Marinobacter goseongensis sp. nov., from seawater

Seong Woon Roh,[1,2] Zhe-Xue Quan,[3] Young-Do Nam,[1,2] Ho-Won Chang,[2] Kyoung-Ho Kim,[2] Sung-Keun Rhee,[4] Hee-Mock Oh,[2] Che Ok Jeon,[5] Jung-Hoon Yoon[2] and Jin-Woo Bae[1,2,5]

Correspondence
Jin-Woo Bae
baejw@kribb.re.kr

[1]Korea University of Science and Technology, Daejeon 305-333, Republic of Korea

[2]Biological Resource Center, KRIBB, Daejeon 305-806, Republic of Korea

[3]School of Life Sciences, Fudan University, Shanghai 200433, PR China

[4]Department of Microbiology, Chungbuk National University, Cheongju 361-763, Republic of Korea

[5]Environmental Biotechnology National Core Research Center, Gyeongsang National University, Jinju 660-701, Republic of Korea

A Gram-negative marine bacterium, designated strain En6^T, was isolated from seawater of the East Sea of Korea. The organism grew in 1–25 % (w/v) NaCl and at 10–37 °C and pH 5.3–9.3, with optimal growth occurring in 4–5 % NaCl and at 25–30 °C and pH 7.5. Phylogenetic analysis of the 16S rRNA gene sequence of strain En6^T placed this bacterium in the clade *Marinobacter* within the class *Gammaproteobacteria*. The 16S rRNA gene sequence similarity between strain En6^T and *Marinobacter lipolyticus* SM19^T, the most closely related species, was 98.4 %, and the level of DNA–DNA relatedness between the two strains was 22 %. On the basis of the phylogenetic analysis and phenotypic and chemotaxonomic data, strain En6^T is considered to represent a novel species of the genus *Marinobacter*. The name *Marinobacter goseongensis* sp. nov. is proposed, with strain En6^T (=KCTC 12515^T=DSM 19471^T) as the type strain.

The genus *Marinobacter*, within the class *Gammaproteobacteria*, was first proposed by Gauthier *et al.*, (1992) to accommodate a Gram-negative, aerobic, rod-shaped bacterium that was isolated from Mediterranean seawater. Since this genus was proposed in 1992, more than 15 species of *Marinobacter* have been isolated from various environments, including: seawater (Antunes *et al.*, 2007; Gauthier *et al.*, 1992; Shivaji *et al.*, 2005; Yoon *et al.*, 2003, 2004), oil-producing well (Huu *et al.*, 1999), marine sediment (Gorshkova *et al.*, 2003; Romanenko *et al.*, 2005), saline soil (Gu *et al.*, 2007; Martin *et al.*, 2003), coastal hot spring (Shieh *et al.*, 2003), wastewater (Liebgott *et al.*, 2006), laboratory cultures of dinoflagellates (Green *et al.*, 2006) and sea sand (Kim *et al.*, 2006). In this paper, the taxonomic position of strain En6^T was established by means of phenotypic, genetic and chemotaxonomic analyses.

A single novel strain, designated En6^T, was isolated from coastal seawater of the East Sea of Korea at a depth of 100 m, by using a dilution-plating technique on marine agar 2216 (MA; Difco). The colonies were repeatedly restreaked to obtain a pure culture and the 16S rRNA gene of the strain was sequenced. Growth at various temperatures (4–45 °C) and pH values (pH 3.0–12.0) was examined using marine broth (MB; Difco). The requirement and tolerance of various NaCl concentrations (0–30 %) 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 and in various concentrations of NaCl was determined in triplicate and sequencing of the 16S rRNA gene was performed in triplicate to check the purity of the novel strain. Cell morphology was examined by using light microscopy (ECLIPSE 80*i*; Nikon) and electron microscopy. The Gram-reaction was determined by using a Gram Stain kit (Difco), according to the manufacturer's instructions. Motility was examined by using the wet-mount method and spore formation was determined using the staining method (Schaeffer & Fulton, 1933). Hydrolysis of gelatin and starch was determined as described by Smibert & Krieg (1994). The heat resistance of cells was determined as described by Guo *et al.* (2007). API ZYM test strips with suspension medium (0.85 % NaCl in demineralized water) (bioMérieux) were used to analyse traits of strain En6^T in triplicate, according to the manufacturer's instructions. Utilization of organic substrates was determined using Biolog GN2 plates consisting of 95 substrates and GN (Gram-negative)/GP (Gram-positive) inoculating fluid (0.40 % NaCl, 0.03 % Pluronic F-68 and 0.02 % Gellan gum) with the salinity adjusted to 5 % (w/v) NaCl, according to the manufacturer's instructions. Catalase activity was determined by using bubble

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain En6^T is EF660754.

