***Catellatospora koreensis* sp. nov., a novel actinomycete isolated from a gold-mine cave**

Soon Dong Lee, Sa-Ouk Kang and Yung Chil Hah

Author for correspondence: Yung Chil Hah. Tel: +82 2 880 6700. Fax: +82 2 888 4911.
e-mail: hahyungc@snu.ac.kr

Department of
Microbiology, College of
Natural Sciences and
Research Center for
Molecular Microbiology,
Seoul National University,
Seoul 151-742,
Republic of Korea

A new actinomycete strain, LM 042^T, which was isolated from a gold-mine cave in Kongju, Republic of Korea, is described by phenotypic and genotypic characters. The organism formed short chains of non-motile spores and globose bodies from substrate mycelium. An aerial mycelium was absent. This organism was chemotaxonomically characterized by the presence of meso-diaminopimelic acid, rhamnose, xylose, glucose, mannose and ribose in whole-cell hydrolysates (a type II cell wall and a variant of sugar pattern D), a glycolyl type of muramic acid, DNA G+C content of 70.4 mol%, a type PII phospholipid pattern (phosphatidylethanolamine as a diagnostic nitrogenous phospholipid), a tetrahydrogenated menaquinone with 10 isoprene units as a major menaquinone, and fatty acid profiles predominated by iso-branched hexadecanoic acid, iso-branched pentadecanoic acid and heptadecanoic acid. A comparative analysis of 16S rDNA sequences indicated that this organism formed a distinct clade within the evolutionary radiation of the family Micromonosporaceae and clustered with members of the genus *Catellatospora*. The 16S rDNA similarity values between the isolate and its phylogenetic neighbours, the two subspecies of *Catellatospora citrea* and *Catellatospora tsunoense*, were 95.0–95.2% and 94.9%, respectively. An equidistant relationship was observed among the isolate, *Catellatospora ferruginea* and all other members of the *Micromonosporaceae* genera (levels of similarity 93.0–94.0%). The combination of physiological, chemotaxonomic and DNA–DNA hybridization data supported that this organism is a novel species of the genus *Catellatospora*, for which the name *Catellatospora koreensis* sp. nov. is proposed. The type strain is LM 042^T (= IMSNU 50729^T).

Keywords: *Catellatospora koreensis*, soil bacteria, actinomycetes, phylogeny

INTRODUCTION

The genus *Catellatospora* was described by Asano & Kawamoto (1986) for aerobic, Gram-positive and mesophilic actinomycetes which belong to the family *Micromonosporaceae* of the order *Actinomycetales* and are morphologically characterized by short chains of non-motile spores borne directly on the substrate mycelium without the formation of aerial mycelium. This genus has a type II/D cell wall (meso-diaminopimelic acid, 3-hydroxy-diaminopimelic acid, arabinose and xylose) (Lechevalier & Lechevalier, 1970), the glycolyl type of muramic acid (Uchida & Aida, 1984) and a type PII phospholipid pattern

(phosphatidylethanolamine as a diagnostic phospholipid) (Lechevalier *et al.*, 1981), and originally contained four species, *Catellatospora citrea* (with *Catellatospora citrea* subsp. *methionotrophica*), *Catellatospora ferruginea*, *Catellatospora matsumotoense* and *Catellatospora tsunoense*. Based on the type of a major menaquinone and the presence or absence of the cell-wall sugar, 3-*O*-methylrhamnose, members of the genus are divided into two groups. *Catellatospora citrea* with the subspecies *Catellatospora citrea* subsp. *methionotrophica* and *Catellatospora tsunoense* are characterized by the presence of menaquinones with nine isoprene units and the absence of 3-*O*-methylrhamnose, whereas *Catellatospora ferruginea* and *Catellatospora matsumotoense* have menaquinones with ten isoprene units and 3-*O*-methylrhamnose is present (Asano & Kawamoto, 1986, 1988;

The GenBank accession number for the 16S rDNA sequence of strain LM 042^T is AF171700.

Asano *et al.*, 1989). Comparative analysis of 16S rDNA sequences indicated that members of the genus *Catellatospora*, except for *Catellatospora matsumotoense*, were true members of the family *Micromonosporaceae* but were phylogenetically heterogeneous (Koch *et al.*, 1996) as represented by the chemotaxonomic groupings mentioned above. It has been recently proposed that *Catellatospora matsumotoense* be transferred to the genus *Micromonospora* as *M. matsumotoense* based on its 16S rDNA sequence and phenotypic characters (Lee *et al.*, 1999).

The family *Micromonosporaceae* was emended by Koch *et al.* (1996) based on 16S rDNA sequence studies and contains the genera *Actinoplanes*, *Catellatospora*, *Catenuloplanes*, *Couchioplanes*, *Dactylosporangium* and *Pilimelia* in addition to the type genus *Micromonospora*. Recently, the genera *Spirilliplanes* (Tamura *et al.*, 1997) and *Verrucosispora* (Rheims *et al.*, 1998) were described as new members of this family. The family *Micromonosporaceae* forms a coherent group in the actinobacterial phylogenetic tree and has been placed in the suborder *Micromonosporineae* of the order *Actinomycetales* (Stackebrandt *et al.*, 1997), with description of the family-specific 16S rDNA signatures. The genera of this family are readily differentiated from each other on the basis of chemotaxonomic, morphological and physiological properties (Asano & Kawamoto, 1986; Goodfellow *et al.*, 1990; Rheims *et al.*, 1998; Tamura *et al.*, 1994, 1997; Yokota *et al.*, 1993).

