Isoptericola dokdonensis sp. nov., isolated from soil

Jung-Hoon Yoon,¹ Peter Schumann,² So-Jung Kang,¹ Seo-Youn Jung¹ and Tae-Kwang Oh¹

Correspondence

Jung-Hoon Yoon
jhyoon@kribb.re.kr

¹Korea Research Institute of Bioscience and Biotechnology (KRIBB), Laboratory of Microbial Function, PO Box 115, Yusong, Taejeon, Korea

²DSMZ – Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Mascheroder Weg 1b, D-38124 Braunschweig, Germany

A Gram-positive, non-motile, rod- or coccoid-shaped *Isoptericola*-like bacterium, strain DS-3^T, was isolated from a soil sample from Dokdo, Korea, and its taxonomic position was investigated by a polyphasic approach. The organism grew optimally at 30 °C and pH 7.0–8.0. Strain DS-3^T had the peptidoglycan type based on L-Lys–D-Asp, and galactose, glucose, rhamnose and ribose as the whole-cell sugars. It contained MK-9(H₄) as the predominant menaquinone and anteiso-C_{15:0} and iso-C_{15:0} as the major fatty acids. The major polar lipids were diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol and two unidentified glycolipids. The DNA G + C content was 74.1 mol%. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain DS-3^T was most closely related to members of the genus *Isoptericola*. Similarity values between the 16S rRNA gene sequences of strain DS-3^T and the type strains of *Isoptericola* species ranged from 98.0 to 98.4 %. DNA–DNA relatedness values (11–23 %) and differential phenotypic properties demonstrated that strain DS-3^T was distinguishable from recognized *Isoptericola* species. On the basis of phenotypic properties and phylogenetic and genetic distinctiveness, strain DS-3^T represents a novel species in the genus *Isoptericola*, for which the name *Isoptericola dokdonensis* sp. nov. is proposed. The type strain is DS-3^T (=KCTC 19128^T =CIP 108921^T).

The genus *Isoptericola* was proposed by reclassification of *Cellulosimicrobium variabile* (Bakalidou *et al.*, 2002) as *Isoptericola variabilis* (Stackebrandt *et al.*, 2004). Currently, the genus *Isoptericola* comprises three species with validly published names: *Isoptericola variabilis* (Stackebrandt *et al.*, 2004), *Isoptericola hypogaeus* (Groth *et al.*, 2005) and *Isoptericola halotolerans* (Zhang *et al.*, 2005). In this study, we report on the taxonomic characterization of an *Isoptericola*-like bacterial strain, DS-3^T, which was isolated from a soil sample from Dokdo in Korea.

Strain DS-3^T was isolated using the standard dilution plating technique at 25 °C on nutrient agar (Difco). The type strains of three *Isoptericola* species were used as reference strains: *I. variabilis* DSM 10177^T and *I. hypogaeus* DSM 16849^T were obtained from the DSMZ (Braunschweig, Germany) and *I. halotolerans* KCTC 19046^T was obtained from the Korean Collection for Type Cultures (Taejeon, Korea). The morphological, physiological and biochemical characteristics of strain DS-3^T were investigated using routine cultivation on trypticase soy agar (TSA; Difco) at 30 °C. Cell morphology was examined by light microscopy (Nikon E600) and

transmission electron microscopy. The presence of flagella was determined by transmission electron microscopy using cells from exponentially growing cultures. For transmission electron microscopic observation, cells were negatively stained with 1 % (w/v) phosphotungstic acid and the grids were air-dried and examined with a Philips CM-20 transmission electron microscope. The Gram reaction was determined using the bioMérieux Gram stain kit according to the manufacturer's instructions. Growth at various temperatures (4–45 °C) was measured on TSA. To investigate tolerance of NaCl, trypticase soy broth was prepared according to the formula of the Difco medium and NaCl concentrations were varied (0, 0.5 and 1.0–10.0 %, w/v, at intervals of 1.0 %). The pH range for growth was determined in nutrient broth (Difco) adjusted to various pH values (pH 4.5–10.5 at intervals of 0.5 pH units) 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. Catalase and oxidase activities and hydrolysis of casein, gelatin, hypoxanthine, starch, Tweens 20, 40, 60 and 80, tyrosine, urea and xanthine were determined as described by Cowan & Steel (1965). Hydrolysis of aesculin and nitrate reduction was studied as described previously (Lanyi, 1987). Utilization of

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain DS-3^T is DQ387860.

substrates as sole carbon and energy sources was tested according to the method of Kämpfer *et al.* (1991). Susceptibility to antibiotics was tested on TSA plates using antibiotic discs containing the following concentrations: 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 properties and enzyme activities were tested using API 20E and API ZYM systems (bioMérieux).

