Dokdonella soli sp. nov., a gammaproteobacterium isolated from soil

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An aerobic, Gram-negative, yellow-coloured, rod-shaped bacterial strain, designated KIS28-6^T, was isolated from soil from Ulleung, an island located in the East Sea of Korea. A phylogenetic analysis revealed that strain KIS28-6^T was a member of the genus *Dokdonella*, having 16S rRNA gene sequence similarities of 98.1 and 96.9 % with respect to *Dokdonella fugitiva* CIP 108692^T and *Dokdonella koreensis* DSM 17203^T, respectively. Strain KIS28-6^T showed DNA–DNA hybridization values of 38 and 32 % with respect to *D. fugitiva* CIP 108692^T and *D. koreensis* DSM 17203^T, respectively. The major fatty acids (>10 %) were iso-C_{17:1ω9c} (35.7 %), iso-C_{17:0} (26.9 %) and iso-C_{15:0} (11.7 %), the major respiratory quinone was Q-8 and the DNA G+C content was 73.0 mol%. On the basis of the results obtained in this polyphasic taxonomic analysis, strain KIS28-6^T represents a novel species of the genus *Dokdonella*, for which the name *Dokdonella soli* sp. nov. is proposed. The type strain is KIS28-6^T (=KACC 12741^T =JCM 15421^T).

Ulleung is a South Korean island in the East Sea. The island consists primarily of trachyandesite (extrusive igneous rock) generated from volcanic eruptions that occurred about 9350 years ago. During an investigation of the presence of cultivable bacterial communities in the soils of Ulleung, we isolated a strain, designated KIS28-6^T, that was found to be phylogenetically related (albeit at rather low levels of sequence similarity) to members of the genus *Dokdonella* of the family *Xanthomonadaceae*. In this study, we describe the physiological, chemotaxonomic and phylogenetic characteristics of this organism. These polyphasic taxonomic studies have revealed that strain KIS28-6^T represents a novel species of the genus *Dokdonella*.

At the time of writing, the genus *Dokdonella* contains two species, namely *Dokdonella koreensis* and *Dokdonella fugitiva*; the type strains of these species were isolated from soil from Dokdo (a group of South Korean islets in

the East Sea) and composted potting soils in Portugal, respectively (Yoon *et al.*, 2006; Cunha *et al.*, 2006).

Strain KIS28-6^T was isolated from soil samples collected from Ulleung (37° 30' N 130° 52' E) by using the standard dilution plating technique and R2A medium (Difco) at pH 7.2 ± 0.2, as described previously (Reasoner & Geldreich, 1985). One of the isolates that appeared on the R2A medium, designated strain KIS28-6^T, was distinguishable by its yellow colonies; this strain was subjected to a taxonomic investigation.

The strain was cultivated at 30 °C on R2A agar for observation of the cell morphology under a light microscope and in a transmission electron microscope. All of the basic physiological and biochemical tests were performed at 30 °C as described by Gerhardt *et al.* (1994). The Gram reaction was determined by using a Gram-stain kit according to the instructions of the manufacturer (bioMérieux). Catalase activity was determined by assessing bubble production in 3 % (v/v) H₂O₂ and oxidase activity was determined using 1 % (w/v) tetramethyl-*p*-phenylenediamine. The pH range for growth was determined in R2A broth from pH 4.0 to 10.0 (in increments of 1.0 pH unit): the pH was adjusted, prior to sterilization, by

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of KIS28-6^T is EU685334.

A supplementary table showing the substrates utilized by strain KIS28-6^T and representatives of *Dokdonella* species is available with the online version of this paper.

adding HCl or Na₂CO₃. Growth at various NaCl concentrations (1–5 %) and at various temperatures (4–50 °C) was investigated in R2A broth and on R2A agar, respectively. Growth under anaerobic conditions was determined on R2A and on R2A supplemented with nitrite (prepared anaerobically using nitrogen) with incubation in an anaerobic chamber.

