Sphingosinicella soli sp. nov., isolated from an alkaline soil in Korea

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A Gram-negative, motile, rod-shaped bacterial strain, KSL-125^T, was isolated from an alkaline soil from Kwangchun, Korea, and its taxonomic position was investigated in a polyphasic study. Strain KSL-125^T grew optimally at 30 °C, at pH 7.5–8.0 and in the presence of 0.5 % (w/v) NaCl.

A neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showed that strain KSL-125^T joins the cluster comprising the two *Sphingosinicella* species at a bootstrap resampling value of 100 %. The values for similarity between the 16S rRNA gene sequence of strain KSL-125^T and those of the type strains of the two *Sphingosinicella* species were 98.9–99.0 %. Strain KSL-125^T contained Q-10 as the predominant ubiquinone and C_{17:1}ω6c, C_{16:1}ω7c and/or iso-C_{15:0} 2-OH and C_{18:1}ω7c as the major fatty acids. The major polar lipids were sphingoglycolipid, diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylmonomethylethanolamine and an unidentified phospholipid. The DNA G + C content was 65.1 mol%. Strain KSL-125^T was distinguishable from the two recognized *Sphingosinicella* species on the basis of differential phenotypic properties, DNA–DNA relatedness data and repetitive-sequence-based PCR genomic fingerprinting patterns. The phenotypic, phylogenetic and genetic data showed that strain KSL-125^T represents a novel species of the genus *Sphingosinicella*, for which the name *Sphingosinicella soli* sp. nov. is proposed. The type strain is KSL-125^T (=KCTC 12482^T =DSM 17328^T).

The genus *Sphingosinicella* was proposed by Maruyama *et al.* (2006) with the description of a single species, *Sphingosinicella microcystinivorans*, as a novel member of the family *Sphingomonadaceae*. Subsequently, another *Sphingosinicella* species, *Sphingosinicella xenopeptidilytica*, was described (Geueke *et al.*, 2007). Here we report on the taxonomic characterization of a *Sphingosinicella*-like bacterial strain, KSL-125^T, which was isolated from an alkaline soil in Korea.

An alkaline soil collected from Kwangchun, Korea, was used as the source for the isolation of bacterial strains. Strain KSL-125^T was isolated with the usual dilution plating technique on 10 × diluted nutrient agar (Difco) at

30 °C. The type strains of the two *Sphingosinicella* species were used as reference strains: *S. microcystinivorans* KCTC 12019^T was obtained from the Korean Collection for Type Cultures (Taejeon, Korea) and *S. xenopeptidilytica* 3-2W4^T was obtained from H.-J. Busse (Geueke *et al.*, 2007). To investigate its morphological, physiological and biochemical characteristics, strain KSL-125^T was routinely cultivated at 30 °C on trypticase soy agar (TSA; Difco). The cell morphology was examined using light microscopy (E600; Nikon) and the presence of flagella was investigated using transmission electron microscopy with cells from exponentially growing cultures. The Gram reaction was determined by using the bioMérieux Gram stain kit according to the manufacturer's instructions. Growth in the absence of NaCl and at various NaCl concentrations (0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 %, w/v) was investigated in trypticase soy broth prepared according to the formula of the Difco medium except that NaCl was excluded. Growth at various temperatures (4–45 °C) was measured on TSA. The pH range for growth was determined in nutrient broth

Abbreviation: rep-PCR, repetitive-sequence-based PCR.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain KSL-125^T is DQ087403.

Biolog assimilation data for strain KSL-125^T and the two *Sphingosinicella* species are presented in a supplementary table available with the online version of this paper.

(Difco) adjusted to various pHs (initial pH 4.5–10.5, in increments of 0.5 pH units). The pH was adjusted to various levels prior to sterilization by the addition of HCl or Na₂CO₃. Growth under anaerobic conditions was determined after incubation in an anaerobic chamber on TSA and on TSA supplemented with nitrate, both of which had been prepared anaerobically using nitrogen. Catalase and oxidase activities and the hydrolysis of casein, starch, hypoxanthine, tyrosine, xanthine and Tweens 20, 40, 60 and 80 were determined as described by Cowan & Steel (1965). Hydrolysis of aesculin, gelatin and urea and reduction of nitrate were studied as described previously (Lanyi, 1987). Assimilation of various substrates was determined by using the Biolog GN2 MicroPlate assay as recommended by the manufacturer. Assimilation results were checked after 3 days. Enzyme activity was determined by using the API ZYM system (bioMérieux). Susceptibility to antibiotics was tested on TSA plates using antibiotic discs containing the following: polymyxin B, 100 U; streptomycin, 50 µg; penicillin G, 20 U; chloramphenicol, 100 µg; ampicillin, 10 µg; cephalothin, 30 µg; gentamicin, 30 µg; novobiocin, 5 µg; tetracycline, 30 µg; kanamycin, 30 µg; lincomycin, 15 µg; oleandomycin, 15 µg; neomycin, 30 µg; carbenicillin, 100 µg. Other physiological and biochemical tests were performed with the API 20E system (bioMérieux). The occurrence of genes *mlrA*, *mlrB*, *mlrC*, *mlrD* and *puf* was investigated by using primers and conditions described previously (Saito *et al.*, 2003; Gueke *et al.*, 2007); *S. microcystinivorans* KCTC 12019^T and *S. xenopeptidilytica* 3-2W4^T were used as positive or negative controls.