A polyphasic approach based on phenotypic and genotypic classification methods is required for determining the precise taxonomic position of a new isolate. In this paper we describe the characterization and classification of isolate LM 042^T, and we propose that this organism should be classified as a new species of the genus *Catellatospora*, as *Catellatospora koreensis* sp. nov. Strain LM 042^T has been deposited in the Culture Collection Center of the Institute of Microbiology, Seoul National University, as IMSNU 50729^T.

METHODS

Micro-organisms and culture conditions. Strain LM 042^T was isolated from a soil collected at a gold-mine cave in Kongju, Republic of Korea. The isolation medium contained (per l distilled water); yeast extract (Difco), 0.1 g; glucose, 0.1 g; K₂HPO₄, 0.5 g; Na₂HPO₄, 0.7 g; KNO₃, 0.1 g; NaCl, 0.3 g; MgSO₄·7H₂O, 0.1 g; CaCl₂·2H₂O, 0.02 g and 1 ml trace mineral solution (FeSO₄·7H₂O, 200 mg; ZnSO₄·7H₂O, 180 mg; MnSO₄·4H₂O, 20 mg; CuSO₄·5H₂O, 90 mg; CoSO₄·7H₂O, 10 mg; H₃BO₃, 200 mg and (NH₄)₆Mo₇O₂₄·4H₂O, 5 mg in 1 l distilled water). Isolate LM 042^T was maintained on yeast extract/malt extract agar [medium 2 of the International *Streptomyces* Project (ISP medium 2)] and as mycelial fragments or spores in 20% (v/v) glycerol or as lyophilized cells. For comparative purposes, the following type strains of the genus *Catellatospora* were used in this study: *Catellatospora citrea* subsp. *citrea* IFO 14495^T (= IMSNU 22008^T), *Catel-*

atospora citrea subsp. *methionotrophica* IFO 14553^T (= IMSNU 22006^T), *Catellatospora ferruginea* IFO 14496^T (= IMSNU 22009^T), *Catellatospora matsumotoense* IFO 14550^T (= IMSNU 22003^T) and *Catellatospora tsumoense* IFO 14552^T (= IMSNU 22005^T).

Morphology and pigmentation. For examining the degree of growth, pigmentation and aerial mycelia, isolate LM 042^T was grown on ISP medium 2, oatmeal agar (ISP medium 3) and inorganic salts/starch agar (ISP medium 4) as described by Shirling & Gottlieb (1966), and on glucose/asparagine agar (Waksman, 1950), yeast extract/glucose agar (Asano & Kawamoto, 1986), Bennett agar, Hickey-Tresner agar (Waksman, 1961), yeast extract agar, nutrient agar and tapwater agar. Production of melanin pigment was examined using tyrosine agar (ISP medium 7) and peptone/yeast extract/iron agar (ISP medium 6). For scanning electron microscopy, strain LM 042^T was grown on ISP medium 3 and ISP medium 4 for 21 d. The agar blocks prepared from the growth medium were fixed with osmium tetroxide and dehydrated using a graded ethanol series. After replacing the ethanol with isoamyl acetate, the samples were critical-point-dried, sputter-coated with gold and observed by a Stereoscan model 260 scanning electron microscope (Cambridge).

Physiological characteristics. The ranges for temperature and pH, and NaCl tolerance were determined on ISP medium 2. Degradation of adenine, hypoxanthine, DL-tyrosine and xanthine was studied as described by Gordon *et al.* (1974). Urease activity was determined by a colour change using Bacto urea broth (Difco). The production of hydrogen sulfide was tested using a lead acetate strip. Catalase activity was determined with a 3% (v/v) hydrogen peroxide solution. Nitrate reductase activity was determined with an equal mixture of 0.5% α-naphthylamine and 0.8% sulfanilic acid. Degradation of hippurate was investigated with 12% acidified ferric chloride on nutrient broth supplemented with 1% sodium hippurate. Hydrolysis of casein, gelatin and starch was studied by previously described methods (Mac Faddin, 1980). Hydrolysis of DNA was determined using Bacto DNase test agar (Difco). Susceptibility to antibiotics and chemical inhibitors at the defined concentrations was tested using nutrient agar. Utilization of carbohydrates as sole carbon source was determined using yeast nitrogen base without amino acids (Difco) as described previously (Asano & Kawamoto, 1986).