production in 3 % (v/v) hydrogen peroxide solution, and oxidase activity was determined using an oxidase reagent (bioMérieux), in triplicate using MA. Cells of strain En6^T were Gram-negative and oxidase- and catalase-positive. Gelatin and starch were not hydrolysed. No spores were produced and the cells were not resistant to heat. Optimal growth occurred at 25–30 °C and pH 7.5, and in 4–5 % (w/v) NaCl. Comparisons of additional characteristics and results of biochemical and physiological assays for strain En6^T and the type strains of its closest relatives in the phylogenetic tree, are shown in Table 1. Strain En6^T was able to grow in 25 % NaCl, but five closely related species were not. On the other hand, strain En6^T did not grow at 40 °C, whereas the other *Marinobacter* species used could. Together, these data clearly differentiate the novel isolate from other closely related species.

Fatty acid methyl esters of cells of strain En6^T and reference strains grown on MA at 30 °C for 2 days were analysed, according to standard protocols provided by the MIDI/Hewlett Packard Microbial Identification System (Sasser, 1990). The fatty acid composition of strain En6^T is shown in Table 2. Strain En6^T had a relatively high content of the fatty acid $C_{18:1}\omega 9c$ (31.5 %), compared with the contents (21.6–24.7 %) of the type strains of its closest relatives, as shown in Table 2.

Chromosomal DNA was extracted and purified using a DNA extraction kit (iNtRON Biotechnology), and was used as a template with two bacterial universal primers for PCR amplification of the 16S rRNA gene, as described previously (Baker *et al.*, 2003). The PCR product was purified using a QIAquick PCR Purification kit (Qiagen) and sequenced as described previously (Roh *et al.*, 2008). DNA–DNA hybridization was performed using the fluorometric method of Ezaki *et al.* (1989). The 16S rRNA gene sequence of the novel isolate was aligned with 20 reference sequences from the NCBI database by using the multiple sequence alignment program CLUSTAL_X (1.8) (Thompson *et al.*, 1997). Calculation of pairwise 16S rRNA gene sequence similarities were achieved using the EzTaxon server (http://www.eztaxon.org/; Chun *et al.*. 2007). Phylogenetic relationships between representatives of the genus *Marinobacter* were determined using the program

Table 1. Phenotypic characteristics that differentiate strain En6^T (*Marinobacter goseongensis* sp. nov.) from phylogenetically related species

Strains: 1, En6^T (data from the present study); 2, *M. lipolyticus* SM19^T (Martin *et al.*, 2003); 3, *M. sediminum* R65^T (Romanenko *et al.*, 2005); 4, *M. flavimaris* SW-145^T (Yoon *et al.*, 2004); 5, *M. algicola* DG893^T (Green *et al.*, 2006); 6, *M. salsuginis* SD-14B^T (Antunes *et al.*, 2007). +, Positive; −, negative; W, weak reaction; ND, not determined.

Characteristic	1	2	3	4	5	6
Cell size (μm)	0.5–0.8 × 1.6–2.0	0.3–0.5 × 2.5–3.5	0.3–0.4 × 1.8–2.5	0.6–0.9 × 1.5–3.0	0.45–0.55 × 1.6–2.5	ND
Growth at/in:						
20 % NaCl	+	−	−	+	−	+
4 °C	−	−	+	+	ND	−
Maximum NaCl for growth (%)	25	15	18	20	12	20
Maximum temperature for growth (°C)	37	40	42	45	40	45
Hydrolysis of:						
Gelatin	−	−	−	−	−	+
Starch	−	−	ND	−	+	−
Utilization of:						
D-Fructose	−	+	−	+	+	−
D-Glucose	−	+	+	−	+	+
Maltose	−	+	−	−*	+	−
D-Mannitol	−	+	−	−	+	−
D-Gluconic acid	−	+	−	+*	+	ND
DL-Lactic acid	+	−	−	−*	+	ND
L-Glutamic acid	+	−	−	−	−	ND
Proline	−	−	−	+*	+	+
API ZYM tests						
Esterase (C4)	+	ND	−	+	ND	+
Lipase (C14)	−	ND	−	+	ND	+
Valine arylamidase	+	ND	−	−	ND	W
Cystine arylamidase	+	ND	−	−	ND	W
Acid phosphatase	+	ND	−	+	ND	W
N-acetyl-β-glucosaminidase	+	ND	−	+	ND	+

*Data from Green *et al.* (2006).

Table 2. Fatty acid contents of strain En6^T (*Marinobacter goseongensis* sp. nov.) and closely related *Marinobacter* species

Strains: 1, En6^T; 2, *M. lipolyticus* SM19^T; 3, *M. sediminum* R65^T; 4, *M. flavimaris* SW-145^T. All data are from this study. Values are percentages of the total fatty acids.