The activities of various enzymes and some physiological and biochemical properties were examined using the API 20NE and API ZYM systems (bioMérieux) according to the manufacturer's instructions. Susceptibility to the antibiotics polymyxin B, streptomycin, chloramphenicol, ampicillin, novobiocin, tetracycline, kanamycin, oleandomycin and trimethoprim (each at 50 µg l⁻¹) was tested on R2A agar plates. Strain KIS28-6^T consisted of strictly aerobic, Gram-negative, rod-shaped bacteria, 0.4–0.5 µm wide and 1.4–4.5 µm long. Colonies were yellow, round and convex with clear margins. The strain did not grow on tryptic soy agar (TSA), nutrient agar or MacConkey agar (Difco). The ability of KIS28-6^T to produce acid from lactose and arabinose serves to differentiate this strain from *D. fugitiva* CIP 108692^T. Also, strain KIS28-6^T and *D. koreensis* DSM 17203^T can be differentiated from *D. fugitiva* CIP 108692^T on the basis of their hydrolysis of tyrosine and aerobic reduction of nitrate. Differential properties for KIS28-6^T and the type strains of the two recognized species of the genus *Dokdonella* are given in Table 1.

The utilization of various substrates was examined in a liquid mineral salts medium containing the following (l⁻¹): NH₄Cl, 1.0 g; K₂HPO₄, 4.0 g; KH₂PO₄, 1.5 g; MgSO₄ · 7H₂O, 0.5 g; trace-element solution, 5 ml; pH 7.0 (Mukhopadhyaya *et al.*, 2000). The substrates (listed in Supplementary Table S1, available in IJSEM Online) were added at a concentration of 0.2 % for carbohydrates and 0.1 % for organic acids, alcohols and amino acids. The substrate-utilization test revealed that *D. fugitiva* CIP 108692^T can be differentiated from *D. koreensis* DSM 17203^T and strain KIS28-6^T on the basis of the utilization of sodium citrate, trehalose and casein. *D. fugitiva* CIP 108692^T and *D. koreensis* DSM 17203^T can be differentiated from strain KIS28-6^T on the basis of the utilization of succinic acid and glycine (Supplementary Table S1). Growth-factor and vitamin requirements were examined in mineral salts medium (Mukhopadhyaya *et al.*, 2000) supplemented with 50 mg yeast extract or 1 ml vitamin mixture (10 mg biotin and 20 mg pyridoxine in 100 ml distilled water) l⁻¹ and amended with the substrates listed in Supplementary Table S1. The results revealed that growth of strain KIS28-6^T increased significantly in medium amended with either yeast extract or vitamin mixture (data not shown). However, the addition of growth factors did not widen the substrate utilization of strain KIS28-6^T.

Genomic DNA was isolated using the method of Ausubel *et al.* (1987), except that the lysates were extracted twice with chloroform to remove residual phenol. The 16S rRNA gene was amplified by using the universal primers fd1 and rP2

Table 1. Differential properties of strain KIS28-6^T and the type strains of *Dokdonella* species

Strains: 1, strain KIS28-6^T; 2, *D. fugitiva* CIP 108692^T (unless indicated, data from Cunha *et al.*, 2006); 3, *D. koreensis* DSM 17203^T (unless indicated, data from Yoon *et al.*, 2006). +, Positive; (+), weakly positive; –, negative; R, resistant; S, sensitive; ND, no data available.

Characteristic	1	2	3
Motility	–	–	+
Catalase/oxidase	+/+	–/(+)	+/+
Motility	–	–	+
Growth temperature (°C)	5–35	25–45	10–39
Optimum growth temperature (°C)	30	40	30
Tolerance of NaCl (%)	≤1	≤2	≤3
Acid production from:*			
α-Lactose	+	–	–
L-Arabinose	+	–	+
Hydrolysis of:			
Tyrosine	+	–*	+
Aesculin	–	+*†	–
DNA	–	+*	ND
Urea	–	+*†	–
Aerobic nitrate reduction*	+	–	+
β-Galactosidase	+	+	–
Enzyme activities (API ZYM)*			
Valine arylamidase	–	+	–
N-Acetyl-β-glucosaminidase	+	–	–
Response to antibiotics (50 µg ml ⁻¹)*			
Polymyxin B	R	S	R
Streptomycin	S	S	R
Chloramphenicol	R	S	S
Ampicillin	S	S	R
Novobiocin	R	S	R
Oleandomycin	R	S	R
Trimethoprim	S	R	R
DNA G + C content (mol%)	73.0	68.3	71.0

*Data from this study.