Chemotaxonomy. The isomer of diaminopimelic acid was determined by the method of Stanek & Roberts (1974). The composition of whole-cell sugars was determined by GC (Saddler *et al.*, 1991). The acyl type of muramic acid was determined by the colorimetric method of Uchida & Aida (1984). Menaquinones and phospholipids were extracted by the integrated method of Minnikin *et al.* (1984). The purified menaquinones were analysed by using HPLC (Kroppenstedt, 1985) and a Quattro mass spectrometer (Micromass). The resultant phospholipids were separated by two-dimensional TLC (Minnikin *et al.*, 1977) and identified as described by Collins *et al.* (1982). The presence of mycolic acids was determined by TLC (Minnikin *et al.*, 1980). Cellular fatty acid methyl esters were prepared from cells grown on yeast extract/glucose broth at 30 °C for 3 d (Minnikin, 1988) and were analysed with a Hewlett Packard model 5890A gas chromatograph equipped with a SPB-1 fused silica capillary column (Supelco) and a flame-ionization detector. The resultant peaks were identified with standard samples (catalogue no. 1114; Matreya).

Table 1. Strains and 16S rDNA accession numbers used in this study

IMSNU, Institute of Microbiology, Seoul National University, Korea; DSM, DSMZ – Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany; IFO, Institute for Fermentation, Osaka, Japan.

Taxon	Strain	Accession no.
<i>Catellatospora koreensis</i>	LM 042 ^T (= IMSNU 50729 ^T)	AF171700
<i>Catellatospora citrea</i> subsp. <i>citrea</i>	IMSNU 22008 ^T	AF152106
<i>Catellatospora citrea</i> subsp. <i>methionotrophica</i>	IMSNU 22006 ^T	AF152107
<i>Catellatospora ferruginea</i>	IMSNU 22009 ^T	AF152108
<i>Catellatospora matsumotoense</i>	IMSNU 22003 ^T	AF152109
<i>Catellatospora tsumoense</i>	IMSNU 22005 ^T	AF152110
<i>Actinoplanes brasiliensis</i>	DSM 43805 ^T	X93185
<i>Actinoplanes cyaneus</i>	DSM 46137 ^T	X93186
<i>Actinoplanes philippinensis</i>	DSM 43019 ^T	X93187
<i>Actinoplanes regularis</i>	DSM 43151 ^T	X93188
<i>Couchioplanes caeruleus</i> subsp. <i>caeruleus</i>	IFO 13939 ^T	D85479
<i>Catenuloplanes japonicus</i>	IFO 14176 ^T	D85476
<i>Dactylosporangium aurantiacum</i>	DSM 43157 ^T	X93191
<i>Dactylosporangium fulvum</i>	DSM 43917 ^T	X93192
<i>Dactylosporangium matsuzakiensis</i>	DSM 43810 ^T	X93193
<i>Dactylosporangium roseum</i>	DSM 43916 ^T	X93914
<i>Dactylosporangium thailandense</i>	DSM 43158 ^T	X92630
<i>Dactylosporangium vinaceum</i>	DSM 32823 ^T	X93196
<i>Micromonospora aurantiaca</i>	DSM 43813 ^T	X92604
<i>Micromonospora brunnea</i>	DSM 43814 ^T	X92605
<i>Micromonospora carbonacea</i> subsp. <i>carbonacea</i>	DSM 43168 ^T	X92599
<i>Micromonospora chalcea</i>	DSM 43026 ^T	X92594
<i>Micromonospora chersina</i>	DSM 44151 ^T	X92628
<i>Micromonospora coerulea</i>	DSM 43143 ^T	X92598
<i>Micromonospora echinospora</i> subsp. <i>echinospora</i>	DSM 43816 ^T	X92607
<i>Micromonospora halophytica</i> subsp. <i>halophytica</i>	DSM 43171 ^T	X92601
<i>Micromonospora inositola</i>	DSM 43819 ^T	X92610
<i>Micromonospora olivasterospora</i>	DSM 43868 ^T	X92613
<i>Micromonospora purpurea</i>	DSM 43036 ^T	X92595
<i>Micromonospora purpureochromogenes</i>	DSM 43821 ^T	X92611
<i>Micromonospora rhodorangea</i>	DSM 1039 ^T	X92612
<i>Micromonospora rosaria</i>	DSM 803 ^T	X92631
<i>Pilimelia anulata</i>	DSM 43039 ^T	X93189
<i>Pilimelia terevasa</i>	DSM 43040 ^T	X93190
<i>Spirilliplanes yamanashiensis</i>	IFO 15828 ^T	D63912
<i>Verrucosipora gifhornensis</i>	DSM 44337 ^T	Y15523

DNA base composition and DNA–DNA hybridization.

Genomic DNA was isolated as described previously (Hopwood *et al.*, 1985). The G + C content of the DNA was determined by HPLC (Mesbah *et al.*, 1989). DNA–DNA hybridization studies were carried out by the previously described methods of Hopwood *et al.* (1985).

Phylogenetic analysis and nucleotide accession numbers.

The cloning of 16S rDNA following PCR-mediated amplification from genomic DNA was carried out as described previously (Lee *et al.*, 2000). The cloned 16S rDNA sequence was determined using the Cy5 AutoRead Sequencing Kit (Pharmacia). The sequence obtained was aligned by using the program CLUSTAL W (Thompson *et al.*, 1994) with those of the type strains of the family *Micromonosporaceae* and

related actinomycete taxa. Evolutionary distance matrices were calculated by using the method of Jukes & Cantor (1969), and a phylogenetic tree was reconstructed by the neighbour-joining method (Saito & Nei, 1987). The reliability of the tree topology was evaluated by using SEQBOOT, DNADIST, NEIGHBOR and CONSENSE programs in the PHYLIP package (Felsenstein, 1985, 1993), based on 1000 bootstrapped trees generated.