Cell biomass for DNA extraction and for analyses of cell-wall components, isoprenoid quinones and polar lipids was obtained from cultures grown by shaking at 150 r.p.m. in trypticase soy broth (Difco) at 30 °C. Chromosomal DNA was isolated and purified according to the method described previously (Yoon *et al.*, 1996), with the exception that RNase T1 was used in combination with RNase A to minimize contamination with RNA. The 16S rRNA gene was amplified by PCR using 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 the method of Tamaoka & Komagata (1984) with the modification that DNA was hydrolysed and the resultant nucleotides were analysed by reverse-phase HPLC. The presence or absence of diaminopimelic acid in the peptidoglycan was determined by the method described by Komagata & Suzuki (1987). Preparation of cell walls and determination of peptidoglycan structure were carried out using modified methods of Schleifer & Kandler (1972) and MacKenzie (1987). Whole-cell sugars were determined as described by Komagata & Suzuki (1987). Isoprenoid quinones were extracted according to the method of Komagata & Suzuki (1987) and analysed using reverse-phase HPLC on a YMC ODS-A (250 × 4.6 mm) column. Polar lipids were extracted according to the procedures described by Minnikin *et al.* (1984) and identified by two-dimensional TLC followed by spraying with appropriate detection reagents (Minnikin *et al.*, 1984; Komagata & Suzuki, 1987). For fatty acid methyl ester analysis, cell mass of strain DS-3^T was harvested from TSA plates after incubation for 3 days at 30 °C. The fatty acid methyl esters were extracted and prepared according to the standard protocol of the MIDI/Hewlett Packard Microbial Identification System (Sasser, 1990). DNA–DNA hybridization was performed fluorometrically by the method of Ezaki *et al.* (1989) using photobiotin-labelled DNA probes and microdilution wells. Hybridization was performed with five replications for each sample. The highest and lowest values obtained in each sample were excluded and the means of the remaining three values were quoted as DNA–DNA relatedness values.

The morphological, cultural, physiological and biochemical characteristics of strain DS-3^T are given in the species description (see below) or are shown in Table 1. The almost

complete 16S rRNA gene sequence of strain DS-3^T determined in this study comprised 1474 nt, representing approximately 96 % of the *Escherichia coli* 16S rRNA gene sequence. Comparative 16S rRNA gene sequence analysis showed that strain DS-3^T was most closely related to members of the genus *Isoptericola*. In a phylogenetic tree based on the neighbour-joining algorithm, strain DS-3^T joined the clade comprising *Isoptericola* species at a bootstrap confidence value of 85.0 % (Fig. 1). Strain DS-3^T exhibited 16S rRNA gene sequence similarity values of 98.0, 98.0 and 98.4 % to the type strains of *I. halotolerans*, *I. hypogaeus* and *I. variabilis*, respectively, and of less than 97.6 % to other species included in the phylogenetic analysis.