Cell biomass for DNA extraction and for the analyses of isoprenoid quinones and polar lipids was obtained from cultivation at 30 °C in trypticase soy broth (Difco) supplemented with 1% (v/v) Hutner's mineral base (Cohen-Bazire *et al.*, 1957). Chromosomal DNA was isolated and purified according to the method described by Yoon *et al.* (1996) except that RNase T1 was used in combination with RNase A. The 16S rRNA gene was amplified by using a PCR with two universal primers as described previously (Yoon *et al.*, 1998). Sequencing of the amplified 16S rRNA gene and phylogenetic analysis were performed as described by Yoon *et al.* (2003). The DNA G+C content was determined by using the method of Tamaoka & Komagata (1984) with the modification that DNA was hydrolysed and the resulting nucleotides were analysed by reversed-phase HPLC. Isoprenoid quinones were extracted according to the method of Komagata & Suzuki (1987) and were analysed using reversed-phase HPLC and a YMC ODS-A (250 × 4.6 mm) column. Polar lipids were extracted according to the procedures described by Minnikin *et al.* (1984) and were identified by using two-dimensional TLC followed by spraying with the appropriate detection reagents (Minnikin *et al.*, 1984; Komagata & Suzuki, 1987). For fatty acid methyl ester analyses, cell mass of strain KSL-125^T, *S. microcystinivorans* KCTC 12019^T and *S. xenopeptidilytica* 3-2W4^T was harvested

from TSA plates after cultivation for 7 days at 30 °C. The fatty acids were extracted and fatty acid methyl esters were prepared according to the standard protocol of the MIDI/Hewlett Packard Microbial Identification System (Sasser, 1990). Repetitive-sequence-based PCR (rep-PCR) genomic fingerprinting using REP, ERIC, BOX, (GTG)₅ and SERE PCR primers was performed as described previously (Versalovic *et al.*, 1994; Rademaker *et al.*, 1998; Rajashekara *et al.*, 1998). Computer-assisted analysis of the genomic fingerprints was performed by using GelCompar II (version 1.5) software (Applied Maths). Similarity among the patterns was calculated using Pearson's similarity coefficient and the dendrogram was constructed using the UPGMA algorithm (Sneath & Sokal, 1973).

Morphological, cultural, physiological and biochemical properties of strain KSL-125^T are shown in Table 1 and Supplementary Table S1 (available in IJSEM Online) or are given in the species description. The almost-complete 16S rRNA gene sequence of strain KSL-125^T determined in this study comprised 1409 nt (representing approximately 96% of the *Escherichia coli* 16S rRNA gene sequence). Comparative 16S rRNA gene sequence analyses showed that strain KSL-125^T is phylogenetically closely related to the family *Sphingomonadaceae* of the *Alphaproteobacteria* (Fig. 1). In the phylogenetic tree based on the neighbour-joining algorithm, strain KSL-125^T joined the cluster comprising the two *Sphingosinicella* species with 100% bootstrap support (Fig. 1). The 16S rRNA gene sequence of strain KSL-125^T shared the same signature nucleotides as those defined for the genus *Sphingosinicella*, as described by Gueke *et al.* (2007). Strain KSL-125^T exhibited 16S rRNA gene sequence similarity values of 98.9 and 99.0% with respect to the type strains of *S. microcystinivorans* and *S. xenopeptidilytica*, respectively, and showed less than 94.2% sequence similarity with respect to other species used in the phylogenetic analysis. Chemotaxonomic properties confirm the phylogenetic affiliation of strain KSL-125^T as a member of the genus *Sphingosinicella*. The predominant isoprenoid quinone detected in strain KSL-125^T was Q-10 (at a peak area ratio of approximately 90%) and minor amounts of Q-8 and Q-9 were also present. Strain KSL-125^T had a cellular fatty acid profile comprising large amounts of unsaturated, hydroxy and straight-chain fatty acids; the major fatty acids (>10% total fatty acids) were C_{17:1}ω6c (27.7%), C_{16:1}ω7c and/or iso-C_{15:0} 2-OH (23.3%) and C_{18:1}ω7c (19.0%). This cellular fatty acid profile was generally similar to those of the two *Sphingosinicella* species, but was distinguishable from them in terms of the contents of some fatty acids, particularly C_{17:1}ω6c, which was present as a major component in strain KSL-125^T but was only a minor component in the two *Sphingosinicella* species (Table 2). The major polar lipids detected in strain KSL-125^T were sphingoglycolipid, diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylmonomethylethanolamine and an unidentified phospholipid; a minor amount of an

