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Longispora albida gen. nov., sp. nov., a novel genus of the family *Micromonosporaceae*

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A novel actinomycete strain was isolated from a soil sample collected in Japan by using gellan gum as a solidifying agent. Spore-chains from the short sporophores were straight and each had more than 20 spores per chain. Spores possessed no motility. Cell-wall peptidoglycan contained *meso*-diaminopimelic acid, glycine, alanine and glutamic acid; whole-cell hydrolysates contained arabinose, galactose and xylose. The acyl type of the peptidoglycan was glycolyl. The predominant menaquinones were MK-10(H_4) and MK-10(H_6); MK-10(H_8) was a minor component. Mycolic acids were not detected. The diagostic phospholipid was phosphatidylethanolamine. Cellular fatty acids included heptadecenoic ($C_{17:1}$), 14-methylpentadecanoic (i-$C_{16:0}$) and octadecenoic ($C_{18:1}$) acids. The G+C content of the DNA was 70 mol%. On the basis of morphogical and chemotaxonomic properties and phylogenetic analysis based on 16S rDNA sequence data, it is proposed that this strain should be classified in a novel genus and species, *Longispora albida* gen. nov., sp. nov., in the family *Micromonosporaceae*. The type strain is K97-0003^T (=NRRL B-24201^T=JCM 11711^T).

INTRODUCTION

Members of the order *Actinomycetales* offer possibilities for the discovery of new bioactive compounds. Many new bioactive metabolites have been found from *Streptomyces* strains that were isolated from soil, in which strains of this genus are distributed at a high frequency. An efficient way to find new bioactive metabolites is by the discovery of new micro-organisms and many approaches have been used for this purpose, e.g. isolation from plants or soil in particular environments, pre-treatments of soil samples and changing the formulation of isolation media. As one of these various approaches, we tried to isolate actinomycete strains by using gellan gum, which was used for selective isolation of the genus *Actinobispora* Jiang *et al.* 1991 by Suzuki *et al.* (1998) as a solidifying agent, instead of agar. As a result, we isolated strain K97-0003^T, which produces actinohivin, a novel anti-HIV (human immunodeficiency virus) protein found in the culture broth of the strain by a syncytium formation assay system (Chiba *et al.*, 2001).

Strain K97-0003^T grew better on gellan gum media than on agar media. Morphological and chemotaxonomic properties of the strain indicated that it belonged to the family *Micromonosporaceae* (Krasil'nikov 1938, emend. Koch *et al.* 1996). Phylogenetic analysis based on 16S rDNA sequence data showed that the strain formed a lineage within the family *Micromonosporaceae*, but not within any existing genus. Therefore, we propose that the strain should be classified as a novel genus and species, *Longispora albida* gen. nov., sp. nov.

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Abbreviation: DAP, diaminopimelic acid.

The GenBank/EMBL/DDBJ accession number for the 16S rDNA sequence of strain K97-0003^T is AB089241.

METHODS

Micro-organisms. Strain K97-0003^T was isolated from a soil sample collected at Suginami, Tokyo, Japan, by culture on water/proline/gellan gum medium [tap water, 1 % proline, 1 % gellan gum (Kanto Chemical); unadjusted pH] at 27 °C.

Morphology. Morphological characteristics of the strain were observed by scanning electron microscopy (model JSM-5600; JEOL) following incubation on 1/10 V8 juice/gellan gum medium [2 % V8 juice (Campbell's soup), 0·03 % $CaCO_3$, 1 % gellan gum, tap water, pH 7·2] that contained 0·06 % $CaCl_2.2H_2O$ for 20 days at 27 °C and fixation by 4 % osmium tetroxide vapour.

Cultural and physiological characteristics. Cultural and physiological characteristics of the strain were determined following incubation for 2 weeks at 27 °C on media recommended by Waksman (1961) and the International *Streptomyces* Project (Shirling & Gottlieb, 1966). Colour names and hue numbers were determined according to the *Color Harmony Manual* (Taylor *et al.*, 1958). The ability of the strain to grow on a range of sole carbon sources at 1 % (w/v) was determined in carbon utilization media (Pridham &

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Gottlieb, 1948) with agar or gellan gum and agar medium of yeast nitrogen base without amino acids (Difco), as described by Asano & Kawamoto (1986). NaCl tolerance and pH and temperature ranges for growth were determined on yeast extract/malt extract agar (ISP medium 2). Media for spore formation were as follows: ISP media (2, 3 and 7), glucose/peptone, nutrient, water/proline (1 % proline, tap water), 1/10 V8 and 1/10 V8 that contained 0·06 % $CaCl_2.2H_2O$; 1·5 % agar or 1 % gellan gum was used as the solidifying agent.

