Marinomonas basaltis sp. nov., a marine bacterium isolated from black sand

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A Gram-negative, aerobic, slightly halophilic, rod-shaped bacterium was isolated from black sand in Soesoggak, Jeju island, Korea. The strain, designated J63^T, was oxidase- and catalase-positive and arginine dihydrolase-negative. The isolate required Na^+ for growth and differed from phenotypically related species by being able to utilize sucrose and D-galactose as a carbon source. Phylogenetic analysis based on the sequence of the 16S rRNA gene revealed that strain J63^T belongs to the genus *Marinomonas*. It exhibited 16S rRNA gene sequence similarities of 97.6–98.7 % to the closely related species *Marinomonas communis*, *Marinomonas ostreistagni*, *Marinomonas aquimarina* and *Marinomonas vaga*. The phylogenetic analysis revealed that strain J63^T comprised a relatively long subline of descent, shared a branch point with the outlying species *Marinomonas communis* and occupied a phylogenetically distant position on the main *Marinomonas* branch. Based on DNA–DNA hybridization, the levels of relatedness between strain J63^T and *M. communis* NBRC 102224^T, *M. aquimarina* CIP 108405^T and *M. vaga* JCM 20774^T were 56.2, 45.1 and 51.3 %, respectively. On the basis of the phenotypic, genetic and phylogenetic data, strain J63^T should be placed in the genus *Marinomonas* as representing a novel species, for which the name *Marinomonas basaltis* sp. nov. is proposed. The type strain is J63^T (=KCTC 22118^T=JCM 14948^T).

The genus *Marinomonas* was created to accommodate two reclassified *Alteromonas* species, namely *Alteromonas communis* and *Alteromonas vaga* (Baumann *et al.*, 1972). These species formed a separate and distinct branch when compared with other species of *Alteromonas* (Van Landschoot & De Ley, 1983). At the time of writing, the genus *Marinomonas* comprised 10 species with validly published names (http://www.bacterio.cict.fr/m/marinomonas.html), which are phenotypically heterogeneous. Species assigned to the genus *Marinomonas* are rod-shaped and motile by polar or peritrichous flagella, have DNA G+C contents in the range 46.9–48.0 mol% and the ability to metabolize *m*-hydroxybenzoate, *p*-hydroxybenzoate and quinate and to utilize acetate. The recognized species of the genus *Marinomonas* were *M. aquimarina, M. communis, M. dokdonensis, M. mediterranea, M. ostreistagni, M. polaris, M. pontica, M. primoryensis, M. ushuaiensis* and *M. vaga* (Baumann *et al.*, 1972; Gupta *et al.*, 2006; Ivanova *et al.*, 2005; Lau *et al.*, 2006; Macian *et al.*, 2005; Prabagaran *et al.*, 2005; Romanenko *et al.*, 2003; Solano & Sanchez-Amat, 1999; Van Landschoot & De Ley, 1983; Yoon *et al.*, 2005). A *Marinomonas*-like, Gram-negative, rod-shaped bacterial strain, J63^T, was isolated recently from black sand from Soesoggak, Jeju island, Korea, by using the dilution-plating technique. In this report, we describe the morphological, biochemical and phylogenetic characteristics of strain J63^T.

Soesoggak is a coastal area, surrounded by basalt on three sides, in the south of Jeju island (33° 15′ 16″ N 126° 37′ 52″ E). A seawater sample with black sand (50 ml), collected in a sterile tube, was placed directly on 10 % marine agar 2216 (MA; Difco). Several representative colonies appeared on 10 % MA after incubation at 30 °C for 1 day. One of the representative colonies, designated strain J63^T, was grown routinely at 30 °C for 3 days on MA or in marine broth (MB; Difco). Cell morphology was examined by using light microscopy (E600; Nikon) and transmission electron microscopy. Cellular motility of the novel isolate was observed in fresh wet-mounts of young

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain J63^T is EU143359.

