at 94 °C for 1 min, annealing at 50 °C for 1 min and extension at 72 °C for 1.5 min, with a final extension step at 72 °C for 2 min. The PCR products were purified by Prep-A-Gene (Bio-Rad) and were sequenced directly with an ABI model 373A automatic DNA sequencer and a PRISM Ready Reaction Dye Primer cycle sequencing kit (Applied Biosystems). Sequences of the other members of the genus *Kitasatospora* used for alignment and calculation of similarity levels were obtained from the DDBJ database. The sequences were checked manually and ambiguous positions were removed. CLUSTAL W (Thompson *et al.*, 1994) was then used to generate evolutionary distances (the K_{nuc} value of Kimura, 1980) and similarity values and to reconstruct the phylogenetic tree by the neighbour-joining method (Saitou & Nei, 1987). The topology of the tree was evaluated by performing a bootstrap analysis (Felsenstein, 1985) using 1000 resamplings. The phylogenetic tree was drawn using the TREEVIEW software.

Nucleotide sequence accession numbers. The 16S rDNA sequences of the five isolates, five known *Kitasatospora* species and *Streptomyces kifunensis* determined in this study were deposited in the DDBJ nucleotide sequence database; these accession numbers (with an AB prefix) are shown in Fig. 3.

RESULTS

Morphological characteristics

Fig. 1 shows scanning electron micrographs of aerial spores of strains SK-3255^T and SK-3406^T. The other

Table 2. Physiological characteristics that differentiate strains SK-3255^T and SK-3406^T from related *Kitasatospora* species

Taxa are identified as: 1, strain SK-3255^T; 2, strain SK-3406^T; 3, *K. setae* KM-6054^T; 4, *K. phosalacinea* KA-338^T; 5, *K. griseola* AM-9660^T; 6, *K. cochleata* IFO 14768^T; 7, *K. cystarginea* IFO 14836^T; 8, *K. paracochleata* IFO 14769^T. Data were taken from this study (strains SK-3255^T and SK-3406^T), Omura *et al.* (1982) (*K. setae* KM-6054^T), Takahashi *et al.* (1984b) (*K. phosalacinea* KA-338^T and *K. griseola* AM-9660^T), Nakagaito *et al.* (1992a) (*K. cochleata* IFO 14768^T and *K. paracochleata* IFO 14769^T) and Nakagaito *et al.* (1992b) (*K. cystarginea* IFO 14836^T). Characters are scored as: +, positive; −, negative; ND, not determined. All strains are positive for peptonization of milk, hydrolysis of starch and utilization of D-glucose. All strains are negative for liquefaction of gelatin and utilization of inositol and D-mannitol.

Characteristic	1	2	3	4	5	6	7	8
Fermentation of melanoid pigment	−	−	−	−	−	+	−	+
Reduction of nitrate	−	+	−	+	−	−	−	+
Coagulation of milk	−	−	+	−	+	−	−	−
Utilization of:								
L-Arabinose	+	+	+	+	+	+	−	−
D-Xylose	+	+	+	+	+	−	−	−
Raffinose	+	−	−	+	+	−	−	−
Melibiose	+	−	−	−	−	ND	ND	ND
D-Fructose	−	−	−	+	−	−	−	+
D-Rhamnose	+	−	−	+	−	−	−	−
Sucrose	−	−	−	+	−	−	−	−
Cellulose	−	−	−	−	−	ND	ND	ND
Temperature for growth (°C)	15–37	15–41	15–37	15–42	15–37	13–38	17–40	11–38
DNA G+C content (mol%)	73.7	73.5	73.1	73.4	73.1	72.4	70.6	73.1

novel isolates formed similar spores. These strains produced aerial mycelia that consisted of long straight chains of 20 or more spores with smooth surfaces. No fragmentation of the vegetative mycelium, sporangium, zoospore or sclerotium was observed. Fig. 2 shows a light micrograph of strain SK-3255^T in a submerged culture. The strain formed submerged spores in yeast extract/dextrose medium. The other four novel isolates also formed submerged spores (data not shown).