†Negative according to Cunha *et al.* (2006).

(Weisburg *et al.*, 1991) and was sequenced as described by Weon *et al.* (2005). Alignment of 16S rRNA gene sequences was performed with the CLUSTAL W program (Thompson *et al.*, 1994) and a phylogenetic analysis was performed using MEGA, version 3.1 (Kumar *et al.*, 2004). Distances were calculated using the distance options of Kimura's two-parameter model (Kimura, 1983) and clustering was determined using the neighbour-joining and maximum-parsimony methods, with bootstrapping based on 1000 replicates. To determine genomic relatedness, the filter hybridization method was performed as described by Seldin & Dubnau (1985). Probe labelling was conducted by using the non-radioactive DIG High Prime system (Roche); hybridized DNA was visualized using the DIG luminescent detection kit (Roche). DNA–DNA relatedness was quantified by using a densitometer (Bio-Rad).

The almost-complete 16S rRNA gene sequence (1450 bp) was determined for strain KIS28-6^T. Phylogenetic analysis of 16S rRNA gene sequences revealed that strain KIS28-6^T was a member of the genus *Dokdonella* (Fig. 1). In the neighbour-joining phylogenetic tree (Fig. 1), the strain clustered with *D. fugitiva* A3^T and *D. koreensis* DS-123^T with 100 % bootstrap support. The branching order in the phylogenetic trees calculated using the neighbour joining, maximum-likelihood and maximum-parsimony methods were congruent for members of the genus *Dokdonella*. Strain KIS28-6^T showed the highest 16S rRNA gene sequence similarities with respect to *D. fugitiva* A3^T (98.1 %) and *D. koreensis* DS-123^T (96.9 %), while the levels of genomic DNA relatedness with *D. fugitiva* CIP 108692^T and *D. koreensis* DSM 17203^T were 38 and 32 %, respectively. A bacterium that shows 16S rRNA gene sequence similarity ≥ 97 % with respect to its closest relatives is generally considered to represent a different species when the DNA–DNA reassociation level is < 70 % (Wayne *et al.*, 1987). It was concluded, therefore, that strain KIS28-6^T is genotypically distant from these type strains.

Cellular fatty acid methyl esters were prepared using bacteria grown in R2A medium for 2 days at 30 °C and were analysed using GC according to the instructions of the Microbial Identification System (MIDI). Isoprenoid quinones were analysed by means of HPLC, as described by Groth *et al.* (1996). DNA G + C contents were determined by means of HPLC analysis of deoxyribonucleosides, as described by Mesbah *et al.* (1989), using a reversed-phased column (Supelcosil LC-18-S; Supelco). The fatty acid profiles of *D. fugitiva* CIP 108692^T and *D. koreensis* DSM 17203^T were determined previously on buffered charcoal-yeast extract and TSA, respectively (Yoon *et al.*, 2006; Cunha *et al.*, 2006). The novel strain was unable to grow on TSA, so we determined the fatty acid profile for KIS28-6^T, *D. fugitiva* CIP 108692^T and *D. koreensis* DSM 17203^T cultivated on R2A medium. The fatty acid profile of strain KIS28-6^T when grown on R2A is shown in Table 2. The results revealed the presence of iso-C_{18:0} and summed feature 3 (iso-C_{15:0} 2-OH and/or C_{16:1}ω7c) in *D. fugitiva* CIP 108692^T cultivated on R2A. However, these fatty acids were not expressed on buffered charcoal-yeast extract

medium (Cunha *et al.*, 2006). C_{17:1}ω5c was not present when *D. koreensis* DSM 17203^T was grown on TSA but was observed when this strain was grown on R2A (Table 2). This lends further support to the idea that the fatty acid profiles of bacteria vary according to the growth medium (Abu Hatab & Gaugler, 1997; Bartholomeusz *et al.*, 1998). The major quinone in strain KIS28-6^T was Q-8. The DNA G + C content of the strain was 73.0 mol%, which is close to those reported for *D. fugitiva* CIP 108692^T (68.3 mol%) and *D. koreensis* DSM 17203^T (71.0 mol%).