bacterial cultures in MB by using the hanging drop method. The presence of flagella was investigated by transmission electron microscopy, with cells from an exponentially growing culture. The Gram-reaction was determined using a Gram Stain kit (Difco), according to the manufacturer's instructions. The reference strains used, *M. communis* NBRC 102224^T, *M. aquimarina* CIP 108405^T and *M. vaga* JCM 20774^T, were the closest relatives based on 16S rRNA gene similarity. The strains were obtained from NBRC, CIP and JCM and were grown under the same conditions. Cultures of the isolate and the reference strains were stored at −80 °C in MB containing 20 % glycerol. For morphological and physiological characterization, strain J63^T was generally cultivated in MB, incubated by shaking at 30 °C. The requirement and tolerance of various NaCl concentrations (0–10 %; in increments of 1 %) were determined in broth medium that comprised all of the constituents of MB except NaCl, supplemented with appropriate concentrations of NaCl. Growth at various temperatures and pH values was determined using MB. Growth under anaerobic conditions was determined after incubation for 7 days in anaerobic Gaspak jars (BBL) containing 80 % N_2, 10 % CO_2 and 10 % H_2. Catalase activity was determined by bubble production in a 3 % (v/v) H_2O_2 solution and oxidase activity by using an oxidase reagent (bioMérieux). API 20NE and API ZYM test strips (bioMérieux) with inoculating fluid (0.85 % NaCl) and Biolog GN metabolic fingerprinting plates with GN/GP inoculating fluid were used to analyse the biochemical and physiological traits of the bacterial strains; additional biochemical tests were performed using methods and media described previously by Gordon *et al.* (1973). Gelatin and starch hydrolysis were investigated as described by Smibert & Krieg (1994). Bacterial strains grown on MA for 3 days at 30 °C were used for the analysis of fatty acid methyl esters. The fatty acid methyl esters were extracted and prepared according to standard protocols provided by the MIDI/Hewlett Packard Microbial Identification System (Sasser, 1990). Chromosomal DNA was extracted and purified, according to the method of Sambrook *et al.* (1989). The 16S rRNA gene was amplified by PCR using PCR Master mix solution (iNtRON Biotechnology, Korea) and two universal primers, as described previously (Stackebrandt *et al.*, 1993). Sequencing of the amplified 16S rRNA gene and phylogenetic analysis were performed as described previously (Roh *et al.*, 2008). The DNA G+C content was determined using real-time PCR, as described by Gonzalez & Saiz-Jimenez (2002). DNA–DNA hybridization was performed fluorometrically according to the method of Ezaki *et al.* (1989), using photobiotin-labelled DNA probes and microwell plates. The 16S rRNA gene sequence of strain J63^T was aligned with ten reference sequences from the RDP database (Fig. 1) using the multiple sequence alignment program CLUSTAL_X (1.8) (Thompson *et al.*, 1997). Phylogenetic relationships between representatives of the genus *Marinomonas* were determined using MEGA version 2.1 software. Distance matrices were determined following the assumptions described by Kimura (1980). These matrices were used to elaborate dendrograms using the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Kluge & Farris, 1969) methods. A bootstrap analysis for investi-

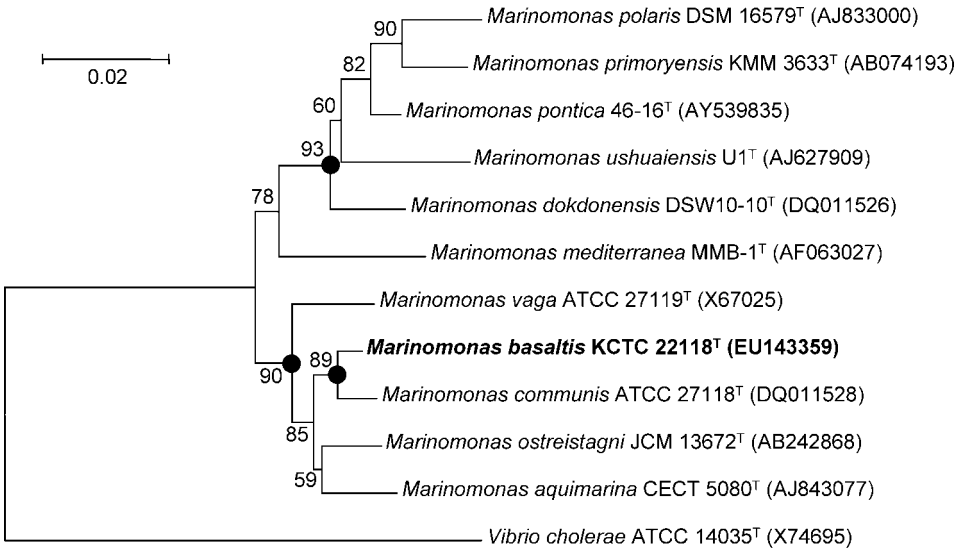

Fig. 1. Consensus phylogenetic tree based on 16S rRNA gene sequences showing the relationship between strain J63^T and type strains of recognized *Marinomonas* species.. The tree was constructed based on the neighbour-joining method and p-distances. Filled circles indicate generic branches that were also recovered by using the maximum-parsimony algorithm. Bootstrap analyses were performed with 1000 repetitions; only values >50 % are shown. GenBank accession numbers are given in parentheses. Bar, 0.02 % sequence divergence.

gating the stability of the trees was performed by obtaining a consensus tree based on 1000 randomly generated trees.

