Algoriphagus terrigena sp. nov., isolated from soil

Jung-Hoon Yoon, Mi-Hwa Lee, So-Jung Kang and Tae-Kwang Oh

Correspondence

Jung-Hoon Yoon
jhyoon@kribb.re.kr

Tae-Kwang Oh
otk@kribb.re.kr

Korea Research Institute of Bioscience and Biotechnology (KRIIBB), PO Box 115, Yusong, Taejeon, Korea

A Gram-negative, non-motile, non-spore-forming bacterial strain, DS-44^T, was isolated from soil from Dokdo in Korea, and its taxonomic position was investigated by using a polyphasic approach. It grew optimally at 25 °C and in the presence of 2 % (w/v) NaCl. Strain DS-44^T contained MK-7 as the predominant menaquinone and iso-C_{15:0} and C_{16:1ω7c} and/or iso-C_{15:0} 2-OH as the major fatty acids. The DNA G + C content was 49.0 mol%. Phylogenetic analyses based on 16S rRNA gene sequences revealed that strain DS-44^T belongs to the genus *Algoriphagus* of the phylum *Bacteroidetes*. Similarity values between the 16S rRNA gene sequences of strain DS-44^T and those of the type strains of recognized *Algoriphagus* species were in the range 93.8–95.7 %, making it possible to categorize strain DS-44^T as a species that is separate from previously described *Algoriphagus* species. On the basis of its phenotypic properties and phylogenetic distinctiveness, strain DS-44^T (=KCTC 12545^T = CIP 108837^T) was classified in the genus *Algoriphagus* as the type strain of a novel species, for which the name *Algoriphagus terrigena* sp. nov. is proposed.

The genus *Algoriphagus* was first described by Bowman *et al.* (2003). At the time of writing, the genus consists of eight species with validly published names: *Algoriphagus ratkowskyi* (the type species; Bowman *et al.*, 2003), *A. aquimarinus*, *A. chordae* and *A. winogradskyi* (Nedashkovskaya *et al.*, 2004), *A. halophilus* (Yi & Chun, 2004; Nedashkovskaya *et al.*, 2004), *A. antarcticus* (Van Trappen *et al.*, 2004), *A. yeomjeoni* (Yoon *et al.*, 2005a) and *A. locisalis* (Yoon *et al.*, 2005b). *Algoriphagus* species have been isolated from marine environments, Antarctic lakes and salterns (Bowman *et al.*, 2003; Nedashkovskaya *et al.*, 2004; Van Trappen *et al.*, 2004; Yoon *et al.*, 2005a, b). In this study, we report on the taxonomic characterization of an *Algoriphagus*-like bacterial strain, DS-44^T, which was isolated from soil from Dokdo in Korea.

Strain DS-44^T was isolated by using the standard dilution plating technique on 10 × diluted nutrient agar (Difco) with distilled water at 25 °C. The morphological, physiological and biochemical characteristics of strain DS-44^T were investigated using routine cultivation on marine agar 2216 (MA; Difco) at 25 °C. The cell morphology was examined by using light microscopy (E600; Nikon) and transmission electron microscopy. The presence of flagella was determined using a transmission electron microscope with cells from exponentially growing cultures. Gliding motility was investigated as

described by Bowman (2000). The Gram reaction was determined by using the bioMérieux Gram stain kit according to the manufacturer's instructions. The pH range for growth was determined in marine broth 2216 (MB; Difco) adjusted to various pH values (pH 4.5–10.5 at increments of 0.5 pH units). Before sterilization, the pH was adjusted to various levels by the addition of HCl or Na₂CO₃. Growth in the absence of NaCl was investigated in trypticase soy broth prepared according to the formula of the Difco medium except that no NaCl was used. Growth at various NaCl concentrations [0.5 % (w/v) and 1.0–10.0 % (w/v) at increments of 1.0 %] was investigated in MB or trypticase soy broth (Difco). Growth at various temperatures (4–40 °C) was measured on MA. Growth under anaerobic conditions was determined after incubation in an anaerobic chamber on MA and on MA supplemented with nitrate, both of which had been prepared anaerobically under nitrogen. Catalase and oxidase activities and the hydrolysis of casein, gelatin, hypoxanthine, starch, Tweens 20, 40, 60 and 80, L-tyrosine, urea and xanthine were determined as described by Cowan & Steel (1965). The hydrolysis of aesculin and the reduction of nitrate were studied as described previously (Lanyi, 1987). H₂S production was tested as described previously (Bruns *et al.*, 2001). The presence of flexirubin-type pigments was investigated as described by Reichenbach (1992). Acid production from carbohydrates was determined as described by Leifson (1963). Utilization of substrates as sole carbon and energy sources was tested as described by Baumann & Baumann (1981), using supplementation with 2 % (v/v) Hutner's mineral base (Cohen-Bazire *et al.*, 1957) and 1 % (v/v) vitamin solution (Staley, 1968). Susceptibility to antibiotics was tested on MA plates

Published online ahead of print on 16 December 2005 as DOI 10.1099/ijs.0.64092-0.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain DS-44^T is DQ178979.