Chemotaxonomic characterization. Isomers of diaminopimelic acid (DAP) in whole-cell hydrolysates were determined by TLC following standard methods (Becker *et al.*, 1965; Hasegawa *et al.*, 1983) and the *N*-acyl types of muramic acid were determined by the method of Uchida & Aida (1977). Purified cell wall was obtained by the method of Kawamoto *et al.* (1981) and the amino acid composition of hydrolysed cell walls was determined by TLC. Whole-cell sugars were analysed after Becker *et al.* (1965), presence of mycolic acids was examined by TLC following Tomiyasu (1982) and phospholipids were extracted and identified following the method of Minnikin *et al.* (1977). Menaquinones were extracted and purified by the method of Collins *et al.* (1977), then analysed by HPLC (model 802-SC; Jasco) on a chromatograph equipped with a CAPCELL PAK C18 column (Shiseido) (Tamaoka *et al.*, 1983). Methyl esters of cellular fatty acids were prepared by direct transmethylation with methanolic hydrochloride and analysed by using GLC (model GC-17A; Shimadzu) with a DB-23 capillary column (0·25 mm × 30 m; J&W Scientific) (Suzuki & Komagata, 1983).

DNA base composition. Chromosomal DNA was prepared by using the procedure of Marmur (1961) and the G+C content of the DNA preparations was determined by the HPLC method of Tamaoka & Komagata (1984).

Analysis of 16S rDNA sequence. Chromosomal DNA was prepared by using the same method as above. 16S rDNA was PCR-amplified by using previously described methods (Takahashi *et al.*, 2002) and was sequenced directly on an ABI model 377A automatic DNA sequencer by using a PRISM Ready Reaction Dye Primer Cycle Sequencing kit (Applied Biosystems). CLUSTAL W software (Thompson *et al.*, 1994) was used for multiple alignment of selected sequences, for calculating evolutionary distances (Kimura, 1980) and similarity values and for constructing a phylogenetic tree based on the neighbour-joining method (Saitou & Nei, 1987). Data were resampled with 1000 bootstrap replications (Felsenstein, 1985). For the phylogenetic tree by the maximum-likelihood method, PAUP* version 4.0 beta 8 (Swofford, 2001) was used.

RESULTS AND DISCUSSION

Cultural and morphological characteristics

After incubation for 2 weeks at 27 °C, strain K97-0003^T grew well on yeast extract/malt extract agar, oatmeal agar, peptone/yeast extract/iron agar and nutrient agar, but aerial mycelia did not grow (Table 1). After substitution of agar by gellan gum, aerial mycelia grew slightly on a few media and spores were produced well on 1/10 V8 gellan gum medium that contained $CaCl_2$ (Table 2).

Short sporophores branched from the vegetative mycelia; spore-chains from the sporophores were straight and each had more than 20 spores per chain. Spores were cylindrical, 0·4–0·5 × 1·0–1·4 μm and had a smooth surface (Fig. 1). Whirls, sclerotic granules, sporangia and flagellated spores were not observed.

Physiological characteristics

Strain K97-0003^T grew at 12–37 °C and the optimum range was 21–33 °C. No melanoid pigment was produced; tests for reduction of nitrate, liquefaction of gelatin and coagulation and peptonization of milk were positive. Hydrolysis of starch was negative on gellan gum medium and the strain did not grow on agar medium. The strain utilized D-glucose, but not L-arabinose, D-fructose, *myo*-inositol, D-mannitol, melibiose, raffinose, L-rhamnose, sucrose or D-xylose, on agar and gellan gum media. Cellulose was not decomposed. The pH range for growth was 6–9. The strain did not grow in the presence of 2 % NaCl.

Chemotaxonomic characteristics

Strain K97-0003^T contained arabinose, galactose and xylose in whole-cell hydrolysates. Cell-wall peptidoglycan contained *meso*-DAP, glycine, alanine and glutamic acid. The acyl type of the peptidoglycan was glycolyl. Predominant menaquinones were MK-10(H_4) and MK-10(H_6) and a minor menaquinone component was MK-10(H_8). Mycolic acids were not detected. The only phospholipid detected

Table 1. Growth and cultural characteristics of strain K97-0003^T

Strain K97-0003^T did not grow on inorganic salts/starch agar (ISP medium 4), sucrose/nitrate agar, glucose/nitrate agar or glycerol/calcium malate agar. No aerial mycelia were produced on any medium.

