Tsukamurella soli sp. nov., isolated from soil

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A Gram-positive, rod-shaped, catalase-positive, oxidase-negative, white-coloured bacterium, designated strain JS18-1^T, was isolated from a soil sample collected from Halla mountain, Jeju island, Korea. 16S rRNA gene sequence analysis revealed that the strain was most closely related to members of the genus *Tsukamurella* with levels of sequence similarity of 95.4–96.5 %. Strain JS18-1^T shared highest 16S rRNA gene sequence similarity with *Tsukamurella strandjordii* DSM 44573^T (96.5 %), *Tsukamurella carboxydivorans* Y2^T (96.4 %) and *Tsukamurella tyrosinosolvens* DSM 44234^T (96.4 %). The G + C content of the total DNA of strain JS18-1^T was 70 mol%. The cell-wall peptidoglycan type was A1γ and mycolic acids were also detected. The predominant polar lipids were diphosphatidylglycerol, phosphatidylethanolamine and phosphatidylinositol. The major quinone was menaquinone-9 (MK-9) and major cell-wall sugars were arabinose, ribose and glucose. The major fatty acids (>10 % of the total fatty acids) were C_{16:0}, C_{18:1ω9c}, C_{18:0} 10-methyl and summed feature 3 (C_{16:1ω7c} and/or iso-C_{15:0} 2-OH). Phylogenetic analysis based on 16S rRNA gene sequences and chemotaxonomic, biochemical and physiological characteristics indicate that strain JS18-1^T represents a novel species of the genus *Tsukamurella*, for which the name *Tsukamurella soli* sp. nov. is proposed. The type strain is JS18-1^T (=KACC 20764^T=DSM 45046^T).

The genus *Tsukamurella* was proposed by Collins *et al.* (1988), the type species being *Tsukamurella paurometabola*. At the time of writing, the genus comprises 10 recognized species. *T. paurometabola* (Collins *et al.*, 1988), *T. incheonensis* (Yassin *et al.*, 1995), *T. pulmonis* (Yassin *et al.*, 1996), *T. tyrosinosolvens* (Yassin *et al.*, 1997) and *T. strandjordii* (Kattar *et al.*, 2001) were recovered from clinical specimens. *T. spumae* (Nam *et al.*, 2003), *T. sunchonensis* (Seong *et al.*, 2003), *T. pseudospumae* (Nam *et al.*, 2004), *T. spongiae* (Olson *et al.*, 2007) and *T. carboxydivorans* (Park *et al.*, 2009) were isolated from activated sludge, deep-water marine sponge and soil. Members of the genus *Tsukamurella* have been characterized based on the presence of mycolic acids, menaquinone-9 and A1γ-type peptidoglycan. Phylogenetically, the genus is closely related to the genus *Dietzia*. During the course of a study on cultivable bacterial

communities of forest soils of Halla mountain, Jeju island, Republic of Korea, a novel strain, designated JS18-1^T, was isolated by the spread-plate technique on R2A (Difco) agar after incubation at 30 °C for 7 days. The purified strain was routinely cultivated and maintained on R2A medium. The aim of the present study was to elucidate the taxonomic position of strain JS18-1^T by using a polyphasic approach.

Cell morphology was observed by light microscopy (Axio; Zeiss). The effects of various NaCl concentrations (0, 1, 2, 3, 4 and 5 %, w/v), pH (4.0–10.0, at intervals of 1.0 pH units) and temperature (5–45 °C, at intervals of 5 °C) on growth of strain JS18-1^T were investigated in R2A medium. Gram staining, catalase and oxidase activities, and hydrolysis of carboxymethylcellulose (CMC), casein, chitin, DNA, hypoxanthine, pectin, starch, Tween 80, tyrosine and xanthine were tested according to Smibert & Krieg (1994). The ability of the strain to grow on various substrates as sole carbon source was checked by using the basal medium of Boiron *et al.* (1993) and the methods employed by Nam *et al.* (2003, 2004). In addition, various physiological and biochemical

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain JS18-1^T is FJ917743.

A table showing differential phenotypic characteristics between strain JS18-1^T and the type strains of closely related species of the genus *Tsukamurella* is available with the online version of this paper.

properties were determined by using the API ZYM, API 20NE and API ID 32GN (bioMérieux) systems. Tests in these commercial systems were generally performed according to the manufacturer's instructions. Strain JS18-1^T was able to grow on R2A agar, nutrient agar (Difco) and trypticase soy agar (Difco), but not on MacConkey agar (Difco). Cells of strain JS18-1^T were Gram-positive, rod-shaped, non-spore-forming, catalase-positive and oxidase-negative. Colonies were white, dry and matt with irregular spreading margins and did not form capsules or aerial hyphae. Comparisons of the phenotypic characteristics of strain JS18-1^T and the type strains of closely related species of the genus *Tsukamurella* are presented in Table 1 and Supplementary Table S1 (available in IJSEM Online).