Cultural and physiological characteristics

Table 1 shows the cultural characteristics of the novel isolates. The five strains grew well on both synthetic and complex media. Table 2 shows physiological characteristics of strains SK-3255^T and SK-3406^T. Strains SK-3412 and SK-3421 showed the same cultural characteristics and physiological properties as SK-3406^T. The spore mass of strain SK-3255^T was oyster-white to silver-grey and no soluble pigment was produced. The strain utilized L-arabinose, D-xylose, raffinose, melibiose and D-rhamnose. The temperature range for growth was 15–37 °C. The spore mass of strain SK-3406^T was brownish white to brownish grey and no soluble pigment was produced. The strain utilized L-arabinose and D-xylose. The temperature range for growth was 15–41 °C. The spore mass of strain OM-5023 was light grey to shadow-grey and yellowish soluble pigments were produced on oatmeal agar and glycerol/asparagine agar.

Chemotaxonomy

The five strains contained similar amounts of LL-DAP and *meso*-DAP in whole-cell hydrolysates of cells in submerged culture. Aerial spores on solid culture on inorganic salts/starch agar and submerged spores in liquid culture in yeast extract/dextrose medium contained LL-DAP. The vegetative mycelium and submerged mycelium mainly contained *meso*-DAP.

Galactose, mannose and ribose, but no arabinose or xylose, were detected in whole-cell hydrolysates. The diagnostic phospholipids phosphatidylethanolamine and phosphatidylinositol were detected, but phosphatidylcholine was not. This pattern corresponds to phospholipid type II (Lechevalier *et al.*, 1977). MK-9(H₆) and MK-9(H₈) were detected as predominant menaquinones. The *N*-acyl type of muramic acid in peptidoglycan was the acetyl type. No mycolic acid was detected.

16S rDNA analysis

Almost-complete 16S rDNA sequences were determined for the five novel isolates and for five previously described *Kitasatospora* species and *Streptomyces kifunensis* IFO 15206^T using our newly designed primers. The lengths of the 16S rDNA sequences of *Kitasatospora* strains ranged from 1515 to 1523 bp. The sequences of both the 5'- and 3'-terminal regions were conserved among the genus *Kitasatospora* strains.

Fig. 3. A neighbour-joining dendrogram based on 1400 bp of the 16S rRNA gene sequences. The tree was validated by a bootstrap analysis (1000 replications) and values greater than 50% are indicated at the nodes. Bar, 0.01 substitutions per nucleotide position.

Table 3. DNA homologies among strains SK-3255^T, SK-3406^T, SK-3412, SK-3421 and OM-5023 and related *Kitasatospora* species

Strain	Source of photobiotin-labelled DNA:				
	SK-3255 ^T	SK-3406 ^T	SK-3412	SK-3421	OM-5023
SK-3255 ^T	100				
SK-3406 ^T	37	100			
SK-3412	21	71	100		
SK-3421	63	97	85	100	
OM-5023	23	48	47	37	100
<i>K. setae</i> KM-6054 ^T	53	35	50	44	54
<i>K. phosalacinea</i> KA-338 ^T	32	40	57	49	46
<i>K. griseola</i> AM-9660 ^T	35	53	69	47	73
<i>K. cochleata</i> IFO 14768 ^T	15	3	15	7	31
<i>K. cystarginea</i> IFO 14836 ^T	26	10	29	12	36
<i>K. paracochleata</i> IFO 14769 ^T	42	12	22	8	22

Fig. 3 shows a neighbour-joining tree generated from about 1400 bp, which is the length after removing gaps. The five novel isolates formed a clade with the members of the genus *Kitasatospora*. These strains showed high similarities, with values of 93.0–98.5%, to the 16S rDNA sequences of the previously described *Kitasatospora* species.

DNA–DNA hybridization

Table 3 shows the levels of DNA relatedness among the novel isolates and the other members of the genus *Kitasatospora*. DNA relatedness values between strain

SK-3255^T and the other *Kitasatospora* strains ranged from 15 to 63%. The DNA relatedness values between strain SK-3406^T and strains SK-3412 and SK-3421 ranged from 71 to 97%, but those between strain SK-3406^T and the other strains were 3–48%. The level of DNA relatedness between strain OM-5023 and *K. griseola* AM-9660^T was 73%.

