Marinobacter koreensis sp. nov., isolated from sea sand in Korea

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A marine, Gram-negative, aerobic, motile, straight-rod-shaped, moderately halophilic bacterium, designated strain DD-M3^T, was isolated from sea sand in Pohang, Korea. A phylogenetic tree based on 16S rRNA gene sequences showed that the strain fell within the evolutionary radiation encompassed by the genus *Marinobacter*. The levels of 16S rRNA gene sequence similarity between the novel strain and the type strains of recognized *Marinobacter* species ranged from 94.2 to 97.6%, the highest values being with *Marinobacter flavimaris* SW-145^T (97.6%) and *Marinobacter lipolyticus* SM19^T (96.8%). The values for DNA–DNA relatedness between isolate DD-M3^T and the type strains of the most closely related species, *M. flavimaris* and *M. lipolyticus*, were 41 and 36%, respectively. Strain DD-M3^T was characterized as having Q-9 as the predominant respiratory quinone and 16:0, summed feature 3 and 18:1 ω 9c as the main fatty acids. The DNA G + C content was 54.1 mol%. On the basis of its phenotypic and genotypic characteristics, DD-M3^T represents a novel species of the genus *Marinobacter*, for which the name *Marinobacter koreensis* sp. nov. is proposed, with DD-M3^T (= KACC 11513^T = DSM 17924^T) as the type strain.

The genus *Marinobacter* was first proposed, by Gauthier *et al.* (1992), as comprising halophiles from marine-related environments. The genus *Marinobacter* is a member of the *Gammaproteobacteria*, and currently contains 11 species with validly published names. The type species is *Marinobacter hydrocarbonoclasticus*.

Sea-shore sand was collected at Homi Cape (Pohang, Korea). The sample was serially diluted using 0.85% (w/v) saline. The diluted solution was spread on marine agar 2216 (MA; Difco) and incubated for 7 days at 28 °C. The isolate was routinely grown on MA for 2 days at 28 °C and maintained as a glycerol suspension (20%, w/v) at –80 °C.

The cell morphology was observed using cells grown for 48 h on MA. For observation using transmission electron

microscopy (model 912AB; LEO), cells were negatively stained with 0.5% (w/v) uranyl acetate (see Supplementary Fig. S1 available in IJSEM Online). Growth was tested on MacConkey agar, nutrient agar, R2A and trypticase soy agar (all from Difco). Tests for catalase, oxidase and hydrolysis of casein, cellulose, DNA, gelatin, Tween 80 and starch were conducted according to the methods of Smibert & Krieg (1994). Hydrolysis of alginic acid (0.5%, w/v), chitin (1%, w/v), pectin (0.5%, w/v) and tyrosine (0.5%, w/v) was also tested by observing the appearance of clear zones around colonies. The urease test was performed using the method described by MacFaddin (2000). Growth under anaerobic conditions was determined in BBL anaerobic agar (Becton Dickinson). Tolerance of various NaCl concentrations was determined using CASO broth (15.0 g peptone from casein, 5.0 g peptone from soymeal in 1000 ml deionized water) supplemented with appropriate NaCl concentrations (0, 0.5, 1, 3, 5, 8, 10, 12, 15, 18, 20 and 25%, w/v). The pH range (pH 4.0–10.0, using increments of 1.0 pH unit) for growth was determined in marine broth buffered with citrate-phosphate buffer or Tris/HCl buffer (Breznak & Costilow, 1994). API 20NE, API 50 CH and API ZYM test strips

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain DD-M3^T is DQ325514.

A transmission electron micrograph of strain DD-M3^T and a table showing the cellular fatty acid composition of strain DD-M3^T and *Marinobacter* strains are available as supplementary material in IJSEM Online.

Fig. 1. Neighbour-joining tree, based on 16S rRNA gene sequences of *Marinobacter* species, showing the relationship between strain DD-M3^T and members of the genus *Marinobacter*. Bootstrap values (expressed as percentages of 1000 replications) greater than 50% are shown at branch points. *O. linum* ATCC 11336^T was used as an outgroup. Bar, 0.01 substitutions per site.

(bioMérieux) were used according to the manufacturer's instructions except for the following: as inocula for the API 20NE and API 50 CH test strips, colonies suspended in a solution of sea salts (Sigma) and 0.85% NaCl (w/v) were used. The utilization of organic substrates was tested using Biolog GN2 microplates; colonies suspended in half-strength sea-salts solution were used as the inocula.