Medium	Growth	Reverse colour
Yeast extract/malt extract agar (ISP 2)	Moderate; light ivory (2ca)	Cream (1½ca)
Oatmeal agar (ISP 3)	Moderate; alabaster tint (13ba)	Blue tint (15ba)
Glycerol/asparagine agar (ISP 5)	Poor; cream (1½ca) to butter yellow (1½ga)	Cream (1½ca) to light yellow (1½ea)
Glucose/asparagine agar	Poor; pearl (2ba)	Yellow tint (1ba)
Peptone/yeast extract/iron agar (ISP 6)	Moderate; pearl pink (3ca)	Colonial yellow (1ga)
Tyrosine agar (ISP 7)	Poor; light ivory (2ca)	Cream (1½ca)
Glucose/peptone agar	Poor; cream (1½ca)	Cream (1½ca)
Nutrient agar	Moderate; light wheat (2ea)	Butter yellow (1½ga)

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Table 2. Aerial mycelium formation of strain K97-0003^T on various media

−, Negative; $+^t$, trace aerial mycelium observed with a light microscope; +, poor aerial mycelium; ++, aerial mycelium produced (better than +).

Medium	Agar		Gellan gum	
	Growth	Aerial mycelium	Growth	Aerial mycelium
Yeast extract/malt extract (ISP 2)	+	$+^t$	++	$+^t$
Oatmeal (ISP 3)	+	−	+	−
Tyrosine (ISP 7)	+	−	+	+
Glucose/peptone	+	−	++	−
Nutrient	+	−	+	−
Water/proline	+	−	++	−
1/10 V8	+	−	+	+
1/10 V8 + $CaCl_2$	+	$+^t$	+	++

was phosphatidylethanolamine; phosphatidylcholine, phosphatidylglycerol and an unidentified phospholipid that contains glucosamine were absent. This corresponds to phospholipid pattern II *sensu* of Lechevalier *et al.* (1977). Predominant cellular fatty acid components were heptadecenoic ($C_{17:1}$, 24 %), 14-methylpentadecanoic (i-$C_{16:0}$, 20 %) and octadecenoic ($C_{18:1}$, 16 %) acids (Table 3). The G + C content of the DNA was 70 mol%.

The acyl type of strain K97-0003^T was glycolyl; mycolic acids were absent. These two characteristics show that this strain is a member of the family *Micromonosporaceae*, the family *Glycomycetaceae* or the genus *Microbacterium* (Orla-Jensen, 1919) in the family *Microbacteriaceae* (Table 4). However, its morphology and chemotaxonomic characters differed from those of members of the genus *Microbacterium* and of the only genus, *Glycomyces* (Labeda *et al.*, 1985), of the family *Glycomycetaceae*. As a result, this strain was placed in the family *Micromonosporaceae*.

At present, the family *Micromonosporaceae* consists of eleven genera: *Actinoplanes* (Couch, 1950), *Asanoa* (Lee & Hah, 2002), *Catellatospora* (Asano & Kawamoto, 1986), *Catenuloplanes* (Yokota *et al.*, 1993), *Couchioplanes* (Tamura *et al.*, 1994), *Dactylosporangium* (Thiemann *et al.*, 1967), *Micromonospora* (Ørskov, 1923), *Pilimelia* (Kane, 1966), *Spirilliplanes* (Tamura *et al.*, 1997), *Verrucosispora* (Rheims

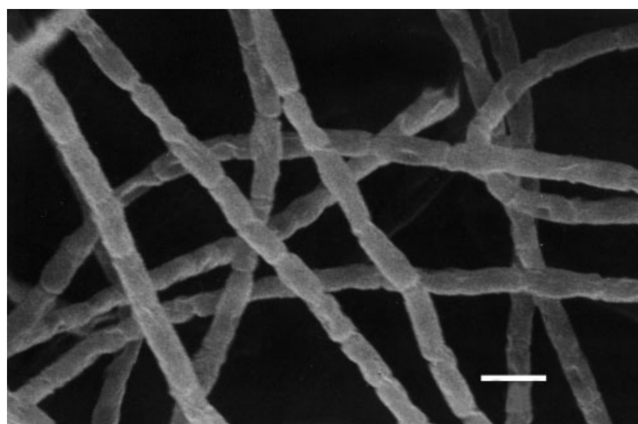

Fig. 1. Scanning electron micrograph of spore-chains of strain K97-0003^T, grown on 1/10 V8 + $CaCl_2$ gellan gum medium for 20 days at 27 °C. Bar, 1 μm.