The total hydrolysate of the peptidoglycan of strain DS-3^T contained the amino acids alanine and glutamic acid and smaller amounts of lysine and aspartic acid. In addition, L-Lys–D-Asp was present, which is stable under conditions of total hydrolysis (4 M HCl, 16 h at 100 °C). From quantitative analysis of the peptidoglycan amino acids by gas chromatography, strain DS-3^T contained alanine, aspartic acid, glutamic acid and lysine in an approximate ratio of 1.4:0.3:1.0:0.2. The reduced amounts of lysine and aspartic acid were due to the occurrence of the stable peptide L-Lys–D-Asp. From these data, it was concluded that strain DS-3^T showed the peptidoglycan type A4_α, based on L-Lys–D-Asp, as described by Schleifer & Kandler (1972). The peptidoglycan structure of strain DS-3^T was the same as that of *I. variabilis* and *I. halotolerans*, but different from that of *I. hypogaeus*, which has the peptidoglycan type based on L-Lys–D-Glu (Stackebrandt *et al.*, 2004; Groth *et al.*, 2005; Zhang *et al.*, 2005). Strain DS-3^T contained MK-9(H₄), at peak area ratio of approximately 85 %, as the predominant menaquinone; smaller amounts (approx. 6–9 %) of MK-9(H₂) and MK-8(H₄) were also present. This menaquinone profile was similar to those of *I. variabilis* and *I. halotolerans*, but different from that of *I. hypogaeus*, which has major amounts of MK-9(H₂) and MK-9 and a minor amount of MK-9(H₄) (Stackebrandt *et al.*, 2004; Groth *et al.*, 2005; Zhang *et al.*, 2005). Major polar lipids detected in strain DS-3^T were diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol and two unidentified glycolipids. This polar lipid profile was similar to those of *Isoptericola* species, although it differed from that of *I. hypogaeus* in that phosphatidylinositol mannoside was absent (Stackebrandt *et al.*, 2004; Groth *et al.*, 2005; Zhang *et al.*, 2005). The fatty acid profile of strain DS-3^T comprised (>0.5 % of total fatty acids) branched fatty acids anteiso-C_{15:0} (58.6 %), iso-C_{15:0} (11.5 %), iso-C_{14:0} (4.2 %), anteiso-C_{17:0} (4.1 %), iso-C_{16:0} (3.0 %), iso-C_{13:0} (1.1 %) and iso-C_{17:0} (0.5 %); and straight-chain fatty acids C_{16:0} (9.0 %), C_{14:0} (3.3 %), C_{15:0} (2.6 %) and C_{17:0} (0.6 %). This fatty acid profile was similar to those of *Isoptericola* species in that anteiso-C_{15:0} was the major fatty acid (Stackebrandt *et al.*, 2004; Groth *et al.*, 2005; Zhang *et al.*, 2005). The DNA G + C content of strain DS-3^T was 74.1 mol%. Based on the results of the phylogenetic and chemotaxonomic analyses, it seems appropriate that strain

Table 1. Differential phenotypic characteristics of strain DS-3^T and *Isoptericola* species

Species/strains: 1, strain DS-3^T (*I. dokdonensis* sp. nov.); 2, *I. variabilis* (unless indicated, data from Bakalidou *et al.*, 2002; Groth *et al.*, 2005; Zhang *et al.*, 2005); 3, *I. hypogeus* (Groth *et al.*, 2005); 4, *I. halotolerans* (Zhang *et al.*, 2005). +, Positive; –, negative; w, weakly positive; ND, no data available; v, variable. All taxa are positive for Gram-staining, catalase (data from this study for *I. hypogeus*), nitrate reduction, hydrolysis of aesculin (data from this study for *I. halotolerans*), utilization of D-glucose, D-galactose, D-cellobiose, D-mannose, D-xylose, L-arabinose and salicin and activity of esterase (C4), esterase lipase (C8), leucine arylamidase, naphthol-AS-BI-phosphohydrolase, β -galactosidase, α -glucosidase and β -glucosidase and are susceptible (data from this study) to neomycin (30 μ g), tetracycline (30 μ g) and chloramphenicol (100 μ g). All species are negative for motility, hydrolysis of Tween 80 (data from this study for *I. hypogeus*) and activity of α -chymotrypsin, β -glucuronidase, α -mannosidase and α -fucosidase and are not susceptible (data from this study for *I. halotolerans*) to ampicillin (10 μ g) or kanamycin (30 μ g).

Characteristic	1	2	3	4
Oxidase	+	–	–*	–
Growth at:				
10 °C	+	–	+	+
42 °C	–	+	–	+
Growth in the presence of 10 % (w/v) NaCl	–	+	–	+
Hydrolysis of:				
Casein	–	+†	+	–
Gelatin	+	+	+	–
Hypoxanthine	–	+	+	–*
Starch	–	+†	+	–
Tyrosine	–	+	+	+*
Urea	+	+†	–	–
Xanthine	–	+	+	–*
Utilization of:				
D-Fructose	–	+	+	+
D-Trehalose	+	+	–	+
Sucrose	+	+	–	+
Maltose	+	+	w	+
Acetate	–	+	–	ND
L-Glutamate	–	+	–	ND
API ZYM tests				
Alkaline phosphatase	–	+	+	–*
Lipase (C14)	–	w	–	–*
Valine arylamidase	–	w	w	–*
Cystine arylamidase	–	+	+	–*
Trypsin	–	v	–	–*
Acid phosphatase	–	w	+	–*
α -Galactosidase	+	+	–	–*
N-Acetyl- β -glucosaminidase	+	+	+	–*
Susceptibility to:				
Gentamicin (30 μ g)	–	+*	+*	+*
Novobiocin (5 μ g)	+	–*	+*	+*
Penicillin G (20 U)	+	–	–	+*
Streptomycin (50 μ g)	+	–	+	+*
Polymyxin B (100 U)	+	w	+	w*
Cephalothin (30 μ g)	+	+*	–*	+*
Carbenicillin (100 μ g)	+	+*	–*	–*
Lincomycin (15 μ g)	+	–	–	–*
Oleandomycin (15 μ g)	–	–*	–*	+*
Cell-wall sugars‡	Gal, Glc, Rha, Rib	Gal, Glc, Rha	Gal, Glc, Rha, (Man)	Gal
Peptidoglycan type	L-Lys–D-Asp	L-Lys–D-Asp	L-Lys–D-Glu	L-Lys–D-Asp
DNA G+C content (mol%)	74.1	70–72	73.8	72.8