DISCUSSION

Based on the phenotypic, morphological and chemotaxonomic characteristics and genotypic data for the five novel isolates, it is concluded that they belong to

the genus *Kitasatospora*. Nine *Kitasatospora* species have been described previously, but *K. mediodidica*, *K. azatica* and *K. cheerisanensis* were clearly distinguished from our strains by the production of soluble pigment, the morphology of the aerial mycelia and the fragmentation of the vegetative mycelia. Therefore, the five novel strains were compared with the related *Kitasatospora* species *K. setae*, *K. phosalacinea*, *K. griseola*, *K. cochleata*, *K. paracochleata* and *K. cystarginea* by phenotypic characteristics and DNA–DNA hybridization.

A comparison of the physiological properties of strains SK-3255^T and SK-3406^T and related *Kitasatospora* species is shown in Table 2. *K. cochleata* and *K. paracochleata* differ from strains SK-3255^T and SK-3406^T in melanin formation and carbon source utilization. *K. cystarginea* differs from the two strains in carbon source utilization. *K. phosalacinea* utilizes D-fructose and sucrose, but the two novel strains do not. Only strain SK-3255^T utilizes melibiose. Strain SK-3255^T differs from the previously described species in physiological characteristics and levels of DNA relatedness. Strains SK-3406^T, SK-3412 and SK-3421 showed that they belong to the same species by their cultural and physiological characteristics and the level of DNA relatedness. They had high DNA–DNA reassociation values with each other, in the range 71–97 %, while they had low values with representative species. Strain SK-3406^T was similar to *K. setae*, but the DNA relatedness value was low. Strains SK-3255^T and SK-3406^T were clearly distinguished from previously described species. Strain OM-5023 is regarded as belonging to the species *K. griseola* by virtue of its cultural characteristics and the level of DNA relatedness. We therefore propose that strains SK-3255^T and SK-3406^T should be described as new species of the genus *Kitasatospora*, *Kitasatospora cineracea* sp. nov. for strain SK-3255^T and *Kitasatospora niigatensis* sp. nov. for strain SK-3406^T.

As *Streptomyces kifunensis* formed a clade with the members of the genus *Kitasatospora* in phylogenetic analysis and had the phenotypic characteristics of the genus *Kitasatospora*, this micro-organism should be transferred to the genus *Kitasatospora*.

Description of *Kitasatospora cineracea* sp. nov.

Kitasatospora cineracea (ci.ne.ra'ce.a. L. fem. adj. *cineraceus* ash-grey, referring to the colour of the aerial mycelium).

A Gram-positive, aerobic, non-acid-fast actinomycete that produces yellowish brown vegetative mycelium and oyster-white to silver-grey aerial mycelium on yeast extract/malt extract agar, inorganic salts/starch agar, glycerol/asparagine agar and sucrose/nitrate agar. The spore chains are straight and flexuous, with 20 or more rod-shaped, smooth-surfaced spores (0.9–1.0 × 0.6 µm) per chain. Submerged spores are formed in liquid culture. Soluble pigments, including melanin, are not produced. The cell wall contains both *meso*-

DAP and LL-DAP. Whole-cell hydrolysates contain galactose, mannose and ribose but lack arabinose and xylose. Phosphatidylethanolamine and phosphatidyl-inositol are contained in the polar lipid fraction. MK-9(H₆) and MK-9(H₈) are predominant menaquinones. The *N*-acyl type is the acetyl type. The strain is positive for peptonization of milk and hydrolysis of starch. D-Glucose, L-arabinose, D-xylose, raffinose, melibiose and D-rhamnose are utilized for growth, but D-mannitol, D-fructose, inositol, sucrose and cellulose are not utilized. The temperature range for growth is 15–37 °C. The strain is resistant to novobiocin at 100 µg ml⁻¹. The G+C content of the DNA is 73.7 mol %.

The type strain is SK-3255^T (= IFO 16452^T = JCM 10915^T = NRRL B-23134^T).

Description of *Kitasatospora niigatensis* sp. nov.

Kitasatospora niigatensis (ni.i.gat.en'sis. N.L. fem. adj. *niigatensis* of Niigata, the city in Japan where the species originated).