The 16S rRNA gene was amplified by using a PCR with primers fd1 and rp2 (Weisburg *et al.*, 1991) on colonies; the entire PCR fragment was directly sequenced (Hiraishi, 1992). For the phylogenetic analyses, the 16S rRNA gene sequences of all the type strains of the genus *Marinobacter* (and *Oceanospirillum linum* ATCC 11336^T as an outgroup) were used. The 16S rRNA gene sequences were aligned by using the MEGALIGN program (DNASTAR). A phylogenetic tree was reconstructed using the neighbour-joining method of Saitou & Nei (1987) on MEGA, version 2.1 (Kumar *et al.*, 2001). The stability of relationships was assessed by performing bootstrap analyses of the neighbour-joining data, based on 1000 resamplings. The phylogenetic analyses were also assessed by using maximum-parsimony analysis.

DNA–DNA hybridization was determined using a membrane filter technique with the DIG High Prime DNA labelling and detection starter kit II (Roche Molecular Biochemicals) (Kwon *et al.*, 2003). Isoprenoid quinones were analysed by HPLC as described previously (Groth *et al.*, 1996). For quantitative analysis of the whole-cell fatty acids, strain DD-M3^T was cultivated on MA at 28 °C for 48 h. For preparation and analysis of the fatty acid methyl esters, the standard protocol of the MIDI/Hewlett Packard Microbial Identification System was used (Sasser, 1990). The DNA G+C content was determined according to Mesbah *et al.* (1989), using a reversed-phase column (Supelcosil LC-18-S; Supelco).

The cells of strain DD-M3^T were straight rods, 0.3–0.5 µm in diameter and 1.5–3.0 µm in length (Supplementary

Fig. S1). Optimum growth was obtained at 28 °C, pH 6.0–8.0 and 3–8% NaCl. Strain DD-M3^T showed poor growth on trypticase soy agar, and no growth was observed on R2A, nutrient agar or MacConkey agar. In the API 50 CH test strip, no colour changes were detected for any of the substrates. Details of the physiological and biochemical properties are given in Table 1 and in the species description.

The major isoprenoid quinone of strain DD-M3^T was Q-9. The main fatty acids were 16:0 (28.8%), summed feature 3 (iso-15:0 2-OH/16:1ω7c, 15.2%) and 18:1ω9c (12.2%) (see Supplementary Table S1 available in IJSEM Online). The DNA G+C content of DD-M3^T was 54.1 mol%, lying within the range (53–60 mol%) for the genus *Marinobacter* (Table 1).

In the phylogenetic tree (Fig. 1) constructed using almost-complete 16S rRNA gene sequences (1444 nt for strain DD-M3^T) on the basis of the neighbour-joining algorithm, strain DD-M3^T clustered with members of the genus *Marinobacter*. The sequence similarities between isolate DD-M3^T and the type strains of the genus *Marinobacter* ranged from 94.2 to 97.6%. Strain DD-M3^T showed the highest levels of 16S rRNA gene sequence similarity with *Marinobacter flavimaris* SW-145^T (97.6%) and *Marinobacter lipolyticus* SM19^T (96.8%). In the light of the high sequence similarities, DNA–DNA hybridization was performed between strain DD-M3^T and reference strains (*M. flavimaris* SW-145^T and *M. lipolyticus* SM19^T). The values for DNA relatedness between the isolate and the type strains of the two most closely related species, *M. flavimaris* and *M. lipolyticus*, were 41 and 36%, respectively, which means that this isolate is genotypically distinct from the type strains of these species (Wayne *et al.*, 1987).

On the basis of the data presented, strains DD-M3^T should be classified in the genus *Marinobacter* as a member of a novel species, for which the name *Marinobacter koreensis* sp. nov. is proposed.

Description of *Marinobacter koreensis* sp. nov.

Marinobacter koreensis (ko.re.en'sis. N.L. masc. adj. *koreensis* pertaining to Korea, where the type strain was isolated).

Cells are Gram-negative, aerobic, motile by means of a single polar flagellum, moderately halophilic, straight-rod-shaped (0.3–0.5 × 1.5–3.0 µm) and do not form spores. Colonies on MA are creamy in colour, circular (1–2 mm) and convex with entire edges after 48 h at 28 °C. Temperature, salt and pH ranges for growth are 10–45 °C, 0.5–20% NaCl and pH 5–9. Tween 80 is hydrolysed, but alginic acid, casein, chitin, CM-cellulose, DNA, gelatin, pectin, tyrosine, starch and urea are not. Tweens 40 and 80, pyruvic acid methyl ester, acetic acid, β-hydroxybutyric acid, α-ketoglutaric acid, α-ketovaleric acid, DL-lactic acid, succinic acid, bromosuccinic acid, L-glutamic acid and L-proline are utilized, and

Table 1. Phenotypic comparison of strain DD-M3^T and phylogenetically related *Marinobacter* species