Table 3. Fatty acid composition of strain K97-0003^T

Fatty acid	Composition (%)
Saturated:	
$C_{14:0}$	0·1
$C_{15:0}$	1·2
$C_{16:0}$	1·1
$C_{17:0}$	4·6
$C_{18:0}$	0·5
$C_{19:0}$	2·1
Unsaturated:	
$C_{16:1}\omega9$	0·1
$C_{16:1}\omega A$*	0·5
$C_{17:1}\omega B$*	8·5
$C_{17:1}\omega C$*	1·2
$C_{17:1}\omega D$*	14·3
$C_{18:1}\omega9$	2·3
$C_{18:1}\omega11$	0·3
$C_{18:1}\omega E$*	13·1
$C_{19:1}\omega F$*	6·5
$C_{19:1}\omega G$*	2·2
Branched:	
iso-$C_{14:0}$	0·5
iso-$C_{15:0}$	3·1
anteiso-$C_{15:0}$	0·7
iso-$C_{16:0}$	20·1
iso-$C_{17:0}$	7·0
anteiso-$C_{17:0}$	7·5
$C_{18:0}$†	2·5

*A–G: Double-bond positions of these fatty acids are not known.
†This fatty acid is either iso- or anteiso-branched.

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Table 4. Comparison of morphological and chemotaxonomic characteristics of strain K97-0003T and genera that contain *N*-glycolyl muramic acid in the peptidoglycan and no mycolic acids

Taxa: 1, K97-0003T; 2, *Micromonospora*; 3, *Actinoplanes*; 4, *Asanoa*; 5, *Catellatospora*; 6, *Catenuloplanes*; 7, *Couchioplanes*; 8, *Dactylosporangium*; 9, *Pilimelia*; 10, *Spirilliplanes*; 11, *Verrucosispora*; 12, *Virgisporangium*; 13, *Glycomyces*; 14, *Microbacterium*. Data were taken from Labeda *et al.* (1985), Stackebrandt & Kroppenstedt (1987), Vobis (1989), Goodfellow *et al.* (1990), Evtushenko *et al.* (1991), Tamura *et al.* (1994, 1997, 2001), Rheims *et al.* (1998), Takeuchi & Hatano (1998), Kudo *et al.* (1999), Lee *et al.* (2000), Lee & Hah (2002) and Zlamala *et al.* (2002). ND, Not determined; D, a substantial proportion of species differ.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Spore-chains	+	−	−	+	+	+	+	−	−	+	−	−	+	−
Spore motility	−	−	+	−	−	+	+	+	+	+	−	+	−	D
Diamino acid	*meso*-DAP	*meso*-DAP	*meso*-DAP	*meso*-DAP	*meso*-DAP	L-Lys	L-Lys	*meso*-DAP	*meso*-DAP	*meso*-DAP	*meso*-DAP	*meso*-DAP	*meso*-DAP	L-Lys/D-Orn
Major menaquinones (MK-)	$10(H_{4,6})$	$10(H_{4,6})$, $9(H_{4,6})$	$9(H_4)$	$10(H_{6,8})$	$9(H_{4,6})$ or $10(H_4)$	$10(H_4)$, $11(H_4)$	$9(H_4)$	$9(H_{6,8})$	$9(H_{4,2})$	$10(H_4)$	$9(H_4)$	$10(H_{4,6})$	D	11,12,13,14
Phospholipid type*	PII	PII	PII	PII	PII	PIII	PII	PII	PII	PII	PII	PII	PI	DPG+, PG+‡
Fatty acid type†	2d	3b	2d	2d	3b	2c	2c	3b	2d	2d	2b	2d	2c	2c
Characteristic whole-cell sugars	Ara, Gal, Xyl	Ara, Xyl	Ara, Xyl	Ara, Gal, Xyl	Ara, Gal, Xyl/Xyl only	Xyl	Ara, Gal, Xyl	Ara, Xyl	Ara, Xyl	Ara, Xyl	Man, Xyl	Ara, Gal, Xyl	Ara, Xyl	ND
G+C content (mol%)	70	71–72	72–73	71–72	71–73	70–72	69–73	71–73	ND	69	70	71	71–73	66–72

*According to the classification of Lechevalier *et al.* (1977).
†According to the classification of Kroppenstedt (1985).
‡DPG, Diphosphatidylglycerol; PG, phosphatidylglycerol.