Details of the fatty acid compositions of *Algoriphagus* species are available in a supplementary table in IJSEM Online.

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 and biochemical tests were performed with the API 20E and API ZYM systems (bioMérieux).

Cell biomass of strain DS-44^T for DNA extraction and for isoprenoid quinone analysis was obtained by cultivation for

3 days in MB at 25 °C. Chromosomal DNA was isolated and purified according to a method described previously (Yoon *et al.*, 1996), except that RNase T1 was used in combination with RNase A to minimize contamination with RNA. 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 analyses were performed as described by Yoon *et al.* (2003). Isoprenoid quinones were extracted according to the method of Komagata & Suzuki (1987) and analysed using reversed-phase HPLC with a YMC ODS-A

Table 1. Differential phenotypic characteristics of *Algoriphagus* species

Species: 1, *A. terrigena* sp. nov.; 2, *A. ratkovskyi*; 3, *A. aquimarinus*; 4, *A. chordae*; 5, *A. winogradskyi*; 6, *A. halophilus*; 7, *A. antarcticus*; 8, *A. yeomjeoni*; 9, *A. locisalis*. Data are from Bowman *et al.* (2003), Yi & Chun (2004), Nedashkovskaya *et al.* (2004), Van Trappen *et al.* (2004) and Yoon *et al.* (2005a, b). +, Positive; –, negative; v, variable reaction; ND, not determined. Data in parentheses are for the type strain. All species are Gram-negative and rod-shaped and positive for catalase and oxidase. All species are negative for gliding motility, flexirubin-type pigment production, H₂S and indole production, utilization of citrate and susceptibility to ampicillin, benzylpenicillin, gentamicin, kanamycin, neomycin, polymyxin B and streptomycin.

Characteristic	1	2	3	4	5	6	7	8	9
Nitrate reduction	+	–	–	–	+	–	–	–	–
NaCl requirement for growth	+	+	–	+	–	–	ND	+	+
Optimal temperature for growth (°C)	25	16–19	23–25	23–25	25–28	35	20	25–30	30
Growth at/in:									
10 % NaCl	–	–	+	+	–	–	–	–	–
37 °C	–	–	–	–	+	+	–	–	–
Hydrolysis of:									
Agar	–	–	+	+	+	–	–	–	–
Casein	+	v	+	–	–	–	–	+	–
Gelatin	–	–	+	–	+	+	–	v (+)	–
Starch	–	v	–	–	+	+	–	–	–
Tween 80	+	–	+	–	–	+	ND	+	+
Acid production from:									
L-Arabinose	+	+	–	–	–	–	–	–	+
D-Cellobiose	+	+	+	+	+	–	ND	+	+
D-Galactose	+	+	–	+	+	–	ND	+	+
D-Glucose	+	+	+	+	+	+	–	+	+
Lactose	+	–	+	+	+	–	ND	+	+
Maltose	+	+	–	+	+	+	ND	+	+
D-Melibiose	+	+	+	+	+	–	–	+	+
L-Rhamnose	–	+	+	+	+	–	–	+	+
Sucrose	+	–	+	+	+	+	–	+	+
D-Xylose	+	+	+	+	+	+	ND	+	+
Utilization of:									
L-Arabinose	–	+	+	+	+	+	–	–	–
D-Glucose	+	+	+	+	+	+	–	+	+
D-Mannose	+	+	+	+	+	+	–	+	+
Malate	–	v	–	–	–	–	–	–	–
Susceptibility to:									
Carbenicillin	–	+	+	–	v	+	ND	+	–
Lincomycin	–	+	+	+	v	+	ND	+	+
Oleandomycin	–	+	+	–	+	+	ND	+	+
Tetracycline	–	+	+	–	v	+	ND	–	–
DNA G + C content (mol%)	49.0	35–37	41	37–40	39–42	37	40–41	41	42

(250 × 4.6 mm) column. For fatty acid methyl ester analysis, cell mass of strain DS-44^T was harvested from MA plates after incubation for 7 days at 25 °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). 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 reversed-phase HPLC.

The morphological, cultural, physiological and biochemical characteristics of strain DS-44^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-44^T, comprising 1479 nt (approx. 96 % of the *Escherichia coli* 16S rRNA gene sequence), was determined in this study. 16S rRNA gene sequence analyses showed that strain DS-44^T was most closely affiliated to the genus *Algoriphagus* of the phylum *Bacteroidetes* (Fig. 1). In the neighbour-joining tree based on 16S rRNA gene sequences, strain DS-44^T fell within the radiation of the cluster comprising *Algoriphagus* species (Fig. 1). The same tree topology was found in the trees generated with the maximum-likelihood and maximum-parsimony algorithms (Fig. 1). Strain DS-44^T exhibited 16S rRNA gene sequence similarity values of 93.8–95.7 % with respect to the eight recognized *Algoriphagus* species, 93.2–93.4 % with respect to *Hongiella* species and less than 89.8 % with respect to the other species used in the phylogenetic analysis.