A Gram-positive, aerobic, non-acid-fast actinomycete that produces ivory to brownish grey vegetative mycelium and brownish white to purplish grey aerial mycelium on yeast extract/malt extract agar, oatmeal agar, glycerol/asparagine agar and sucrose/nitrate agar. The spore chains are straight and flexuous, with 20 or more rod-shaped, smooth-surfaced spores (1.0–1.1 × 0.5 µm) per chain. Submerged spores are formed in liquid culture. Soluble pigments, including melanin, are not produced. The cell wall contains both *meso*-DAP and LL-DAP. Whole-cell hydrolysates contain galactose, mannose and ribose, but lack arabinose and xylose. Phosphatidylethanolamine and phosphatidyl-inositol are contained in the polar lipid fraction. MK-9(H₆) and MK-9(H₈) are predominant menaquinones. The *N*-acyl type is the acetyl type. The strain is positive for reduction of nitrate, peptonization of milk and hydrolysis of starch. D-Glucose, L-arabinose and D-xylose are utilized for growth, but raffinose, melibiose, D-mannitol, D-fructose, D-rhamnose, inositol, sucrose and cellulose are not utilized. The temperature range for growth is 15–41 °C. The strain is resistant to novobiocin at 100 µg ml⁻¹. The G+C content of the DNA is 73.5 mol %.

The type strain is SK-3406^T (= IFO 16453^T = JCM 10916^T = NRRL B-24135^T).

ACKNOWLEDGEMENTS

We thank Mayumi Shinose, Atsuko Matsumoto and Xu Ping for their help. Hideo Utsuno is gratefully acknowledged for revision of the English text. This study was supported by funds from the Japan Keirin Association.

REFERENCES

- Becker, B., Lechevalier, M. P. & Lechevalier, H. A. (1965). Chemical composition of cell-wall preparation from strains of various form-genera of aerobic actinomycetes. *Appl Microbiol* **13**, 236–243.