The morphological, cultural, physiological and biochemical characteristics of strain J63^T and other related species are shown in Table 1, and are described below. Cells of strain J63^T were Gram-negative, motile, slightly halophilic and rod-shaped, grew at temperatures of 10–45 °C, but not below 10 °C or above 45 °C, and at pH 5–10, but not at pH values below 5 or above 10. Growth occurred in the presence of 1–7 % NaCl; very weak growth occurred at 1–2 % NaCl. No growth was detected in less than 1 % or more than 7 % NaCl. The isolate did not grow under anaerobic conditions, and showed catalase and oxidase activities. The fatty acid methyl esters of strain J63^T and closely related species are shown in Table 2. The fatty acid composition of strain J63^T exhibited a similar trend to those of members of the genus *Marinomonas* and was similar to those of the reference strains. However, strain J63^T had a slightly higher percentage of hydroxy fatty acids than *M. communis* and *M. aquimarina* and a lower percentage of unsaturated fatty acids compared with the other reference strains. Strain J63^T differed from *M. communis* and *M. vaga* in having a higher percentage of straight-chain fatty acids.

Phylogenetic analysis based on the sequences of the 16S rRNA genes of members of different genera of *Oceanospirillaceae* that included *Marinomonas* species revealed that strain J63^T fell within a cluster comprised of *Marinomonas* species (Fig. 1). Strain J63^T was 93.6–98.7 % similar in its 16S rRNA gene sequence to strains of other recognized *Marinomonas* species. On the basis of the 16S rRNA gene sequence, strain J63^T was closely related to *M. communis*, *M. ostreistagni*, *M. aquimarina* and *M. vaga* with 98.7, 98.4, 97.8 and 97.6% similarity, respectively. DNA–DNA hybridization studies were performed to determine the genetic relationships among strain J63^T and *M. communis* NBRC 102224^T, *M. aquimarina* CIP 108405^T and *M. vaga* JCM 20774^T, its closest relatives in terms of 16S rRNA gene similarity. The DNA–DNA hybridization values between strain J63^T, *M. communis* NBRC 102224^T, *M. aquimarina* CIP 108405^T and *M. vaga* JCM 20774^T were 56.2, 45.1 and 51.3 %, respectively. Taken together, the phylogenetic and DNA–DNA hybridization results suggest that strain J63^T represents a novel species of the genus *Marinomonas*, for which the name *Marinomonas basaltis* sp. nov. is proposed.

Description of *Marinomonas basaltis* sp. nov.

Marinomonas basaltis (ba.sal′tis. L. masc. gen. n. *basaltis* of basalt, pertaining to the source of isolation).

Cells are Gram-negative, aerobic and rod-shaped, with overall dimensions of 0.5–0.6 μm (width) by 1.0–2.5 μm

Table 1. Taxonomic characteristics of strain J63^T and the type strains of related species of the genus *Marinomonas*

Strains: 1, strain J63^T (*Marinomonas basaltis* sp. nov.); 2, *M. communis* NBRC 102224^T (data from Baumann *et al.*, 1972); 3, *M. ostreistagni* JCM 13672^T (Lau *et al.*, 2006); 4, *M. aquimarina* CIP 108405^T (Macian *et al.*, 2005); 5, *M. vaga* JCM 20774^T (Baumann *et al.*, 1972); 6, *M. pontica* 46-16^T (Ivanova *et al.*, 2005). All strains do not reduce nitrate to nitrite and are able to utilize L-alanine as a carbon source. +, Positive; –, negative; ND, data not available; V, variable; W, weakly positive.