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et al., 1998) and *Virgisporangium* (Tamura *et al.*, 2001). Although these genera are similar to each other, their cultural, morphological and chemotaxonomic characteristics differ somewhat. Genera that have spore-chains similar to those of strain K97-0003^T are *Asanoa*, *Catellatospora*, *Catenuloplanes*, *Couchioplanes* and *Spirilliplanes*. The latter three possess motile spores; the two genera that do not possess them, *Asanoa* and *Catellatospora*, share this trait with strain K97-0003^T. However, the genera *Asanoa* and *Catellatospora* make distinctive spore-chains that are borne directly from the vegetative hyphae that grow on the surface of agar media and do not produce true aerial mycelia. This indicates that strain K97-0003^T does not belong to any known genus.

Phylogenetic analysis

The almost-complete 16S rDNA sequence (1496 nt) [positions 10–1506, according to the *Escherichia coli* numbering system of Brosius *et al.* (1978)] was determined for strain K97-0003^T; a 1443 nt fragment, between positions 35 and 1475, was used for phylogenetic analysis and compared with 16S rDNA database sequences of members of the class *Actinobacteria*. Phylogenetic analysis based on this large dataset revealed that strain K97-0003^T fell within the cluster of the family *Micromonosporaceae* and was clearly separated from members of the genera *Glycomyces* and *Microbacterium* (data not shown). Fig. 2 shows the phylogenetic tree constructed by the neighbour-joining method; this tree revealed that strain K97-0003^T branched deeply within the family *Micromonosporaceae* and belonged to no previously known genera in this family. The tree constructed by the maximum-likelihood method supported this result.

The pattern of 16S rDNA signature nucleotides (Stackebrandt *et al.*, 1997) differed only from that of the family *Micromonosporaceae* at position 502 (A). Sequence similarity values with other members of the family *Micromonosporaceae* were also low: *Catellatospora citrea* subsp. *citrea*, *Virgisporangium aurantiacum*, *Asanoa ferruginea*, *Dactylosporangium aurantiacum*, *Pilimelia terevasa*, *Catenuloplanes japonicus*, *Verrucosispora gifhornensis*, *Spirilliplanes yamanashiensis*, *Micromonospora chalcea*, *Couchioplanes caeruleus* subsp. *caeruleus* and *Actinoplanes philippinensis* (91·8–93·0 %).

From the above chemotaxonomic and morphological characteristics and phylogenetic analysis, we propose that strain K97-0003^T should be classified in a novel genus and species, *Longispora albida* gen. nov., sp. nov.