Fatty acid	1	2	3	4
$C_{10:0}$	0.5	1.3	1.8	0.3
$C_{12:0}$	6.5	6.1	4.0	6.2
$C_{12:0}$ 3-OH	9.2	9.0	7.4	8.0
$C_{14:0}$	1.1	0.5	1.0	0.9
$C_{15:0}$	0.5	0.4	0.1	0.4
$C_{16:0}$	27.2	24.5	21.2	25.4
$C_{16:1}\omega 9c$	13.6	8.6	6.8	8.0
$C_{16:1}\omega 7c$/15 iso 2-OH	2.5	6.8	8.8	9.7
$C_{16:0}$ 10-methyl	0.9	3.2	1.7	0.3
$C_{17:1}\omega 8c$	1.0	2.5	2.0	2.7
$C_{17:0}$	1.3	2.2	1.7	2.2
$C_{18:1}\omega 9c$	31.5	23.6	21.6	24.7
$C_{18:1}\omega 7c$	0.7	4.1	5.6	1.6
$C_{18:0}$	1.9	3.3	5.8	3.9

MEGA3 (Kumar *et al.*, 2004). Distance matrices were determined according to the assumptions described by Kimura (1980) and were used to develop dendrograms with the neighbour-joining method (Saitou & Nei, 1987). Bootstrap analysis of the stability of the predicted trees was performed by compiling a consensus tree based on 1000 randomly generated trees. Fig. 1 shows that strain En6^T fell within the cluster of *Marinobacter* species. According to the 16S rRNA gene sequences, strain En6^T showed a high level of similarity with *Marinobacter lipolyticus* SM19^T (98.4 %), *Marinobacter gudaonensis* SL014B61A^T (97.8 %), *Marinobacter sediminum* R65^T (97.5 %), *Marinobacter algicola* DG893^T (97.1 %), *Marinobacter salsuginis* SD-14B^T (97.1 %) and *Marinobacter flavimaris* SW-145^T (97.0 %). DNA–DNA relatedness studies were performed to determine the genomic relationship between strain En6^T and the type strains of its closest relatives in the phylogenetic tree. Levels of DNA–DNA relatedness of 22, 17 and 26 % between strain En6^T and *M. lipolyticus* SM19^T, *M. sediminum* R65^T and *M. flavimaris* SW-145^T were obtained, respectively.

Therefore, based on the comparison of the phenotypic, genetic and chemotaxonomic data for previously described