The ability of KIS28-6^T to grow at 5 °C and the presence of a large amount (26.9 %) of fatty acid iso-C_{17:0} are features that serve to differentiate this strain from *D. fugitiva* CIP 108692^T. The absence of motility and the presence of β-galactosidase and N-acetyl-β-glucosaminidase activities are features of the novel strain that serve to differentiate it from *D. koreensis* DSM 17203^T. On the basis of the information obtained in this polyphasic investigation, including data relating to biochemical properties, the fatty acid composition, the 16S rRNA gene sequence and DNA–DNA hybridization, strain KIS28-6^T represents a novel species within the genus *Dokdonella*, for which the name *Dokdonella soli* sp. nov. is proposed.

Description of *Dokdonella soli* sp. nov.

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

Cells are aerobic, Gram-negative, non-motile rods (0.4–0.5 μm wide and 1.4–4.5 μm long). Oxidase-positive and catalase-negative. Colonies on R2A are yellow, convex and round with clear margins. Growth occurs at 5–35 °C (optimally at 30 °C) and pH 5–8 (optimally at pH 6–7). NaCl is not required for growth but growth occurs at NaCl concentrations up to 1 %. Positive in the methyl red test, negative in the Voges–Proskauer test and negative for H₂S production and phenylalanine deaminase activity. Acid is produced from lactose and arabinose. Growth is stimulated by the addition of yeast extract or vitamin mixture. Susceptible to 50 μg streptomycin, ampicillin, tetracycline, kanamycin or trimethoprim 1⁻¹ but not to 50 μg polymyxin B, chloramphenicol, novobiocin or oleandomycin 1⁻¹. Gelatin, tyrosine and Tween 80 are hydrolysed.

Fig. 1. Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the relationships between strain KIS28-6^T and closely related species. Filled circles indicate that the corresponding branches were also recovered in the maximum-parsimony phylogenetic tree. Bootstrap percentages (based on 1000 replicates) are shown at branch points; values below 50 % are not shown. Bar, 0.01 changes per nucleotide position.

Table 2. Cellular fatty acid compositions of strain KIS28-6^T and type strains of related species

Strains: 1, strain KIS28-6^T; 2, *D. fugitiva* CIP 108692^T; 3, *D. koreensis* DSM 17203^T. Data were obtained in this study from strains cultivated on R2A medium at 30 °C and pH 7.2 ± 0.2 for 2 days. –, Not detected or <1%; ECL, equivalent chain-length.

Fatty acid	1	2	3
iso-C _{11:0}	2.5	1.4	4.2
iso-C _{11:0} 3-OH	5.7	6.5	6.2
anteiso-C _{15:0}	–	–	5.3
iso-C _{15:0}	11.7	16.1	13.4
C _{16:0}	2.0	2.7	7.7
iso-C _{16:0}	9.5	11.2	4.4
iso-C _{17:0}	26.9	18.7	10.6
anteiso-C _{17:0}	–	1.6	6.2
C _{17:1} ω5c	–	–	1.1
iso-C _{17:1} ω9c	35.7	34.6	32.1
iso-C _{18:0}	1.1	1.0	–
Unknown ECL 11.799	1.7	2.9	1.5
Summed feature 3*	1.2	1.7	3.5

*Summed features are groups of two or three fatty acids that cannot be separated by GLC with the Microbial Identification System. Summed feature 3 contained iso-C_{15:0} 2-OH and/or C_{16:1}ω7c.