The reference sequences used in this study are listed in Table 1. The strains used as outgroup members were *Nocardia asteroides* DSM 43757^T (X80606), *Saccharomonospora viridis* NCIMB 9602^T (Z38021), *Streptomyces griseus* KCTC 9080^T (M76388) and *Streptosporangium roseum* DSM 43021^T (X89947). The 16S rDNA sequence of isolate LM 042^T (=

IMSNU 50729^T) has been deposited in the GenBank database under accession number AF171700.

RESULTS

Morphology

Isolate LM 042^T produced a well-developed and branched substrate mycelium. An aerial mycelium was not formed on any media tested. Straight, relatively short chains of non-motile spores were borne directly on substrate mycelium. Each spore was cylindrical and its surface was smooth (Fig. 1a). The substrate mycelium was cream-coloured to light yellow. Globose bodies, which were morphologically similar to the globose bodies of *Catellatospora tsunoense* (Asano *et al.*, 1989), were also observed abundantly on oatmeal agar by strain LM 042^T (Fig. 1b).

Chemotaxonomic characteristics

Isolate LM 042^T contained *meso*-diaminopimelic acid, rhamnose, xylose, glucose, mannose and ribose in whole-cell hydrolysates, indicating that this organism

Fig. 1. Scanning electron micrograph of isolate LM 042^T grown on agar media for 21 d. (a) Spore chains directly formed from substrate mycelium on oatmeal agar (ISP medium 3). (b) Globose bodies on inorganic salts/starch agar (ISP medium 4). Bars, 5 µm.

has a type II cell wall and a variant of sugar pattern D (Lechevalier & Lechevalier, 1970). The acyl type of muramic acid in the cell wall was glycolyl. Mycolic acids were not detected. The polar lipids contained phosphatidylethanolamine, diphosphatidylglycerol and phosphatidylinositol (a type PII phospholipid pattern of Lechevalier *et al.*, 1981). Glucosamine-containing phospholipid and phosphatidylcholine were not detected. The G + C content of the DNA was 70.4 mol%. (Table 2). The major menaquinone of isolate LM 042^T was MK-10(H₄); in addition, small amounts of MK-10(H₆) and MK-9(H₄) were also present. It has been previously reported that members of the genus *Catellatospora* contain major menaquinones, MK-9(H₄) or MK-10(H₆, H₈) depending on the strain (Asano & Kawamoto, 1986, 1988; Asano *et al.*, 1989). All of the data available for the type strains of the genus *Catellatospora* were also examined. The data obtained in this study, except for the menaquinone composition of *Catellatospora tsunoense*, were in agreement with the data published previously. It was shown that the major menaquinone of *Catellatospora tsunoense* was identical to that of the isolate LM 042^T in our analysis (Table 2). The predominant fatty acids of isolate LM 042^T were iso-branched hexadecanoic acid (iso-C_{16:0}), iso-pentadecanoic acid (iso-C_{15:0}) and heptadecenoic acid (C_{17:1}). A significant amount of saturated, unsaturated and anteiso-branched fatty acids were also present, whereas 10-methyl-branched fatty acid was not detected. The cellular fatty acid compositions of isolate LM 042^T and *Catellatospora* reference strains are shown in Table 3.

Physiological characteristics

Isolate LM 042^T grew strictly aerobically. This organism showed catalase and nitrate reductase activity but not urease activity. H₂S production was not observed. The ranges of temperature and pH for growth were 20–37 °C and pH 6.0–9.0, respectively. Growth did not occur at 42 °C and in the presence of 2% NaCl. No soluble pigments were produced in any of the media tested. Strain LM 042^T exhibited good growth on oatmeal agar, inorganic salts/starch agar, glucose/asparagine agar, yeast extract/glucose agar, Hickey–Tresner agar and Bennett agar. However, growth of this organism was poor to moderate on glycerol/asparagine agar, yeast extract agar, nutrient agar, tyrosine agar and tap-water agar. The physiological characteristics differentiating isolate LM 042^T from the reference strains of the genus *Catellatospora* are shown in Table 4.

Phylogenetic analysis

The almost complete 16S rDNA sequence of isolate LM 042^T, consisting of a continuous stretch of 1510 nucleotides (97% of the *Escherichia coli* sequence; Brosius *et al.*, 1978), was determined following cloning and sequencing of the amplified gene. The

Table 2. Chemotaxonomic characteristics of isolate LM 042^T and the reference strains of the genus *Catellatospora*

Abbreviations: *meso*-DAP, *meso*-diaminopimelic acid; 3-OH-DAP, 3-hydroxy-diaminopimelic acid; Xyl, xylose; Ara, arabinose; Gal, galactose; DPG, diphosphatidylglycerol; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PIM, phosphatidylinositol mannoside; PME, phosphatidylmethylethanolamine; PL, unknown phospholipid(s). Abbreviations for menaquinones are exemplified by MK-10(H₄): a tetrahydrogenated menaquinone with ten isoprene units.