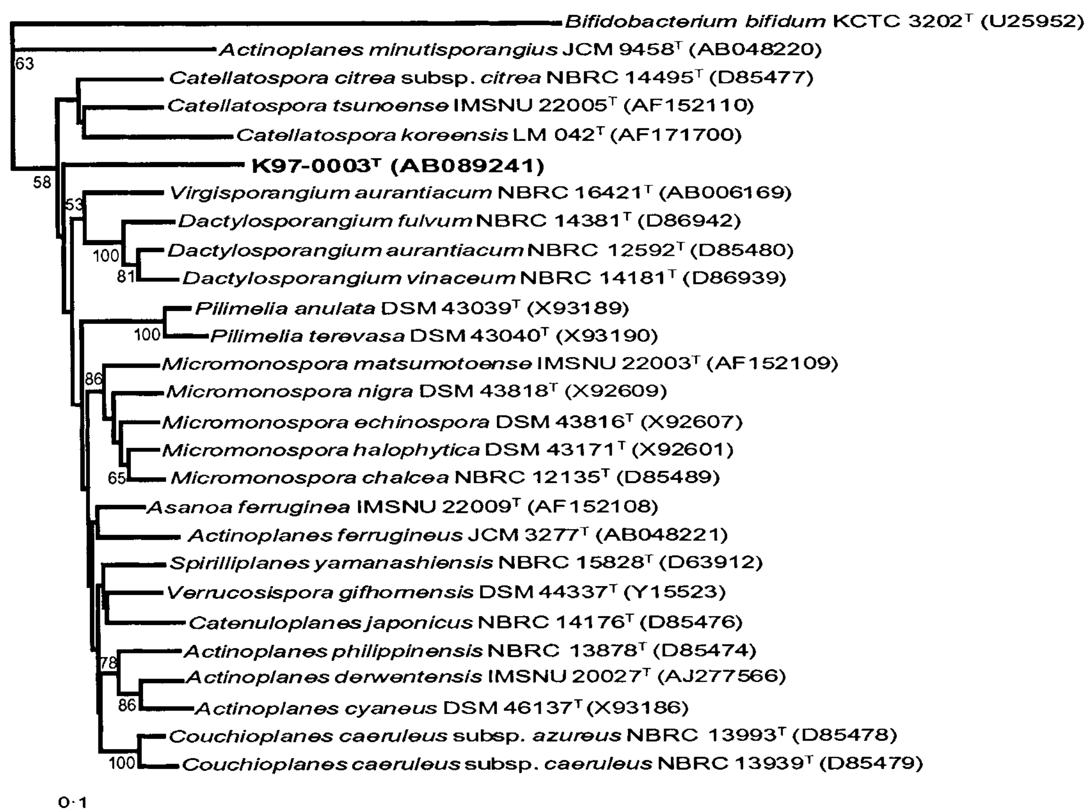

Fig. 2. Phylogenetic tree showing the position of strain K97-0003^T based on 16S rDNA analysis. Numbers at nodes indicate the level (%) of bootstrap support based on neighbour-joining analysis of 1000 resampled datasets. Only values >50 % are shown. Bar, 1 nucleotide substitution per 100 nucleotides.

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Description of *Longispora* gen. nov.

Longispora (Lon.gi.spo′ra. L. adj. *longus* long; Gr. fem. n. *spora* spore; N.L. fem. n. *Longispora* long spore).

Cells are Gram-positive, aerobic, non-acid-fast and non-motile. Spore-chains are straight and have more than 20 spores on the tip of short sporophores that branch from vegetative mycelia. Cell-wall peptidoglycan contains *meso*-DAP, glycine and alanine; arabinose, galactose and xylose are detected in whole-cell hydrolysates. The acyl type is glycolyl. Predominant menaquinones are MK-10(H_4) and MK-10(H_6); a minor component is MK-10(H_8). Mycolic acids are not detected. The diagnostic phospholipid is phosphatidylethanolamine (phospholipid type II). Habitat is soil. Mesophilic. The type species is *Longispora albida*.

Description of *Longispora albida* sp. nov.

Longispora albida (al.bi′da. L. fem. adj. *albida* somewhat white).

General morphological, chemotaxonomic and growth characteristics are as given above for the genus. Spores are cylindrical (0·4–0·5 × 1·0–1·4 μm) and have a smooth surface. Temperature range for growth is 12–37 °C and the optimum range is 21–33 °C. Growth occurs at pH 6–9. Melanoid pigment is not produced; positive for reduction of nitrate, liquefaction of gelatin and coagulation and peptonization of milk. D-Glucose is utilized but L-arabinose, D-fructose, *myo*-inositol, D-mannitol, melibiose, raffinose, L-rhamnose, sucrose and D-xylose are not. Cellulose is not decomposed. Growth is better on gellan gum media than on agar media. No growth in the presence of 2 % NaCl. Predominant cellular fatty acid components are $C_{17:1}$, iso-$C_{16:0}$ and $C_{18:1}$. The G + C content of the DNA is 70 mol%.

The type strain is K97-0003^T (= NRRL B-24201^T = JCM 11711^T).

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REFERENCES

Asano, K. & Kawamoto, I. (1986). *Catellatospora*, a new genus of the *Actinomycetales*. *Int J Syst Bacteriol* **36**, 512–517.

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.

Brosius, J., Palmer, M. L., Kennedy, P. J. & Noller, H. F. (1978). Complete nucleotide sequence of a 16S ribosomal RNA gene from *Escherichia coli*. *Proc Natl Acad Sci U S A* **75**, 4801–4805.

Chiba, H., Inokoshi, J., Okamoto, M. & 11 other authors (2001). Actinohivin, a novel anti-HIV protein from an actinomycete that inhibits syncytium formation: isolation, characterization, and biological activities. *Biochem Biophys Res Commun* **282**, 595–601.

Collins, M. D., Pirouz, T., Goodfellow, M. & Minnikin, D. E. (1977). Distribution of menaquinones in actinomycetes and corynebacteria. *J Gen Microbiol* **100**, 221–230.

Couch, J. N. (1950). *Actinoplanes*. A new genus of the *Actinomycetales*. *J Elisha Mitchell Sci Soc* **66**, 87–92.