Chemotaxonomic studies were conducted with freeze-dried cells after cultivation in R2A medium for 2 days at 30 °C. Analysis of the peptidoglycan was performed according to the procedure described by Schleifer &

Kandler (1972). Polar lipids were extracted and then examined by two-dimensional TLC (Minnikin *et al.*, 1984). Quinones were isolated by using the method of Minnikin *et al.* (1984) and were then separated by HPLC. Analysis of whole-cell sugars was conducted according to the method of Stanek & Roberts (1974). The presence of mycolic acids was examined by using the method described by Minnikin *et al.* (1975). Cellular fatty acid methyl esters were prepared from cells grown in TSA medium for 2 days at 30 °C, and were analysed by GC according to the instructions of the Microbial Identification System (MIDI). The G+C content of the DNA was determined as described by Mesbah *et al.* (1989) by using a reversed-phase column (Supelcosil LC-18 S; Supelco). The peptidoglycan type of strain JS18-1^T was A1_γ based on directly cross-linked *meso*-diaminopimelic acid. Diphosphatidylglycerol, phosphatidylethanolamine and phosphatidylinositol were the major polar lipids. Strain JS18-1^T had a menaquinone pattern consisting of MK-9, MK-8 and MK-10, with MK-9 as the major component. Major cell-wall sugars were arabinose,

Table 1. Phenotypic properties that distinguish strain JS18-1^T from the type strains of recognized species of the genus *Tsukamurella*

Strains: 1, JS18-1^T; 2, *T. carboxydivorans* Y2^T (data from Park *et al.*, 2009); 3, *T. inchonensis* IMMIB D-771^T (Yassin *et al.*, 1995; Nam *et al.*, 2003); 4, *T. paurometabola* DSM 20162^T (Collins *et al.*, 1988; Olson *et al.*, 2007); 5, *T. pseudospumae* N1176^T (Nam *et al.*, 2003, 2004); 6, *T. pulmonis* IMMIB D-1321^T (Yassin *et al.*, 1996; Park *et al.*, 2009); 7, *T. spongiae* K362^T (Olson *et al.*, 2007); 8, *T. spumae* N1171^T (Nam *et al.*, 2003); 9, *T. strandjordii* DSM 44573^T (Kattar *et al.*, 2001; Park *et al.*, 2009); 10, *T. sunchonensis* SCNU5^T (Seong *et al.*, 2003); 11, *T. tyrosinosolvens* DSM 44344^T (Yassin *et al.*, 1997; Park *et al.*, 2009). +, Positive; −, negative; ND, not determined.

Characteristic	1	2	3	4	5	6	7	8	9	10	11
Colony colour	White	White, cream	White, cream	White, cream	Orange, red	White, cream	White, cream	Orange, red	White, cream	Orange	White, cream
Growth at 10 °C	+	−	−	+	+	−	−	−	−	−	−
Assimilation of:											
(+)-D-Arabinose	−	+	+	−	+	+	−	−	−	−	+
(+)-L-Arabinose	−	+	−	−	+	+	+	+	−	−	+
(+)-D-Arabitol	+	+	−	−	+	+	+	+	+	ND	+
(+)-Cellobiose	−	+	−	−	−	+	+	−	−	ND	+
Dulcitol	−	+	−	−	−	+	−	+	−	−	+
<i>meso</i> -Erythritol	−	+	−	−	−	+	−	+	−	−	+
(+)-D-Fructose	+	+	−	+	+	+	+	+	−	+	+
(+)-Maltose	−	+	+	−	+	+	−	+	−	+	+
(−)-D-Mannitol	+	+	+	+	−	−	+	+	+	+	+
(+)-Melezitose	−	+	+	−	+	−	+	+	−	+	+
(+)-Melibiose	−	+	+	+	−	+	+	+	+	−	+
(−)-D-Ribose	+	+	+	+	+	+	+	+	−	ND	+
(+)-D-Salicin	+	+	+	+	−	+	+	−	+	ND	+
(−)-D-Sorbitol	+	+	+	−	−	−	+	+	+	ND	+
Hydrolysis of:											
Aesculin	+	−	+	ND	+	+	ND	−	−	+	+
Casein	−	+	−	−	ND	−	ND	ND	−	−	−
Hypoxanthine	−	+	+	ND	+	+	ND	+	−	+	+
Tyrosine	+	+	−	−	+	−	ND	+	−	+	+
Urea	+	+	+	+	−	+	ND	−	+	−	−
Xanthine	−	+	−	−	−	−	ND	−	−	−	−