Taxon	Cell wall diamino acid(s)	Characteristic sugar(s)	Polar lipids	Major menaquinone(s)	G + C content (mol %)
Isolate LM 042 ^T	<i>meso</i> -DAP	Xyl	DPG, PI, PE	MK-10(H ₄)	70.4
<i>Catellatospora tsunoense</i> IMSNU 22005 ^T	<i>meso</i> -DAP, 3-OH-DAP	Ara, Xyl, Gal	DPG, PI, PG, PME	MK-10(H ₄)	70.6
<i>Catellatospora citrea</i> subsp. <i>citrea</i> IMSNU 22008 ^T	<i>meso</i> -DAP, 3-OH-DAP	Ara, Xyl, Gal	DPG, PI, PIM, PE, PG, PL	MK-9(H ₄ , H ₆)	71.5
<i>Catellatospora citrea</i> subsp. <i>methionotrophica</i> IMSNU 22006 ^T	<i>meso</i> -DAP, 3-OH-DAP	Ara, Xyl, Gal	DPG, PI, PE	MK-9(H ₄ , H ₆)	70.8
<i>Catellatospora ferruginea</i> IMSNU 22009 ^{T†}	<i>meso</i> -DAP, 3-OH-DAP	Ara, Xyl, Gal	DPG, PI, PIM, PE, PG, PL	MK-10 (H ₆ , H ₈)	71.5
<i>Catellatospora matsumotoense</i> IMSNU 22003 ^{T*†}	<i>meso</i> -DAP, 3-OH-DAP	Ara, Xyl, Gal	DPG, PI, PE, PG, PL	MK-10 (H ₆ , H ₈)	71.0

* Contains 3-methylrhamnose as an additional diagnostic sugar.

† According to Asano *et al.* (1989) and Lee *et al.* (1999).

Table 3. Cellular fatty acid compositions (%) of isolate LM 042^T and *Catellatospora* reference strains

Values less than 1 % not shown. Abbreviations: i, iso; ai, anteiso; 10Me, 10-methyl.

Fatty acid	Isolate LM 042 ^T	<i>Catellatospora tsunoense</i> IMSNU 22005 ^T	<i>Catellatospora citrea</i> subsp. <i>citrea</i> IMSNU 22008 ^T	<i>Catellatospora citrea</i> subsp. <i>methionotrophica</i> IMSNU 22006 ^T	<i>Catellatospora ferruginea</i> IMSNU 22009 ^T	<i>Catellatospora matsumotoense</i> IMSNU 22003 ^T
C _{12:0}				1.2		
i-C _{14:0}		1.2		1.0	1.8	
C _{14:0}				1.4		
i-C _{15:0}	21.8	22.0	28.7	19.5	9.5	10.9
ai-C _{15:0}	3.5	4.6	3.4	4.5	21.0	3.8
C _{15:0}			3.0	2.1	4.9	
i-C _{16:1}						11.7
i-C _{16:0}	24.6	11.8	3.1	8.3	18.3	25.3
C _{16:1}	1.1	2.2				
C _{16:0}	3.6	2.2	1.9	8.1	3.1	1.9
i-C _{17:1}	1.5		3.2	1.7		2.2
i-C _{17:0}	8.2	5.7	4.8	3.5	1.1	1.9
C _{17:1}	16.9	18.5	16.9	13.9	15.4	12.0
C _{17:0}	1.3	8.8	14.5	9.8	20.3	1.7
ai-C _{18:1}						2.0
10Me-C _{17:0}		10.4				1.8
C _{18:1}	8.5	0.6	5.2	3.1		12.3
C _{18:0}	6.0	4.0	4.2	13.3	4.0	4.4
10Me-C _{18:0}		5.4				3.1
ai-C _{19:0}			4.7	2.4		
C _{19:1}			2.9	1.1		
C _{19:0}			2.0	1.3		

Table 4. Diagnostic physiological characteristics of isolate LM 042^T and the reference strains of the genus *Catellatospora*