Evtushenko, L. I., Taptykova, S. D., Akimov, V. N., Semyonova, S. A. & Kalakoutskii, L. V. (1991). *Glycomyces tenuis* sp. nov. *Int J Syst Bacteriol* **41**, 154–157.

Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.

Goodfellow, M., Stanton, L. J., Simpson, K. E. & Minnikin, D. E. (1990). Numerical and chemical classification of *Actinoplanes* and some related actinomycetes. *J Gen Microbiol* **136**, 19–36.

Hasegawa, T., Takizawa, M. & Tanida, S. (1983). A rapid analysis for chemical grouping of aerobic actinomycetes. *J Gen Appl Microbiol* **29**, 319–322.

Jiang, C., Xu, L., Yang, Y.-R., Guo, G.-Y., Ma, J. & Liu, Y. (1991). *Actinobispora*, a new genus of the order *Actinomycetales*. *Int J Syst Bacteriol* **41**, 526–528.

Kane, W. D. (1966). A new genus of *Actinoplanaceae*, *Pilimelia*, with a description of two species, *Pilimelia terevasa* and *Pilimelia anulata*. *J Elisha Mitchell Sci Soc* **82**, 220–230.

Kawamoto, I., Oka, T. & Nara, T. (1981). Cell wall composition of *Micromonospora olivoasterospora*, *Micromonospora sagamiensis*, and related organisms. *J Bacteriol* **146**, 527–534.

Kimura, M. (1980). A simple method for estimating evolutionary rates of based substitutions through comparative studies of nucleotide sequences. *J Mol Evol* **16**, 111–120.

Koch, C., Kroppenstedt, R. M., Rainey, F. A. & Stackebrandt, E. (1996). 16S ribosomal DNA analysis of the genera *Micromonospora*, *Actinoplanes*, *Catellatospora*, *Catenuloplanes*, *Couchioplanes*, *Dactylosporangium*, and *Pilimelia* and emendation of the family *Micromonosporaceae*. *Int J Syst Bacteriol* **46**, 765–768.

Krasil'nikov, N. A. (1938). *Ray Fungi and Related Organisms – Actinomycetales*. Moscow: Akademii Nauk SSSR.

Kroppenstedt, R. M. (1985). Fatty acid and menaquinone analysis of actinomycetes and related organisms. In *Chemical Methods in Bacterial Systematics*, pp. 173–199. Edited by M. Goodfellow & D. E. Minnikin. London: Academic Press.

Kudo, T., Nakajima, Y. & Suzuki, K. (1999). *Catenuloplanes crispus* (Petrolini *et al*. 1993) comb. nov.: incorporation of the genus *Planopolyspora* Petrolini 1993 into the genus *Catenuloplanes* Yokota *et al*. 1993 with an amended description of the genus *Catenuloplanes*. *Int J Syst Bacteriol* **49**, 1853–1860.

Labeda, D. P., Testa, R. T., Lechevalier, M. P. & Lechevalier, H. A. (1985). *Glycomyces*, a new genus of the *Actinomycetales*. *Int J Syst Bacteriol* **35**, 417–421.

Lechevalier, M. P., De Bièvre, C. & Lechevalier, H. A. (1977). Chemotaxonomy of aerobic actinomycetes: phospholipid composition. *Biochem Syst Ecol* **5**, 249–260.

Lee, S. D. & Hah, Y. C. (2002). Proposal to transfer *Catellatospora ferruginea* and '*Catellatospora ishikariense*' to *Asanoa* gen. nov. as *Asanoa ferruginea* comb. nov. and *Asanoa ishikariensis* sp. nov., with emended description of the genus *Catellatospora*. *Int J Syst Evol Microbiol* **52**, 967–972.

Lee, S. D., Kang, S.-O. & Hah, Y. C. (2000). *Catellatospora koreensis* sp. nov., a novel actinomycete isolated from a gold-mine cave. *Int J Syst Evol Microbiol* **50**, 1103–1111.

Marmur, J. (1961). A procedure for the isolation of deoxyribonucleic acid from microorganisms. *J Mol Biol* **3**, 208–218.

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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.

Orla-Jensen, S. (1919). *The Lactic Acid Bacteria*. Copenhagen, Denmark: Høst & Son.

Ørskov, J. (1923). *Investigations into the Morphology of the Ray Fungi*. Copenhagen, Denmark: Levin & Munksgaard.

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.

Rheims, H., Schumann, P., Rohde, M. & Stackebrandt, E. (1998). *Verrucosispora gifhornensis* gen. nov., sp. nov., a new member of the actinobacterial family *Micromonosporaceae. Int J Syst Bacteriol* **48**, 1119–1127.