*Data for the type strain from this study.

†Data from Groth *et al.* (2005); the opposite result was reported by Zhang *et al.* (2005).

‡Abbreviations: Gal, galactose; Glc, glucose; Man, mannose; Rha, rhamnose; Rib, ribose. Minor components are indicated in parentheses. Data for strain DS-3^T and *I. hypogeus* were obtained from whole-cell hydrolysates.

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the positions of strain DS-3^T, *Isoptericola* species and some other related taxa. Bootstrap values (expressed as percentages of 1000 replications) of >50% are shown at branch points. *Jonesia denitrificans* DSM 20603^T was used as an outgroup. Bar, 0.01 substitutions per nucleotide position.

DS-3^T be considered as a member of the genus *Isoptericola*, although there are some differences in chemotaxonomic properties between strain DS-3^T and *I. hypogaeus* (Stackebrandt *et al.*, 2004; Groth *et al.*, 2005; Zhang *et al.*, 2005).

DNA–DNA relatedness data demonstrated that strain DS-3^T represents a genomic species that is different from recognized *Isoptericola* species (Wayne *et al.*, 1987). Mean DNA–DNA relatedness values between strain DS-3^T and the type strains of the three recognized *Isoptericola* species were in the range of 11–23%. Strain DS-3^T could also be distinguished from the three recognized *Isoptericola* species by noteworthy differences in several phenotypic properties as shown in Table 1. Therefore, on the basis of the data presented, strain DS-3^T should be placed in the genus *Isoptericola* within a novel species, for which the name *Isoptericola dokdonensis* sp. nov. is proposed.

Description of *Isoptericola dokdonensis* sp. nov.

Isoptericola dokdonensis (dok.do.nen'sis. N.L. masc. adj. *dokdonensis* of Dokdo, Korea, where the organism was first isolated).

Cells are Gram-positive, non-motile rods or cocci (0.8–1.1 × 0.8–4.5 µm). Primary mycelium is formed. Colonies on TSA are circular, convex, smooth, yellow in colour and 1.0–2.0 mm in diameter after 3 days of incubation at 30 °C. Optimal temperature for growth is 30 °C. Growth occurs at 10 and 40 °C, but not at 4 or 41 °C. Optimal pH for growth is between 7.0 and 8.0; growth occurs at pH 6.5, but not at pH 6.0. Growth occurs in the presence of 0–9% (w/v) NaCl. Anaerobic growth occurs on TSA and on TSA supplemented with nitrate. Tweens 20, 40 and 60 are not hydrolysed. H₂S and indole are not produced.

Arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase and tryptophan deaminase are absent. Pyruvate is utilized as a carbon source, but benzoate, citrate, L-malate, succinate and formate are not. The cell-wall peptidoglycan type is L-Lys–D-Asp. The whole-cell sugars are galactose, glucose, rhamnose and ribose. The predominant menaquinone is MK-9(H₄). The major fatty acids (>10% of total fatty acids) are anteiso-C_{15:0} and iso-C_{15:0}. The major polar lipids are diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol and two unidentified glycolipids. The DNA G + C content is 74.1 mol% (determined by HPLC). Other phenotypic characteristics are given in Table 1.

The type strain, DS-3^T (=KCTC 19128^T = CIP 108921^T), was isolated from soil in Dokdo, Korea.