Characteristic	Strain LM 042 ^T	<i>Catellatospora tsunoense</i> IMSNU 22005 ^T	<i>Catellatospora citrea</i> subsp. <i>citrea</i> IMSNU 22008 ^T	<i>Catellatospora citrea</i> subsp. <i>methionotrophica</i> IMSNU 22006 ^T	<i>Catellatospora ferruginea</i> IMSNU 22009 ^T	<i>Catellatospora matsumotoense</i> IMSNU 22003 ^{T*}
Growth at 37 °C	+	—	+	—	+	—
Utilization of carbon source:						
D-Arabinose	—	+	—	+	+	—
Dextran	—	—	—	—	+	—
D-Fructose	+	+	—	—	+	+
Melibiose	—	+	+	—	+	—
Methyl α -D-glucoside	—	—	—	—	+	—
D-Raffinose	+	+	—	—	+	+
L-Rhamnose	—	+	+	+	+	—
D-Ribose	—	—	—	—	+	+
D-Trehalose	—	+	+	+	+	+
Adonitol	—	—	—	—	+	—
D-Mannitol	—	—	—	—	+	—
Growth requirement for:						
Thiamin	—	+	—	—	+	—
Methionine	—	—	—	+	—	—
Enzyme activity:						
Nitrate reductase	+	+	+	+	—	+
Urease	—	—	—	—	—	+
Growth on:						
0.0001 % Crystal violet	+	—	+	+	+	+
0.001 % Brilliant green	+	+	—	—	+	+
Resistance to:						
20 μ g Vancomycin ml ⁻¹	—	—	—	—	+	+
50 μ g Novobiocin ml ⁻¹	—	—	—	—	+	+
50 μ g Gentamicin ml ⁻¹	+	—	—	—	—	—
5 μ g Neomycin ml ⁻¹	+	+	+	—	—	+
10 μ g Tetracycline ml ⁻¹	—	—	—	—	+	+

* According to Asano *et al.* (1989) and Lee *et al.* (1999).

sequence obtained was compared to those of the type strains of the family *Micromonosporaceae* and related taxa. A phylogenetic tree reconstructed by the neighbour-joining method (Saito & Nei, 1987) was based on the comparison of 1403 nucleotides present in all strains between bases 34 and 1495 (*E. coli* numbering system). Our phylogenetic analysis (Fig. 2) indicated that strain LM 042^T formed a cluster with members of the genus *Catellatospora*, which was supported by the bootstrap value of 78 %. Strain LM 042^T showed the highest 16S rDNA similarity values to *Catellatospora citrea* subsp. *citrea* (95.0 %), *Catellatospora citrea* subsp. *methionotrophica* (95.2 %) and *Catellatospora tsunoense* (94.9 %). We observed an equidistant relationship among strain LM 042^T, *Catellatospora ferruginea* and all other members of the *Micromonosporaceae* genera (levels of similarity 93.0–94.0 %). DNA–DNA hybridization studies showed that the isolate LM 042^T exhibited low levels of DNA

relatedness (14–26 %) to the type strains of existing members of the genus *Catellatospora*.

DISCUSSION

Comparative 16S rDNA sequence studies provide a rapid and precise way of determining membership of a new strain in a hierarchic classification system of actinomycete bacteria (Stackebrandt *et al.*, 1997). Analysis of the 16S rDNA sequence indicates that isolate LM 042^T is phylogenetically related to members of the family *Micromonosporaceae* within the actinomycete bacteria, which is supported by the presence of an almost complete set of signature nucleotides previously defined for the family *Micromonosporaceae* (Stackebrandt *et al.*, 1997). The only deviation is the signature at positions 69–122. The sequence of strain LM 042^T revealed an A-T pair at these positions, whereas G-C pairs are found in those of all other

Fig. 2. Phylogenetic tree showing the relationship of isolate LM 042^T to the type strains of the family *Micromonosporaceae*. The species used as outgroups were *Nocardia asteroides*, *Saccharomonospora viridis*, *Streptomyces griseus* and *Streptosporangium roseum*. The tree was reconstructed by the neighbour-joining method. The bootstrap values indicated at the branching points are expressed as percentages of 1000 replications. The scale bar indicates 1 nucleotide substitution per 100 nucleotides.

members of the family. Therefore, this signature should be excluded from the list of family-specific signatures, along with that at position 1113–1141 previously noted by Rheims *et al.* (1998). The binary similarity values of the 16S rDNA sequences between strain LM 042^T and members of the family *Micromonosporaceae* ranged from 92.9 to 95.2%, 1.2–2.7% lower than the range in which members of this family are related among themselves. The phylogenetic tree shown in Fig. 2 reveals that strain LM 042^T forms a cluster with members of the genus *Catellatospora*. Its phylogenetic neighbours are the two subspecies of the type species, *Catellatospora citrea* (sequence similarity 95.0–95.2%), and *Catellatospora tsunoense* (sequence similarity 94.9%). Members of the genus *Catellatospora* have been previously reported to be phylogenetically heterogeneous (Koch *et al.*, 1996). *Catellatospora ferruginea* forms a distinct clade within the evolutionary radiation of the family *Micromonosporaceae* and phylogenetically separates from the cluster encompassing *Catellatospora citrea*, *Catella-*

tospora tsunoense and isolate LM 042^T (93.7–95.9% sequence similarity), and *Catellatospora matsumotoense* (96.9% similarity) which is phylogenetically related to members of the genus *Micromonospora* and members of all other genera of the family *Micromonosporaceae* (95.8–97.5% similarity). *Catellatospora matsumotoense* has been recently proposed to be reclassified as *Micromonospora matsumotoense* on the basis of its 16S rDNA sequence and phenotypic characters (Lee *et al.*, 1999a).