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.

Stackebrandt, E. & Kroppenstedt, R. M. (1987). Union of the genera *Actinoplanes* Couch, *Ampullariella* Couch, and *Amorphosporangium* Couch in a redefined genus *Actinoplanes. Syst Appl Microbiol* **9**, 110–114.

Stackebrandt, E., Rainey, F. A. & Ward-Rainey, N. L. (1997). Proposal for a new hierarchic classification system, *Actinobacteria* classis. nov. *Int J Syst Bacteriol* **47**, 479–491.

Suzuki, K. & Komagata, K. (1983). Taxonomic significance of cellular fatty acid composition in some coryneform bacteria. *Int J Syst Bacteriol* **33**, 188–200.

Suzuki, S., Takahashi, K., Okuda, T. & Komatsubara, S. (1998). Selective isolation of *Actinobispora* on gellan gum plates. *Can J Microbiol* **44**, 1–5.

Swofford, D. (2001). PAUP*: Phylogenetic Analysis Using Parsimony, version 4.0 beta 8. Sunderland, MA: Sinauer.

Takahashi, Y., Matsumoto, A., Seino, A., Ueno, J., Iwai Y. & Ōmura, S. (2002). *Streptomyces avermectinius* sp. nov., an avermectin-producing strain. *Int J Syst Evol Microbiol* **52**, 2163–2168.

Takeuchi, M. & Hatano, K. (1998). Union of the genera *Microbacterium* Orla-Jensen and *Aureobacterium* Collins *et al.* in a redefined genus *Microbacterium. Int J Syst Bacteriol* **48**, 739–747.

Tamaoka, J. & Komagata, K. (1984). Determination of DNA base composition by reverse-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.

Tamura, T., Nakagaito, Y., Nishii, T., Hasegawa, T., Stackebrandt, E. & Yokota, A. (1994). A new genus of the order *Actinomycetales*, *Couchioplanes* gen. nov., with descriptions of *Couchioplanes caeruleus* (Horan and Brodsky 1986) comb. nov. and *Couchioplanes caeruleus* subsp. *azureus* subsp. nov. *Int J Syst Bacteriol* **44**, 193–203.

Tamura, T., Hayakawa, M. & Hatano, K. (1997). A new genus of the order *Actinomycetales*, *Spirilliplanes* gen. nov., with description of *Spirilliplanes yamanashiensis* sp. nov. *Int J Syst Bacteriol* **47**, 97–102.

Tamura, T., Hayakawa, M. & Hatano, K. (2001). A new genus of the order *Actinomycetales*, *Virgosporangium* gen. nov., with descriptions of *Virgosporangium ochraceum* sp. nov. and *Virgosporangium aurantiacum* sp. nov. *Int J Syst Evol Microbiol* **51**, 1809–1816.

Taylor, H. D., Knoche, L. & Granville, W. C. (editors) (1958). *Color Harmony Manual*, 4th edn. Chicago: Container Corporation of America.

Thiemann, J. E., Pagani, H. & Beretta, G. (1967). A new genus of the *Actinoplanaceae*: *Dactylosporangium*, gen. nov. *Arch Mikrobiol* **58**, 42–52.

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. (1977). Acyl type of bacterial cell wall: its simple identification by a colorimetric method. *J Gen Appl Microbiol* **23**, 249–260.

Vobis, G. (1989). Actinoplanetes. In *Bergey's Manual of Systematic Bacteriology*, vol. 4, pp. 2418–2450. Edited by S. T. Williams, M. E. Sharpe & J. G. Holt. Baltimore: Williams & Wilkins.

Waksman, S. A. (1961). Classification, identification, and description of genera and species. In *The Actinomycetes*, vol. 2, pp. 1–363. Baltimore: Williams & Wilkins.

Yokota, A., Tamura, T., Hasegawa, T. & Huang, L. H. (1993). *Catenuloplanes japonicus* gen. nov., sp. nov., nom. rev., a new genus of the order *Actinomycetales. Int J Syst Bacteriol* **43**, 805–812.

Zlamala, C., Schumann, P., Kämpfer, P., Valens, M., Rosselló-Mora, R., Lubitz, W. & Busse, H.-J. (2002). *Microbacterium aerolatum* sp. nov., isolated from the air in the 'Virgilkapelle' in Vienna. *Int J Syst Evol Microbiol* **52**, 1229–1234.

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