ribose and glucose; a minor amount of galactose was also detected. The major fatty acids (>10 % of the total) were C_{16:0} (28.1 %), C_{18:1}ω9c (24.7 %), C_{18:0} 10-methyl (12.9 %) and summed feature 3 (C_{16:1}ω7c and/or iso-C_{15:0} 2-OH; 10.6 %); minor components were C_{14:0} (5.1 %), C_{17:1}ω8c (3.7 %), C_{17:0} (3.7 %), C_{21:1}ω9c (2.6 %), summed feature 6 (C_{19:1}ω9c and/or C_{19:1}ω11c; 1.7 %) and C_{18:0} (1.1 %) (Table 2). The DNA G + C content of strain JS18-1^T was 70 mol%.

The 16S rRNA gene was amplified by colony PCR by using the primers fD1 and rP2 (Weisburg *et al.*, 1991) and the entire PCR fragment was directly sequenced (Hiraishi, 1992). Levels of sequence similarity were calculated by using the EzTaxon server (<http://www.eztaxon.org/>; Chun *et al.*, 2007). An alignment of 16S rRNA gene sequences was performed with the CLUSTAL W program (Thompson *et al.*, 1994), and phylogenetic analysis was performed by using MEGA version 3.1 (Kumar *et al.*, 2004). Phylogenetic dendrograms were constructed based on the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1971) methods with bootstrap values based on 1000 replications. Strain JS18-1^T exhibited levels of 16S rRNA gene sequence similarity of 95.4–

96.5 % to the type strains of recognized species of the genus *Tsukamurella*. It shared highest 16S rRNA gene sequence similarity with *T. strandjordii* DSM 44573^T (96.5 %), *T. carboxydivorans* Y2^T (96.4 %) and *T. tyrosinosolvens* DSM 44234^T (96.4 %). In the neighbour-joining tree, strain JS18-1^T formed a separate phylogenetic lineage from members of the genus *Tsukamurella* that was supported by a high bootstrap value (99 %) (Fig. 1); this result was also found in the maximum-parsimony tree.

The presence of mycolic acids, DNA G + C content (70 mol%), MK-9 as the predominant quinone, A1γ-type peptidoglycan and C_{16:0}, C_{18:1}ω9c, C_{18:0} 10-methyl and summed feature 3 as major fatty acids supported the classification of strain JS18-1^T within the genus *Tsukamurella*. However, the novel strain could be differentiated from recognized species of the genus *Tsukamurella* based on levels of 16S rRNA gene sequence similarity, phylogenetic analysis and several phenotypic characteristics (Table 1 and Supplementary Table S1). Strain JS18-1^T is therefore considered to represent a novel species of the genus *Tsukamurella*, for which the name *Tsukamurella soli* sp. nov. is proposed.

Table 2. Comparison between the fatty acid compositions (%) of strain JS18-1^T and those of members of the genus *Tsukamurella*

Strains: 1, JS18-1^T; 2, *T. carboxydivorans* Y2^T (data from the present study); 3, *T. inchonensis* IMMIB D-771^T (Kattar *et al.*, 2001); 4, *T. paurometabola* DSM 20162^T (Kattar *et al.*, 2001); 5, *T. pulmonis* IMMIB D-1321^T (Nam *et al.*, 2003); 6, *T. spongiae* K362^T (Olson *et al.*, 2007); 7, *T. spumae* N1171^T (Nam *et al.*, 2003); 8, *T. strandjordii* DSM 44573^T (data from the present study); 9, *T. suncheonensis* SCNU5^T (Seong *et al.*, 2003); 10, *T. tyrosinosolvens* DSM 44344^T (data from the present study). –, Less than 1 % or not detected.