Strain DS-44^T contained MK-7, at a peak area ratio of approximately 97 %, as the predominant isoprenoid quinone. The fatty acid profile of strain DS-44^T comprised (>1.0 % of total fatty acids) branched fatty acids iso-C_{15:0} (35.3 %), iso-C_{17:1}ω9c (8.5 %), iso-C_{16:1} H (2.5 %), anteiso-C_{15:0} (2.4 %), anteiso-C_{11:0} (1.6 %), iso-C_{11:0} (1.1 %), iso-C_{14:0} (1.0 %), iso-C_{15:1} G (1.0 %) and iso-C_{16:0} (1.0 %), summed feature 3, comprising C_{16:1}ω7c and/or iso-C_{15:0} 2-OH (24.5 %), and summed feature 4, comprising iso-C_{17:1} I and/or anteiso-C_{17:1} B (2.5 %), hydroxy fatty acids iso-C_{17:0} 3-OH (6.9 %) and iso-C_{15:0} 3-OH (3.2 %) and unsaturated fatty acids C_{16:1}ω5c (2.8 %) and C_{15:1}ω6c (1.0 %). This fatty acid profile was similar with

those of previously analysed *Algoriphagus* species, although there were differences in the proportions of some fatty acids, perhaps because of differences in cultivation conditions (see Supplementary Table S1 in IJSEM Online). The DNA G + C content of strain DS-44^T was 49.0 mol%, which is higher than those of other *Algoriphagus* species (Table 1). These chemotaxonomic properties were in agreement with the result of monothetic phylogenetic classification of strain DS-44^T as a member of the genus *Algoriphagus* (Bowman *et al.*, 2003; Nedashkovskaya *et al.*, 2004; Van Trappen *et al.*, 2004; Yoon *et al.*, 2005a, b). Strain DS-44^T was distinguishable from other *Algoriphagus* species by means of differences in several phenotypic properties, as shown in Table 1. The 16S rRNA gene sequence similarity data were sufficient to allocate strain DS-44^T to a species that is separate from the recognized *Algoriphagus* species (Stackebrandt & Goebel, 1994). Therefore, on the basis of the data presented, strain DS-44^T should be placed in the genus *Algoriphagus* as a member of a novel species, for which the name *Algoriphagus terrigena* sp. nov. is proposed.

Description of *Algoriphagus terrigena* sp. nov.

Algoriphagus terrigena (ter.ri.ge'na. L. masc. or fem. n. *terrigena* child of the earth, referring to the isolation of the type strain from soil).

Cells are Gram-negative, non-spore-forming, non-flagellated short rods or rods (0.4–0.6 × 0.8–2.5 μm); a few cells greater than 50 μm in length are also observed. Colonies on MA are circular, convex, smooth, glistening, light orange in colour and 1.0–2.0 mm in diameter after incubation for 7 days at 25 °C. Optimal growth occurs at 25 °C; growth occurs at 10 and 36 °C, but not at 4 or 37 °C. Optimal pH for growth is 6.5–7.5; growth occurs at pH 5.5, but not at pH 5.0. Optimal growth occurs in the presence of 2 % (w/v) NaCl; growth does not occur in the absence of NaCl or in the presence of >7 % (w/v) NaCl. Growth does not occur under anaerobic conditions on MA or on MA supplemented with nitrate. Aesculin and Tweens 20, 40 and 60 are hydrolysed, but hypoxanthine, xanthine, L-tyrosine and urea are not. H₂S is not produced. Arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase and tryptophan deaminase are absent. In assays with the API ZYM system, alkaline

Fig. 1. Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the positions of strain DS-44^T and some other related taxa. Numbers at nodes are bootstrap values (1000 replications), given as percentages; only values greater than 50 % are shown. *Flavobacterium aquatile* IAM 12316^T was used as outgroup. Dots indicate that the corresponding nodes were also recovered in the trees generated with the maximum-likelihood and maximum-parsimony algorithms. Bar, 0.01 substitutions per nucleotide position.

phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, trypsin, α -chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, β -galactosidase, α -glucosidase, β -glucosidase and *N*-acetyl- β -glucosaminidase are present, α -mannosidase is weakly present, but lipase (C14), cystine arylamidase, α -galactosidase, β -glucuronidase and α -fucosidase are absent. D-Cellobiose, D-fructose, D-galactose, maltose, sucrose, D-trehalose, D-xylose and salicin are utilized as sole carbon and energy sources, but acetate, benzoate, formate, L-glutamate, pyruvate and succinate are not utilized. Acid is produced from D-mannose, D-raffinose and D-trehalose, weakly produced from D-fructose, D-melezitose and D-ribose, but not produced from *myo*-inositol, D-mannitol or D-sorbitol. Susceptible to chloramphenicol and novobiocin, but not to cephalothin. The predominant menaquinone is MK-7. The major fatty acids (>10% of total fatty acids) are iso-C_{15:0} and C_{16:1 ω 7c} and/or iso-C_{15:0} 2-OH. The DNA G+C content is 49.0 mol% (HPLC). Other phenotypic properties are shown in Table 1.

The type strain, DS-44^T (=KCTC 12545^T = CIP 108837^T), was isolated from soil from 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 Ulleung County Administration and the Cultural Heritage Administration of the Republic of Korea for facilitating access to Dokdo.

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