Table 1. Differential phenotypic characteristics of *Sphingosinicella* species

Strains: 1, strain KSL-125^T; 2, *S. microcystinivorans* KCTC 12019^T; 3, *S. xenopeptidilytica* 3-2W4^T. Data for reference strains are from this study unless indicated. All strains were positive for catalase, oxidase, alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase and *N*-acetyl- β -glucosaminidase. All strains were negative for the Gram stain and for urease, arginine dihydrolase, indole production, hydrolysis of aesculin and gelatin, lipase (C14), cystine arylamidase, trypsin, naphthol-AS-BI-phosphohydrolase, α -galactosidase, β -galactosidase, β -glucuronidase, α -glucosidase, β -glucosidase, α -mannosidase and α -fucosidase. The *mlrD* gene was detected by PCR in all strains, but the *mlrC* gene was not detected. +, Positive; –, negative; w, weakly positive.

Characteristic	1	2	3
Nitrate reduction	–	+	+
Growth at 2.5 % (w/v) NaCl	–	– ^a *	+ ^b
Two red pigments in polar lipid extract	–	– ^a	+ ^b
Production of H ₂ S	–	–	+ ^b
Hydrolysis of Tween 80	+	–	– ^b
Detection (by PCR) of:			
<i>mlrA</i>	–	+ ^a	– ^b
<i>mlrB</i>	–	+ ^a	– ^b
<i>puf</i>	–	+ ^a	– ^b
Assimilation of:			
Tween 40	–	+	–
Tween 80	–	+	–
Methyl pyruvate	+	+	–
Monomethyl succinate	–	+	–
D-Galacturonic acid	–	+	–
α -Hydroxybutyric acid	–	+	+
<p>-Hydroxyphenylacetic acid</p>	–	+	–
α -Ketobutyric acid	–	+	+
α -Ketoglutaric acid	–	–	+
α -Ketovaleric acid	+	+	–
DL-Lactic acid	–	+	+
Glycyl L-aspartic acid	–	–	+
L-Ornithine	+	–	–
L-Proline	–	+	+
D-Serine	–	+	–
L-Threonine	–	+	–
DL- α -Glycerol phosphate	–	–	+
Glucose 1-phosphate	–	–	+
Enzyme activity (API ZYM):			
Valine arylamidase	+	–	–
α -Chymotrypsin	–	–	w
Acid phosphatase	–	–	+
Susceptibility to antibiotics			
Penicillin G	+	–	+
Ampicillin	+	–	+
Cephalothin	+	–	–
Carbenicillin	+	–	+
Novobiocin	+	+	–
DNA G + C content (mol%)	65.1	63.6–63.7 ^a , 64.4	65.0

*Data obtained from: *a*, Maruyama *et al.* (2006); *b*, Gueke *et al.* (2007).

Fig. 1. Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the positions of strain KSL-125^T and representatives of some related taxa. Bootstrap percentages (based on 1000 replications) ≥ 50 % are shown at nodes. *Rhodospirillum rubrum* ATCC 11170^T was used as an outgroup. Bar, 0.01 substitutions per nucleotide position.

Table 2. Cellular fatty acid compositions (%) of strain KSL-125^T and the type strains of the two *Sphingosinicella* species

Strains: 1, KSL-125^T; 2, *S. microcystinivorans* KCTC 12019^T; 3, *S. xenopeptidilytica* 3-2W4^T. Data are from this study. –, Not detected.