Acknowledgements

This work was supported by the 21C Frontier program of Microbial Genomics and Applications (grant MG05-0401-2-0) from the Ministry of Science and Technology (MOST) of the Republic of Korea. We are grateful to the Ulleung County Administration and the Cultural Heritage Administration of the Republic of Korea for aiding access to Dokdo.

References

- Bakalidou, A., Kämpfer, P., Berchtold, M., Kuhnigk, T., Wenzel, M. & König, H. (2002). *Cellulosimicrobium variabile* sp. nov., a cellulolytic bacterium from the hindgut of the termite *Mastotermes darwiniensis*. *Int J Syst Evol Microbiol* 52, 1185–1192.
- Cowan, S. T. & Steel, K. J. (1965). *Manual for the Identification of Medical Bacteria*. London: Cambridge University Press.
- Ezaki, T., Hashimoto, Y. & Yabuuchi, E. (1989). Fluorometric deoxyribonucleic acid–deoxyribonucleic acid hybridization in micro-dilution wells as an alternative to membrane filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains. *Int J Syst Bacteriol* 39, 224–229.
- Groth, I., Schumann, P., Schütze, B., Gonzalez, J. M., Laiz, L., Saiz-Jimenez, C. & Stackebrandt, E. (2005). *Isoptericola hypogaeus* sp. nov., isolated from the Roman catacomb of Domitilla. *Int J Syst Evol Microbiol* 55, 1715–1719.
- Kämpfer, P., Steiof, M. & Dott, W. (1991). Microbiological characterization of a fuel-oil contaminated site including numerical identification of heterotrophic water and soil bacteria. *Microb Ecol* 21, 227–251.
- Komagata, K. & Suzuki, K. (1987). Lipids and cell-wall analysis in bacterial systematics. *Methods Microbiol* 19, 161–203.
- Lanyi, B. (1987). Classical and rapid identification methods for medically important bacteria. *Methods Microbiol* 19, 1–67.
- MacKenzie, S. L. (1987). Gas chromatographic analysis of amino acids as the *N*-heptafluorobutyl isobutyl esters. *J Assoc Off Anal Chem* 70, 151–160.
- 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.
- Sasser, M. (1990). *Identification of bacteria by gas chromatography of cellular fatty acids*, Technical Note no. 101. Newark, DE: MIDI Inc.

- Schleifer, K. H. & Kandler, O. (1972).** Peptidoglycan types of bacterial cell walls and their taxonomic implications. *Bacteriol Rev* **36**, 407–477.
- Stackebrandt, E., Schumann, P. & Cui, X.-L. (2004).** Reclassification of *Cellulosimicrobium variabile* Bakalidou *et al.* 2002 as *Isoptericola variabilis* gen. nov., comb. nov. *Int J Syst Evol Microbiol* **54**, 685–688.
- Tamaoka, J. & Komagata, K. (1984).** Determination of DNA base composition by reverse-phase high-performance liquid chromatography. *FEMS Microbiol Lett* **25**, 125–128.
- Wayne, L. G., Brenner, D. J., Colwell, R. R. & 9 other authors (1987).** Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int J Syst Bacteriol* **37**, 463–464.
- Yoon, J.-H., Kim, H., Kim, S.-B., Kim, H.-J., Kim, W. Y., Lee, S. T., Goodfellow, M. & Park, Y.-H. (1996).** Identification of *Saccharomonospora* strains by the use of genomic DNA fragments and rRNA gene probes. *Int J Syst Bacteriol* **46**, 502–505.
- Yoon, J.-H., Lee, S. T. & Park, Y.-H. (1998).** Inter- and intraspecific phylogenetic analysis of the genus *Nocardioides* and related taxa based on 16S rDNA sequences. *Int J Syst Bacteriol* **48**, 187–194.
- Yoon, J.-H., Kang, K. H. & Park, Y.-H. (2003).** *Psychrobacter jeotgali* sp. nov., isolated from jeotgal, a traditional Korean fermented seafood. *Int J Syst Evol Microbiol* **53**, 449–454.
- Zhang, Y.-Q., Schumann, P., Li, W.-J., Chen, G.-Z., Tian, X.-P., Stackebrandt, E., Xu, L.-H. & Jiang, C.-L. (2005).** *Isoptericola halotolerans* sp. nov., a novel actinobacterium isolated from saline soil from Qinghai Province, north-west China. *Int J Syst Evol Microbiol* **55**, 1867–1870.