Fatty acid	1	2	3	4	5	6	7	8	9	10
C _{10:0}	—	—	—	—	—	—	—	1.1	—	—
C _{14:0}	5.1	4.4	4.8	3.8	4.1	3.8	10.3	5.6	2.8	4.1
C _{15:0}	—	—	—	—	—	—	—	—	1.1	—
C _{16:1} ω9c	—	—	—	—	1.5	—	1.8	2.1	—	—
C _{16:0}	28.1	32.4	38.9	36.9	33.7	27.7	26.5	27.1	38.7	35.1
C _{17:1} ω8c	3.7	—	—	—	—	—	—	—	—	—
C _{17:0}	3.7	—	1.0	1.6	1.4	—	—	—	—	—
C _{17:0} 10-methyl	1.0	—	—	—	—	—	—	—	—	—
C _{18:1} ω9c	24.7	29.3	24.8	26.4	30.9	40.7	26.9	30.2	32.6	32.7
C _{18:0}	1.1	1.3	2.8	3.3	5.0	3.0	—	2.2	3.0	1.6
C _{18:0} 10-methyl	12.9	9.3	16.7	16.5	10.1	1.5	17.5	5.6	11.4	12.0
C _{20:1} ω9c	—	1.3	1.6	1.7	5.0	8.5	—	1.4	—	1.5
C _{20:1} ω7c	—	—	—	—	—	—	—	—	—	—
C _{20:0}	—	—	—	—	—	1.1	—	—	—	—
C _{21:1} ω9c	2.6	—	—	—	—	—	—	—	—	—
Summed features*										
3	10.6	16.0	7.1	9.2	6.3	10.3	15.7	23.1	—	12.0
4	—	3.3	—	—	—	—	—	—	—	—
5	—	1.0	—	—	—	—	—	—	—	—
6	1.7	—	1.0	—	—	—	—	1.1	—	—

*Summed features are groups of two or three fatty acids that cannot be separated by GLC with the MIDI system. Summed feature 3 comprises C_{16:1}ω7c and/or iso-C_{15:0} 2-OH; summed feature 4 comprises iso-C_{17:1} I and/or anteiso-C_{17:1} B; summed feature 5 comprises C_{18:2}ω6,9c and/or anteiso-C_{18:0}; summed feature 6 comprises C_{19:1}ω9c and/or C_{19:1}ω11c.

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the position of strain JS18-1^T with respect to the type strains of closely related species. Numbers at nodes indicate bootstrap values, expressed as percentages of 1000 replications; only values >50 % are shown. Filled circles indicate that the corresponding branches were also recovered in the maximum-parsimony tree. Bar, 0.01 changes per nucleotide position.

Description of *Tsukamurella soli* sp. nov.

Tsukamurella soli (so'li. L. neut. gen. n. *soli* of soil, the source of the type strain).

Cells are Gram-positive, rod-shaped (0.9–1.0 µm in length and 2.3–6.0 µm in width), catalase-positive and oxidase-negative. Colonies on R2A medium are white, dry and matt with irregular spreading margins. Growth occurs at 10–35 °C and at pH 4.0–9.0. The optimum temperature and pH for growth are 30 °C and 7.0. Tolerates NaCl concentrations up to 3.0 % (w/v). Hydrolyses Tween 80 and tyrosine, but not casein, chitin, CMC, DNA, hypoxanthine, pectin, starch or xanthine. Positive for urease and aesculin hydrolysis, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase and β-galactosidase (API 20NE). Assimilates D-glucose, D-mannitol, N-acetylglucosamine, trisodium citrate, D-ribose, inositol, sucrose, sodium acetate, lactic acid, L-alanine, glycogen, salicin, D-sorbitol, propionic acid, valeric acid and 3-hydroxybutyric acid, but not L-arabinose, D-mannose, maltose, potassium gluconate, capric acid, adipic acid, malic acid, phenylacetic acid, L-rhamnose, itaconic acid, suberic acid, sodium malonate, potassium 5-ketogluconate, 3-hydroxybenzoic acid, L-serine, melibiose, L-fucose, L-histidine, potassium 2-ketogluconate, 4-hydroxybenzoic acid or L-proline (API 20NE and API ID 32GN). Positive for alkaline phosphatase, esterase lipase (C8),

leucine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-glucosidase and β-glucosidase, weakly positive for esterase (C4) and lipase (C14), and negative for valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase (API ZYM). The cell wall contains A1γ-type peptidoglycan based on directly cross-linked *meso*-diaminopimelic acid. Major cell-wall sugars are arabinose, ribose and glucose; a minor amount of galactose is also present. Polar lipids are diphosphatidylglycerol, phosphatidylethanolamine and phosphatidylinositol. The predominant menaquinone is MK-9; minor amounts of MK-8 and MK-10 are also present. The major fatty acids (>10 % of the total) are C_{16:0}, C_{18:1ω9c}, C_{18:0} 10-methyl and summed feature 3 (C_{16:1ω7c} and/or iso-C_{15:0} 2-OH).

The type strain, JS18-1^T (=KACC 20764^T=DSM 45046^T), was isolated from a soil sample collected from Halla mountain, Jeju island, Republic of Korea. The DNA G+C content of the type strain is 70 mol%.

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