Isolate LM 042^T can be readily distinguished chemically from the type strains of the genus *Catellatospora*. While strain LM 042^T contains meso-diaminopimelic acid, rhamnose, xylose, glucose, mannose and ribose in the whole-cell hydrolysates, all of the *Catellatospora* reference strains contain 3-hydroxy-diaminopimelic acid as an additional diamino acid and arabinose, xylose and galactose as diagnostic sugars (Table 2). *Catellatospora ferruginea* and *Catellatospora matsumotoense* also possess 3-*O*-methylrhamnose as

an additional characteristic sugar (Asano & Kawamoto, 1986). The major menaquinone of strain LM 042^T is MK-10(H₄) (Table 2), whereas other members of the genus contain MK-9(H₄) or MK-10(H₆,H₈). The inclusion of the new isolate to the genus *Catellatospora* increases the heterogeneity of its menaquinone compositions. Considerable quantitative differences are found between isolate LM 042^T and members of the genus *Catellatospora* in cellular fatty acids including the amounts of iso-C_{15:0}, anteiso-C_{15:0}, C_{15:0}, iso-C_{16:1}, iso-C_{16:0}, iso-C_{17:0}, C_{17:0}, C_{18:1} and C_{18:0}. Fatty acids with 19 carbon atoms, which are detected in small amounts in the strains of the type species *Catellatospora citrea*, are not present in isolate LM 042^T and other *Catellatospora* strains. Finally, low levels of DNA–DNA relatedness (<26%) between isolate LM 042^T and the type strains of the genus *Catellatospora* support, along with the chemical characters mentioned above, that this organism should be placed in a new species of the genus *Catellatospora*.

On the basis of physiological, chemotaxonomic and DNA–DNA hybridization data as well as the uniqueness of its 16S rDNA sequence, strain LM 042^T is readily differentiated from the previously validated species of the genus *Catellatospora*, and the name *Catellatospora koreensis* sp. nov. is proposed.

Description of *Catellatospora koreensis* sp. nov.

Catellatospora koreensis [ko.re.en'sis. M.L. adj. *koreensis* of Korea (pertaining to Korean soil from which the organism was isolated)].

Forms a well-developed, branched substrate mycelium. An aerial mycelium is absent. Short chains of spores are borne directly on substrate mycelium. Spores are cylindrical and non-motile. Spore surface is smooth. The colour of substrate mycelium is cream to light yellow. Globose bodies are formed. A soluble pigment is not produced. Good growth on oatmeal agar, inorganic salt/starch agar, glucose/asparagine agar, glucose/yeast extract agar, Hickey–Tresner agar and Bennett agar. Poor to moderate growth on glycerol/asparagine agar, yeast extract agar, nutrient agar, tyrosine agar and tapwater agar. Aerobic. Gram-positive. Catalase-positive. Urease-negative. Nitrate is reduced to nitrite. H₂S is not produced. Grows at pH 6.0–9.0. The temperature range for growth is between 20 and 37 °C. Utilizes L-arabinose, D-cellobiose, D-fructose, D-galactose, D-glucose, D-lactose, maltose, D-mannose, D-raffinose, starch, sucrose and D-xylose as carbon sources. No utilization of D-arabinose, dextran, gluconate, inulin, D-melezitose, melibiose, methyl α-D-glucoside, L-rhamnose, D-ribose, salicin, L-sorbose, D-trehalose, adonitol, dulcitol, butanol, *meso*-erythritol, ethanol, glycerol, *meso*-inositol, 2-propanol, propanol, D-mannitol, D-sorbitol and D-xylitol. Does not degrade adenine, hippurate, hypoxanthine, DL-tyrosine and xanthine. Hydrolyses casein, gelatin and starch but not DNA. Grows on 0.001% brilliant green and 0.0001% crystal violet. No growth on 3% NaCl and 0.01%

lysozyme. Susceptible to 50 µg novobiocin ml⁻¹, 20 µg vancomycin ml⁻¹, 100 µg streptomycin ml⁻¹ and 10 µg tetracycline ml⁻¹, but resistant to 50 µg gentamicin ml⁻¹ and 5 µg neomycin ml⁻¹. Cell wall contains *meso*-diaminopimelic acid, rhamnose, ribose, xylose, mannose and glucose (a type II cell wall and a variant of sugar pattern D). The acyl type of muramic acid is glycolyl. The predominant menaquinone is MK-10(H₄), with minor amounts of MK-9(H₄) and MK-10(H₆). The diagnostic phospholipids contain phosphatidylethanolamine, diphosphatidylglycerol and phosphatidylinositol. Glucosamine-containing phospholipid and phosphatidylcholine are absent. Mycolic acids are absent. The predominant fatty acids (>15.0% of total) are iso-C_{16:0}, iso-C_{15:0} and C_{17:1} acids. A significant amount of saturated, unsaturated and anteiso-branched fatty acids are also present. The G + C content of the DNA is 70.4 mol% (determined by HPLC). Isolated from a gold-mine cave. The type strain is LM 042^T (= IMSNU 50729^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.
- Asano, K. & Kawamoto, I. (1988). *Catellatospora citrea* subsp. *methionotrophica* subsp. nov., a methionine-deficient auxotroph of the *Actinomycetales*. *Int J Syst Bacteriol* **38**, 326–327.
- Asano, K., Masunaga, I. & Kawamoto, I. (1989). *Catellatospora matsumotoense* sp. nov. and *C. tsunoense* sp. nov., actinomycetes found in woodland soils. *Int J Syst Bacteriol* **39**, 309–313.
- Brosius, J., Palmer, J. L., Kennedy, J. P. & Noller, H. F. (1978). Complete nucleotide sequence of a 16S ribosomal RNA gene from *Escherichia coli*. *Proc Natl Acad Sci USA* **75**, 4801–4805.
- Collins, M. D., Goodfellow, M. & Minnikin, D. E. M. (1982). Polar lipid composition in the classification of *Arthrobacter* and *Microbacterium*. *FEMS Microbiol Lett* **15**, 299–302.
- Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.
- Felsenstein, J. (1993). PHYLIP (phylogeny inference package), version 3.51c. Seattle, WA: Department of Genetics, University of Washington.
- 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.
- Gordon, R. E., Barnett, D. A., Handerhan, J. E. & Pang, C. H.-N. (1974). *Nocardia coeliaca*, *Nocardia autotrophica*, and the *Nocardia* strain. *Int J Syst Bacteriol* **24**, 54–63.
- Hopwood, D. A., Bibb, M. J., Chater, K. F. & 7 other authors (1985). *Genetic Manipulation of Streptomyces: a Laboratory Manual*. Norwich: The John Innes Foundation.
- Jukes, T. H. & Cantor, C. R. (1969). Evolution of protein molecules. In *Mammalian Protein Metabolism*, pp. 21–132. Edited by H. N. Munro. New York: Academic Press.