Aesculin, chitin, CM-cellulose, DNA, hypoxanthine, pectin, urea and xanthine are not hydrolysed. Positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase and *N*-acetyl-β-glucosaminidase. Negative for indole production, glucose fermentation, arginine dihydrolase, lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, α-mannosidase and α-fucosidase. The major fatty acids (>5%) when grown on R2A are iso-C_{17:1}ω9c, iso-C_{17:0}, iso-C_{15:0}, iso-C_{16:0} and iso-C_{11:0} 3-OH. The major quinone is Q-8. The DNA G + C content of the type strain is 73.0 mol%.

The type strain, KIS28-6^T (=KACC 12741^T =JCM 15421^T), was isolated from soil from Ulleung, a South Korean island located in the East Sea (Sea of Japan).

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References

- Abu Hatab, M. A. & Gaugler, R. (1997). Growth-mediated variations in fatty acids of *Xenorhabdus* sp. *J Appl Microbiol* **82**, 351–358.
- Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A. & Struhl, K. (editors) (1987). *Current Protocols in Molecular Biology*. New York: Wiley.
- Bartholomeusz, G., Zhu, Y. & Downard, J. (1998). Growth medium-dependent regulation of *Myxococcus xanthus* fatty acid content is controlled by the *esg* locus. *J Bacteriol* **180**, 5269–5272.
- Cunha, S., Tiago, I., Pires, A. L., Da Costa, M. S. & Veríssimo, A. (2006). *Dokdonella fugitiva* sp. nov., a gammaproteobacterium isolated from potting soil. *Syst Appl Microbiol* **29**, 191–196.
- Gerhardt, P., Murray, R. G. E., Wood, W. A. & Krieg, N. R. (editors) (1994). *Methods for General and Molecular Bacteriology*. Washington, DC: American Society for Microbiology.
- Groth, I., Schumann, P., Weiss, N., Martin, K. & Rainey, F. A. (1996). *Agrococcus jenensis* gen. nov., sp. nov., a new genus of actinomycetes with diaminobutyric acid in the cell wall. *Int J Syst Bacteriol* **46**, 234–239.
- Kimura, M. (1983). *The Neutral Theory of Molecular Evolution*. Cambridge: Cambridge University Press.
- Kumar, S., Tamura, K. & Nei, M. (2004). MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. *Brief Bioinform* **5**, 150–163.
- 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.
- Mukhopadhyaya, P. N., Deb, C., Lahiri, C. & Roy, P. (2000). A *soxA* gene encoding a diheme cytochrome *c* and a *sox* locus, essential for sulfur oxidation in a new sulfur lithotrophic bacterium. *J Bacteriol* **182**, 4278–4287.
- Reasoner, D. J. & Geldreich, E. E. (1985). A new medium for the enumeration and subculture of bacteria from potable water. *Appl Environ Microbiol* **49**, 1–7.
- Seldin, L. & Dubnau, D. (1985). Deoxyribonucleic acid homology among *Bacillus polymyxa*, *Bacillus macerans*, *Bacillus azotofixans*, and other nitrogen-fixing *Bacillus* strains. *Int J Syst Bacteriol* **35**, 151–154.
- 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.
- Wayne, L. G., Brenner, D. J., Colwell, R. R., Grimont, P. A. D., Kandler, O., Krichevsky, M. I., Moore, L. H., Moore, W. E. C., Murray, R. G. E. & other authors (1987). International Committee on Systematic Bacteriology. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int J Syst Bacteriol* **37**, 463–464.
- Weisburg, W. G., Barns, S. M., Pelletier, D. A. & Lane, D. J. (1991). 16S ribosomal DNA amplification for phylogenetic study. *J Bacteriol* **173**, 697–703.
- Weon, H. Y., Kim, B. Y., Kwon, S. W., Park, I. C., Cha, I. B., Tindall, B. J., Stackebrandt, E., Trüper, H. G. & Go, S.-J. (2005). *Leadbetterella byssophila* gen. nov., sp. nov., isolated from cotton-waste composts for the cultivation of oyster mushroom. *Int J Syst Evol Microbiol* **55**, 2297–2302.
- Yoon, J. H., Kang, S. J. & Oh, T. K. (2006). *Dokdonella koreensis* gen. nov., sp. nov., isolated from soil. *Int J Syst Evol Microbiol* **56**, 145–150.