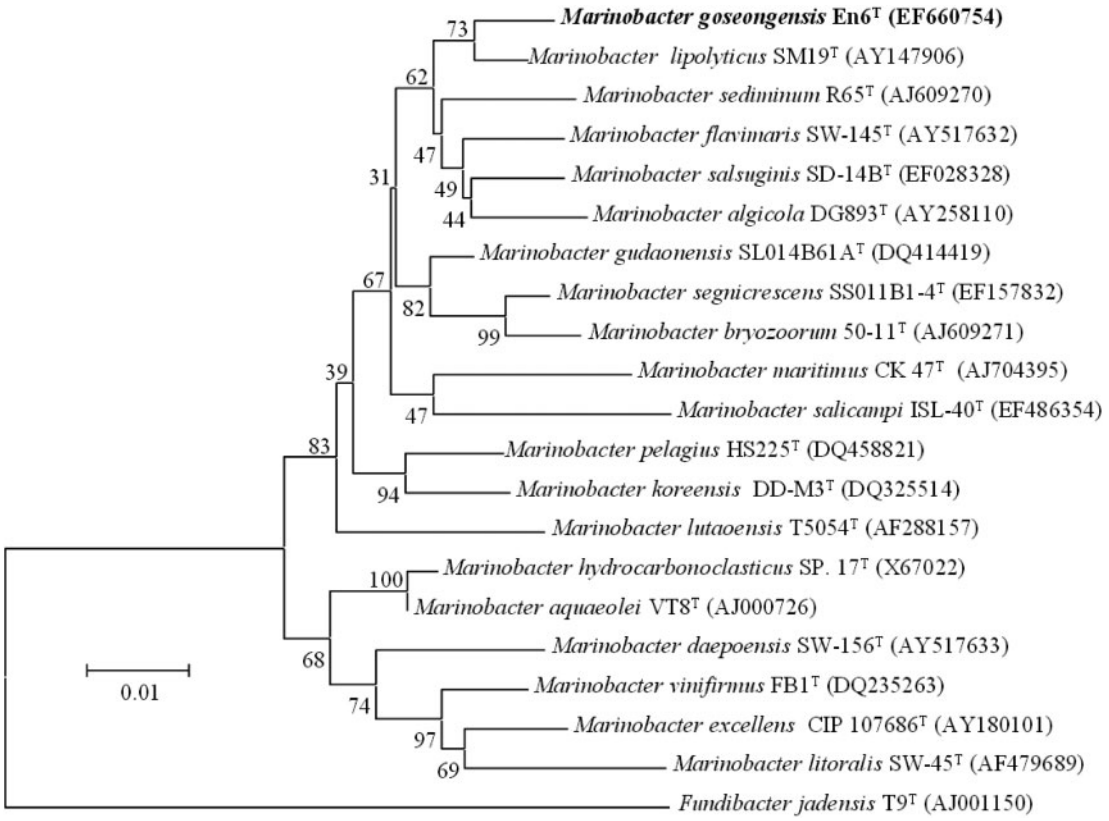

Fig. 1. Phylogenetic dendrogram based on unambiguously aligned base pairs of 16S rRNA gene sequence data, showing the position of strain En6^T (*Marinobacter goseongensis* sp. nov.). The tree was generated using the neighbour-joining method. Numbers at nodes are bootstrap percentages (based on 1000 replications). GenBank/EMBL/DDBJ accession numbers are given in parentheses. Bar, 0.01 accumulated changes per nucleotide.

taxa, strain En6^T is considered to represent a novel species of the genus *Marinobacter*, for which the name *Marinobacter goseongensis* sp. nov. is proposed.

Description of *Marinobacter goseongensis* sp. nov.

Marinobacter goseongensis (go′se.on.gen′sis. N.L. masc. adj. *goseongensis* pertaining to the province Goseong, near the sampling site from which the bacterium was isolated).

Cells are rod-shaped (0.5–0.8 × 1.6–2.0 μm), Gram-negative, non-motile, oxidase- and catalase-positive and non-spore-forming. Cells are not heat resistant. Colonies are cream-coloured, circular and approximately 0.5 mm in diameter after growth for 1 day on MA at 30 °C. Growth occurs at 10–37 °C (optimum, 25–30 °C) and pH 5.3–9.3 (optimum, pH 7.5) and in 1–25 % (w/v) NaCl (optimum, 4–5 % NaCl). Gelatin and starch are not hydrolysed. According to Biolog GN plates, positive for Tween 40, Tween 80, pyruvic acid methyl ester, acetic acid, DL-lactic acid and L-glutamic acid; and negative for α-cyclodextrin, dextrin, glycogen, *N*-acetyl-D-galactosamine, *N*-acetyl-D-glucosamine, adonitol, L-arabinose, D-arabitol, D-cellobiose, i-erythritol, D-fructose, L-fucose, D-galactose, gentiobiose, α-D-glucose, *myo*-inositol, α-D-lactose, lactulose, maltose, D-mannitol, D-mannose, melibiose, methyl β-D-glucoside, D-psicose, raffinose, L-rhamnose, D-sorbitol, sucrose, trehalose, turanose, xylitol, succinic acid monomethyl ester, *cis*-aconitic acid, citric acid, formic acid, D-galactonic acid lactone, D-galacturonic acid, D-gluconic acid, D-glucosaminic acid, D-glucuronic acid, α-hydroxybutyric acid, β-hydroxybutyric acid, γ-hydroxybutyric acid, *p*-hydroxyphenylacetic acid, itaconic acid, α-ketobutyric acid, α-ketoglutaric acid, α-ketovaleric acid, malonic acid, propionic acid, quinic acid, D-saccharic acid, sebacic acid, succinic acid, bromosuccinic acid, succinamic acid, glucuronamide, L-alaninamide, D-alanine, L-alanine, L-alanyl glycine, L-asparagine, L-aspartic acid, glycyl L-aspartic acid, glycyl L-glutamic acid, L-histidine, hydroxy-L-proline, L-leucine, L-ornithine, L-phenylalanine, L-proline, L-pyroglutamic acid, D-serine, L-serine, L-threonine, DL-carnitine, γ-aminobutyric acid, urocanic acid, inosine, uridine, thymidine, phenyethylamine, putrescine, 2-aminoethanol, 2,3-butanediol, glycerol, DL-α-glycerol phosphate, α-D-glucose 1-phosphate and D-glucose 6-phosphate. According to API ZYM test strips, positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, acid phosphatase, naphthol-AS-B1-phosphohydrolase and *N*-acetyl-β-glucosaminidase activities; and negative for lipase (C14), trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, α-mannosidase and α-fucosidase activities. Predominant fatty acids are $C_{12:0}$, $C_{12:0}$ 3-OH, $C_{16:0}$, $C_{16:1}\omega9c$ and $C_{18:1}\omega9c$ (Table 2).

The type strain, En6^T (=KCTC 12515^T=DSM 19471^T), was isolated from seawater of the East Sea of Korea.

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