- 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.
- 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.
- Lechevalier, M. P. & Lechevalier, H. A. (1970). Chemical composition as a criterion in the classification of aerobic actinomycetes. *Int J Syst Bacteriol* **20**, 435–443.
- Lechevalier, M. P., Stern, A. E. & Lechevalier, H. A. (1981). Phospholipids in the taxonomy of actinomycetes. *Zentralblatt Bakteriologie Supplementum* **11**, 111–116.
- Lee, S. D., Goodfellow, M. & Hah, Y. C. (1999). A phylogenetic analysis of the genus *Catellatospora* based on 16S ribosomal DNA sequences, including transfer of *Catellatospora matsumotoense* to the genus *Micromonospora* as *Micromonospora matsumotoense* comb. nov. *FEMS Microbiol Lett* **178**, 349–354.
- Lee, S. D., Kang, S.-O. & Hah, Y. C. (2000). *Hongia* gen. nov., a new genus of the order *Actinomycetales*. *Int J Syst Evol Microbiol* **50**, 191–199.
- Mac Faddin, J. F. (1980). *Biochemical Tests for Identification of Medical Bacteria*, 2nd edn. Baltimore: Williams & Wilkins.
- Mesbah, M., Premachandran, U. & Whitman, W. B. (1989). Precise measurement of the G + C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int J Syst Bacteriol* **39**, 159–167.
- Minnikin, D. E. M. (1988). Isolation and purification of mycobacterial wall lipids. In *Bacterial Cell Surface Techniques*, pp. 125–135. Edited by I. C. Hancock & I. R. Poxton. Chichester: Wiley.
- 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.
- Minnikin, D. E., Hutchinson, I. G., Caldicott, A. B. & Goodfellow, M. (1980). Thin layer chromatography of methanolysates of mycolic acid-containing bacteria. *J Chromatogr* **188**, 221–233.
- Minnikin, D. E., O'Donnell, A. G., Goodfellow, M., Alderson, G., Athalye, M., Schaal, A. & Parlett, J. H. (1984). An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. *J Microbiol Methods* **2**, 233–241.
- Rheims, H., Schumann, P., Rohde, M. & Stackebrandt, E. (1998). *Verrucospora gifhornensis* gen. nov., sp. nov., a new member of the actinobacterial family *Micromonosporaceae*. *Int J Syst Bacteriol* **48**, 1119–1127.
- Saddler, G. S., Tavecchia, P., Lociuolo, S., Zanol, M., Colombo, E. & Selva, E. (1991). Analysis of madurose and other actinomycete whole cell sugars by gas chromatography. *J Microbiol Methods* **14**, 185–191.
- 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., Rainey, F. A. & Ward-Rainey, N. L. (1997). Proposal for a new hierarchical classification system, *Actinobacteria* classis nov. *Int J Syst Bacteriol* **47**, 479–491.
- Staneck, J. L. & Roberts, G. D. (1974). Simplified approach to identification of aerobic actinomycetes by thin-layer chromatography. *Appl Microbiol* **28**, 226–231.
- 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.
- Thompson, J. D., Higgins, D. G. & Gibson, T. J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighing, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* **22**, 4673–4680.
- Uchida, K. & Aida, K. (1984). An improved method for the glycolate test for simple identification of the acyl type of bacterial cell walls. *J Gen Appl Microbiol* **30**, 131–134.
- Waksman, S. A. (1950). *The Actinomycetes: Their Nature, Occurrence, Activities and Importance*. Waltham, MA: Chronica Botanica.
- Waksman, S. A. (1961). Classification, Identification, and Description of Genera and Species. In *The Actinomycetes*, vol. 2. 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.