Fatty acid	1	2	3
Straight-chain fatty acids			
C _{14:0}	–	1.2	1.2
C _{15:0}	4.0	–	–
C _{16:0}	4.1	6.3	7.4
Unsaturated fatty acids			
C _{15:1ω6c}	2.3	–	–
C _{16:1ω5c}	2.2	7.9	5.9
C _{17:1ω6c}	27.7	0.9	1.9
C _{17:1ω8c}	3.0	–	–
C _{18:1ω5c}	–	0.9	1.6
C _{18:1ω7c}	19.0	37.9	38.8
Hydroxy fatty acids			
C _{14:0} 2-OH	6.6	10.7	8.5
C _{15:0} 2-OH	5.6	–	–
iso-C _{16:0} 3-OH	2.3	–	1.8
11-Methyl C _{18:1ω7c}	–	–	0.5
Summed feature 3*	23.3	34.2	32.5

*Summed features represent groups of two or three fatty acids that could not be separated by GLC with the MIDI system. Summed feature 3 contained C_{16:1 ω 7c} and/or iso-C_{15:0} 2-OH.

Fig. 2. Consensus dendrogram, based on similarity values obtained using the Jaccard correlation coefficient and the UPGMA algorithm in an analysis of the combined rep-PCR fingerprints generated by REP, ERIC, BOX, (GTG)₅ and SERE PCR primers, showing relationships among *Sphingosinicella* species.

unidentified glycolipid was also present. The DNA G+C content of strain KSL-125^T was 65.1 mol%.

Strain KSL-125^T could be distinguished from the two recognized *Sphingosinicella* species on the basis of differences in rep-PCR genomic fingerprinting patterns (Fig. 2). Strain KSL-125^T exhibited mean DNA–DNA relatedness values of 27 and 25 % with respect to the type strains of *S. microcystinivorans* and *S. xenopeptidilytica*, respectively. These data indicate that strain KSL-125^T differs genetically from the two recognized *Sphingosinicella* species (Wayne *et al.*, 1987). Strain KSL-125^T is also distinguishable from the two recognized *Sphingosinicella* species by differences in several phenotypic characteristics (Table 1). The phylogenetic and genetic distinctiveness and the differential phenotypic properties are sufficient to allocate strain KSL-125^T to a species that is separate from the recognized *Sphingosinicella* species (Wayne *et al.*, 1987; Table 1). Therefore, on the basis of the data presented, strain KSL-125^T represents a novel species of the genus *Sphingosinicella*, for which the name *Sphingosinicella soli* sp. nov. is proposed.

Description of *Sphingosinicella soli* sp. nov.

Sphingosinicella soli (so'li. L. gen. n. *soli* of soil).

Cells are Gram-negative, non-spore-forming, short rods or rods, 0.3–0.5 × 0.7–4.0 µm. Motile by means of single polar flagella. Colonies are circular, convex, smooth, glistening, yellow in colour and 0.5–1.0 mm in diameter after 7 days cultivation at 30 °C on TSA. The optimal growth temperature is 30 °C; growth occurs at 4 and 37 °C, but not at 38 °C. The optimal pH for growth is 7.5–8.0; growth occurs at pH 6.0 and 9.5, but not at pH 5.5 or 10.0. Optimal growth occurs in the presence of 0.5 % (w/v) NaCl; growth occurs in the absence of NaCl and in the presence of 1 % (w/v) NaCl but not in the presence of >2 % (w/v) NaCl. Growth does not occur under anaerobic conditions on TSA or on TSA supplemented with nitrate. Tweens 20, 40 and 60 are hydrolysed. Casein, starch, hypoxanthine, L-tyrosine and xanthine are not hydrolysed. Lysine decarboxylase, ornithine decarboxylase and tryptophan deaminase are absent. Susceptible to polymyxin B, streptomycin, chloramphenicol, gentamicin, tetracycline,

kanamycin and neomycin, but not to lincomycin or oleandomycin. Acetone/methanol extracts of freeze-dried cells show absorption maxima at 448–449 and 476 nm. The predominant ubiquinone is Q-10. The major fatty acids (>10 % of total fatty acids) are C_{17:1}ω6c, C_{16:1}ω7c and/or iso-C_{15:0} 2-OH and C_{18:1}ω7c. The major polar lipids are sphingoglycolipid, diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylmonomethylethanolamine and an unidentified phospholipid. The DNA G+C content is 65.1 mol% (determined by HPLC). Other phenotypic characteristics are shown in Table 1 and Supplementary Table S1.

The type strain, KSL-125^T (=KCTC 12482^T =DSM 17328^T), was isolated from an alkaline soil from Kwangchun, Korea.

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