Strains: 1, DD-M3^T; 2, *M. hydrocarbonoclasticus* ATCC 49840^T; 3, *M. algicola* DG893^T; 4, *M. bryozoorum* KMM 3840^T; 5, *M. daeponensis* SW-156^T; 6, *M. excellens* KMM 3809^T; 7, *M. flavimaris* SW-145^T; 8, *M. lipolyticus* SM19^T; 9, *M. litoralis* SW-45^T; 10, *M. sediminum* KMM 3657^T; 11, *M. maritimus* CK 47^T; 12, *M. lutaoensis* T5054^T. Data were obtained from Gauthier *et al.* (1992), Gorshkova *et al.* (2003), Shieh *et al.* (2003), Martin *et al.* (2003), Yoon *et al.* (2003, 2004), Romanenko *et al.* (2005), Shivaji *et al.* (2005), Green *et al.* (2006) and this study. All isolates are rod-shaped, motile and positive for catalase and oxidase. ND, No data available.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12
Cell size (µm)	0.3–0.5 × 1.5–3.0	0.3–0.6 × 2–3	0.45–0.55 × 1.6–2.5	0.4–0.5 × 1.0–1.3	0.6–0.8 × 1.5–3.0	0.6–1.4 × 1–8	0.6–0.9 × 1.5–3.0	0.3–0.5 × 2.5–3.5	0.5–0.8 × 1.5–3.0	0.3–0.4 × 1.8–2.5	1.7–2.0 × 6.0–7.0	0.6–1.0 × 2.0–3.5
Colony colour	Cream	White	Cream	White	Cream	Transparent	Cream	Cream	Cream	White	Cream	Off-white
Reduction of nitrate to nitrite	+	+	–	+	–	+	+	–	+	–	–	–
Growth temperature												
Minimum (°C)	10	10	5	7	ND	10	4	15	4	4	4	25
Maximum (°C)	45	45	40	42	45	41	45	40	46	42	37	50
NaCl growth range (%)	0.5–20	1–20	1–12	1–18	ND–18	1–15	1–20	1–15	0.5–18	0.5–18	1–13	0.5–12
Enzyme activities												
Urease	–	–	+	–	–	ND	–	–	+	–	+	ND
Arginine dihydrolase	–	–	+	–	ND	ND	–	–	ND	–	–	–
Utilization of:												
Glycerol	–	–	+	+	–	–	–	–	–	+	–	ND
D-Fructose	–	–	+	–	–	+	+	+	–	–	ND	ND
D-Glucose	–	–	+	–	–	–	–	+	–	+	ND	+
Maltose	–	–	+	–	ND	+	–	+	ND	–	–	ND
D-Mannitol	–	–	+	–	–	+	–	+	–	–	+	+
Sucrose	–	+	–	+	ND	–	–	–	–	+	–	–
Citric acid	–	+	+	–	ND	–	–	–	ND	–	ND	–
DL-Lactic acid	+	+	+	–	ND	–	–	–	ND	–	–	ND
cis-Aconitic acid	–	+	+	–	ND	+	–	–	ND	–	ND	ND
D-Gluconic acid	–	–	+	–	–	–	+	+	–	–	–	ND
L-Alanine	–	–	+	–	–	–	–	–	–	–	+	+
L-Phenylalanine	–	–	+	–	ND	+	–	–	ND	–	ND	+
L-Proline	+	+	+	–	ND	+	+	–	ND	–	+	ND
DNA G+C content (mol%)	54.1	53	55	59.6	57	56	58	57	55	56.5	58	63.5

glycogen, succinic acid monomethyl ester and L-leucine are used weakly (Biolog GN2 microplate). Positive for nitrate reduction, but negative for indole production, glucose fermentation, arginine dihydrolase, urease, aesculin hydrolysis, gelatin hydrolysis and β -galactosidase. Assimilates malic acid and phenylacetic acid, but does not assimilate D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetylglucosamine, D-maltose, potassium gluconate, capric acid, adipic acid or trisodium citrate (API 20NE). Enzymic activity is observed for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase and *N*-acetyl- β -glucosaminidase. No enzymic activity is observed for lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α -chymotrypsin, α -galactosidase, β -galactosidase, β -glucuronidase, α -glucosidase, β -glucosidase, α -mannosidase or α -fucosidase (API ZYM). The predominant isoprenoid quinone is Q-9. The main fatty acids are 16:0, summed feature 3 and 18:1 ω 9c. The DNA G+C content is 54.1 mol% (determined by HPLC).

The type strain, DD-M3^T (=KACC 11513^T=DSM 17924^T), was isolated from sea sand at Homo Cape, Pohang, Korea.

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