- Chung, Y. R., Sung, K. C., Mo, H. K., Son, D. Y., Nam, J. S., Chun, J. & Bae, K. S. (1999). *Kitasatospora cheerisanensis* sp. nov., a new species of the genus *Kitasatospora* that produces an antifungal agent. *Int J Syst Bacteriol* **49**, 753–758.
- Collins, M. D., Pirouz, T., Goodfellow, M. & Minnikin, D. E. (1977). Distribution of menaquinones in actinomycetes and corynebacteria. *J Gen Microbiol* **100**, 221–230.
- Ezaki, T., Hashimoto, Y. & Yabuuchi, E. (1989). 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.
- Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.
- Hasegawa, T., Takizawa, M. & Tanida, S. (1983). A rapid analysis for chemical grouping of aerobic actinomycetes. *J Gen Appl Microbiol* **29**, 319–322.
- Iwai, Y. & Takahashi, Y. (1992). Selection of microbial sources of bioactive compounds. In *The Search for Bioactive Compounds from Microorganisms*, pp. 281–302. Edited by S. Ōmura. New York: Springer.
- Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* **16**, 111–120.
- Kusakabe, H. & Isono, K. (1988). Taxonomic studies on *Kitasatospora cystarginea* sp. nov., which produces a new antifungal antibiotic cystargin. *J Antibiot* **41**, 1758–1762.
- Labeda, D. P. (1988). *Kitasatospora mediocidica* sp. nov. *Int J Syst Bacteriol* **38**, 287–290.
- Lechevalier, M. P., Bievre, C. D. & Lechevalier, H. A. (1977). Chemotaxonomy of aerobic actinomycetes: phospholipid composition. *Biochem Syst Ecol* **5**, 249–260.
- Marmur, J. (1961). A procedure for the isolation of deoxyribonucleic acid from micro-organisms. *J Mol Biol* **3**, 208–218.
- Minnikin, D. E., Patel, P. V., Alshamaony, L. & Goodfellow, M. (1977). Polar lipid composition in the classification of *Nocardia* and related bacteria. *Int J Syst Bacteriol* **27**, 104–117.
- Nakagaito, Y., Yokota, A. & Hasegawa, T. (1992a). Three new species of the genus *Streptomyces*: *Streptomyces cochleatus* sp. nov., *Streptomyces paracochleatus* sp. nov., and *Streptomyces azaticus* sp. nov. *J Gen Appl Microbiol* **38**, 105–120.
- Nakagaito, Y., Shimazu, A., Yokota, A. & Hasegawa, T. (1992b). Proposal of *Streptomyces atroaurantiacus* sp. nov. and *Streptomyces kifunensis* sp. nov. and transferring *Kitasatospora cystarginea* Kusakabe and Isono to the genus *Streptomyces* as *Streptomyces cystargineus* comb. nov. *J Gen Appl Microbiol* **38**, 627–633.
- Ōmura, S., Iwai, Y., Takahashi, Y., Kojima, K., Otoguro, K. & Oiwa, R. (1981). Type of diaminopimelic acid different in aerial and vegetative mycelia of setamycin-producing actinomycete KM-6054. *J Antibiot* **34**, 1633–1634.
- Ōmura, S., Takahashi, Y., Iwai, Y. & Tanaka, H. (1982). *Kitasatospora*, a new genus of the order *Actinomycetales*. *J Antibiot* **35**, 1013–1019.
- Ōmura, S., Takahashi, Y., Iwai, Y. & Tanaka, H. (1985). Revised nomenclature of *Kitasatospora setalba*. *Int J Syst Bacteriol* **35**, 221.
- Pridham, T. G. & Gottlieb, D. (1948). The utilization of carbon compounds by some *Actinomycetales* as an aid for species determination. *J Bacteriol* **56**, 107–114.
- Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Shirling, E. B. & Gottlieb, D. (1966). Methods for characterization of *Streptomyces* species. *Int J Syst Bacteriol* **16**, 313–340.
- Takahashi, Y., Kuwana, T., Iwai, Y. & Ōmura, S. (1984a). Some characteristics of aerial and submerged spores of *Kitasatospora*. *J Gen Appl Microbiol* **30**, 223–229.
- Takahashi, Y., Iwai, Y. & Ōmura, S. (1984b). Two new species of the genus *Kitasatospora*, *Kitasatospora phosalacinea* sp. nov. and *Kitasatospora griseola* sp. nov. *J Gen Appl Microbiol* **30**, 377–387.
- Takahashi, Y., Iwai, Y. & Ōmura, S. (1991). Mode of submerged spore formation in *Kitasatospora setae*. *J Gen Appl Microbiol* **37**, 261–266.
- Takahashi, Y., Iwai, Y. & Ōmura, S. (1996). Physiological regulation of sporulation of *Kitasatospora setae* in submerged culture. *Actinomycetologica* **10**, 43–49.
- Takahashi, Y., Seino, A., Iwai, Y. & Ōmura, S. (1999). Taxonomic study and morphological differentiation of an actinomycete genus, *Kitasatospora*. *Zentbl Bakteriol* **289**, 265–284.
- Tamaoka, J. & Komagata, K. (1984). Determination of DNA base composition by reversed-phase high-performance liquid chromatography. *FEMS Microbiol Lett* **25**, 125–128.
- Tamaoka, J., Katayama-Fujimura, Y. & Kuraishi, H. (1983). Analysis of bacterial menaquinone mixtures by high performance liquid chromatography. *J Appl Bacteriol* **54**, 31–36.
- Taylor, H. D., Knoche, L. & Granville, W. C. (editors) (1958). *Color Harmony Manual*, 4th edn. Chicago: Container Corporation of America.
- Thompson, J. D., Higgins, D. G. & Gibson, T. J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* **22**, 4673–4680.
- Tomiyasu, I. (1982). Mycolic acid composition and thermally adaptative changes in *Nocardia asteroides*. *J Bacteriol* **151**, 828–837.
- Uchida, K. & Aida, K. (1984). An improved method for the glycolate test for simple identification of acyl type of bacterial cell walls. *J Gen Appl Microbiol* **30**, 131–134.
- Wellington, E. M. H., Stackebrandt, E., Sanders, D., Wolstrup, J. & Jorgensen, N. O. G. (1992). Taxonomic status of *Kitasatospora*, and proposed unification with *Streptomyces* on the basis of phenotypic and 16S rRNA analysis and emendation of *Streptomyces* Waksman and Henrici 1943, 339^{AL}. *Int J Syst Bacteriol* **42**, 156–160.
- Zhang, Z., Wang, Y. & Ruan, J. (1997). A proposal to revive the genus *Kitasatospora* (Omura, Takahashi, Iwai, and Tanaka 1982). *Int J Syst Bacteriol* **47**, 1048–1054.