Characteristic	1	2	3	4	5	6
Cell shape*	SR	CR	SR	SR	SR	Rod
Growth at/in:						
4 °C	–	–	–	–	–	+
37 °C	+	+	+	+	–	–
8 % NaCl	–	+	+	+	+	+
Oxidase	+	+	+	+	–	+
Phosphatase	+	–	+	ND	ND	+
Carbon source utilization						
Succinate	–	+	–	+	+	–
D-Mannitol	+	+	+	–	+	+
D-Sorbitol	–	+	–	+	+	+
D-Galactose	+	ND	–	–	ND	–
Maltose	–	V	–	–	V	+
D-Mannose	–	+	ND	ND	+	–
Glycerol	+	+	–	–	+	+
Citrate	+	+	+	+	+	–
Sucrose	+	–	+	–	V	+
Malate	–	+	–	+	+	–
L-Histidine	–	V	–	+	V	+
L-Threonine	+	+	–	–	ND	–
DNA G+C content (mol%)	48.8	45.9–48.0	49.8±0.5	ND	46.4–49.3	46.5

*CR, Curved rod; SR, straight rod.

Table 2. Fatty acid content (%) of strain J63^T and related species of the genus *Marinomonas*

Strains: 1, strain J63^T (*Marinomonas basaltis* sp. nov.); 2, *M. communis* NBRC 102224^T; 3, *M. aquimarina* CIP 108405^T; 4, *M. vaga* JCM 20774^T. Values are percentages of total fatty acids. ECL, Equivalent chain-length. −, Not detected.

Fatty acid	1	2	3	4
Straight-chain fatty acids				
10 : 0	2.3	1.4	3.9	2.5
12 : 0	5.5	5.1	4.5	2.6
14 : 0	2.8	2.1	2.8	2.6
16 : 0	14.5	10.4	14.9	14.7
18 : 0	1.8	3.5	2.2	2.7
Unsaturated fatty acids				
15 : 1ω8*c*	0.7	0.6	0.3	−
18 : 1ω7*c*	42.1	51.8	46.9	47.1
Hydroxy fatty acids				
10 : 0 3-OH	7.6	7.6	5.7	8.9
12 : 0 3-OH	0.4	0.4	0.4	−
16 : 0 3-OH	0.4	−	−	−
Summed feature				
16 : 1ω7*c*/15 : 0 iso 2-OH	20.7	16.7	18.2	17.5
Unknown ECL 11.7999	0.3	0.4	0.2	1.4

(length), as assessed in 3 day cultures grown at 30 °C on MA plates. Cells are motile by monopolar flagella. Colonies are circular to slightly irregular, opaque, smooth, low-convex, ivory-coloured and 1–3 mm in diameter after incubation for 3 days on MA. Growth occurs in the presence of 1–7 % NaCl, but not below 1 % or above 7 % NaCl. Growth occurs at 10–45 °C and within the pH range 5–10. No growth is detected below pH 5 or above pH 10. Growth does not occur in an anaerobic chamber on MA. Catalase- and oxidase-positive and arginine dihydrolase-negative. Gelatin and starch are not hydrolysed. According to API 20NE, indole production is negative. The following substrates are utilized as sole carbon and energy sources: D-mannitol, D-galactose, glycerol, citrate, sucrose, L-alanine, L-threonine, α-cyclodextrin, *N*-acetyl-D-glucosamine, adonitol, L-arabinose, D-fructose, α-D-glucose, *myo*-inositol, trehalose, turanose, pyruvic acid methyl ester, citric acid, D-gluconic acid, DL-lactic acid, succinic acid, L-asparagine, L-aspartic acid, L-glutamic acid, L-proline and L-pyroglutamic acid, but not succinate, D-sorbitol, cellobiose, maltose, D-mannose, malate, L-histidine, dextrin, glycogen, Tween 40, Tween 80, L-erythritol, L-fucose, gentiobiose, lactulose, melibiose, L-rhamnose, xylitol, acetic acid, formic acid, D-glucuronic acid and propionic acid. Using the API ZYM system, exhibits alkaline phosphatase, leucine arylamidase, valine arylamidase, trypsin, acid phosphatase, α-glucosidase, β-glucosidase and *N*-acetyl-β-glucosaminidase activities. Does not exhibit esterase (C4), esterase lipase (C8), lipase (C14), cysteine arylamidase, α-chymotrypsin, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, β-glucuronidase, α-mannosidase or α-fucosidase activity. Predominant fatty acids are 18 : 1ω7*c*, 16 : 1ω7*c*/15 : 0 iso 2-OH and 16 : 0. The DNA G+C content of the type strain is 48.8 mol%.

The type strain, J63^T (=KCTC 22118^T=JCM 14948^T), was isolated from black sand in Soesoggak, Jeju island, Korea.

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