Shewanella spongiae sp. nov., isolated from a marine sponge

Sung-Hyun Yang, Kae Kyoung Kwon, Hee-Soon Lee and Sang-Jin Kim

Correspondence

Sang-Jin Kim
s-jkim@kordi.re.kr

Marine and Extreme Genome Research Center, Korea Ocean Research and Development Institute, PO Box 29, Ansan, 425-600, Republic of Korea

A psychrophilic bacterium, designated strain HJ039^T, was isolated from a marine sponge collected in the East Sea of Korea (also known as the Sea of Japan). Cells were Gram-negative, motile and rod-shaped (1.8–3.54 µm × 0.27–0.73 µm). Growth was observed between 5 and 26 °C (optimum 15 °C), at pH 5.0–8.5 (optimum pH 6.0–6.5) and in the presence of 0–6.0 % NaCl (optimum 2.0 %). The 16S rRNA gene sequence of strain HJ039^T showed high levels of similarity (93.7–95.4 %) with members of the genus *Shewanella*, especially with *Shewanella gaetbuli* TF-27^T (95.2 %), *Shewanella decolorationis* S12^T (94.9 %), *Shewanella putrefaciens* LMG 26268^T (94.6 %), *Shewanella hafniensis* P010^T (94.6 %), *Shewanella algae* ATCC 51192^T (94.5 %) and *Shewanella kaireitica* c931^T (94.5 %). However, phylogenetic analysis revealed that strain HJ039^T shared a phyletic line with *S. algae* and *Shewanella amazonensis*. The major respiratory quinone was Q-8. The DNA G + C content was 52.8 mol%. The major fatty acids were i-13:0 (8.5 %), 15:0 (4.2 %), i-15:0 (23.2 %), i-15:1 (7.9 %), 16:0 (8.7 %), 16:1 ω 7 (21.0 %) and 17:1 ω 8 (6.4 %). From this polyphasic taxonomic evidence, strain HJ039^T is considered to represent a novel species of the genus *Shewanella*, for which the name *Shewanella spongiae* sp. nov. is proposed. The type strain is HJ039^T (= KCCM 42304^T = JCM 13830^T).

The genus *Shewanella* MacDonell and Colwell 1985 comprises a group of Gram-negative, motile, rod-shaped, oxidase-positive, non-fermentative and facultatively anaerobic aquatic and marine bacteria with genomic DNA G + C contents of 42–55 mol% (Bowman, 2005; Gauthier *et al.*, 1995; MacDonell & Colwell, 1985; Venkateswaran *et al.*, 1999). The genus *Shewanella* is a member of the *Gammaproteobacteria* (Anzai *et al.*, 2000) and at the time of writing comprised 34 recognized species (see <http://www.bacterio.cict.fr/s/shewanella.html> and <http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi>). Strains belonging to members of the genus *Shewanella* have been isolated from a variety of sources including marine environments (Bozal *et al.*, 2002; Ivanova *et al.*, 2001; Nealson *et al.*, 1991), clinical samples (Brink *et al.*, 1995; Nozue *et al.*, 1992; Venkateswaran *et al.*, 1999), sediments (Myers & Nealson, 1988; Zhao *et al.*, 2005) and oilfield fluids (Semple & Westlake, 1987). They have also been strongly implicated as opportunistic pathogens of humans and aquatic animals (Aguirre *et al.*, 1994; Brink *et al.*, 1995) and as the causal agents of proteinaceous food spoilage

(Jorgensen & Huß, 1989). *Shewanella* species have also been known to be important in the context of bioremediation, because of their considerable potential for co-metabolic bioremediation of halogenated organic pollutants (Petrovskis *et al.*, 1994), destructive souring of crude petroleum (Semple & Westlake, 1987), and dissimilatory reduction of manganese and iron oxides (Myers & Nealson, 1988), uranium (Lovley & Phillips, 1988) and other compounds (Kostka *et al.*, 1996; Perry *et al.*, 1993). In this study, we report the taxonomic properties of a novel psychrophilic species of *Shewanella* isolated from marine sponge.

Strain HJ039^T was isolated from a marine sponge living at 20 m water depth of the East Sea, Korea (also known as the Sea of Japan). A sponge sample was homogenized and diluted with sterilized seawater, spread onto solid marine agar 2216 (MA; Difco) and then incubated at 10 °C for 1 week. Individual colonies were isolated from MA and the morphologically distinct strain HJ039^T was selected for further characterization.

Unless otherwise stated, physiological and morphological characterization was conducted according to the methods of Sohn *et al.* (2004) and Kwon *et al.* (2005). The bacterial suspension used to inoculate the API 20E, API ZYM (bioMérieux) and Microlog GN2 (Biolog) systems was prepared in a 2 % sea salt (Sigma) solution. To confirm anaerobic growth, cells were inoculated into marine broth

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain HJ039^T is DQ167234.

A table detailing the fatty acid content of strain HJ039^T and closely related species of the genus *Shewanella* is available as supplementary material in IJSEM Online.

2216 in a serum vial capped with an aluminium seal and cultivated at 15 °C for 2 days. The physiological, biochemical and morphological characteristics of strain HJ039^T are given in the species description and in Table 1.

The cellular fatty acid profile of strain HJ039^T was determined from 2-day-old culture at 15 °C according to the procedure described by Sohn *et al.* (2004). Dominant fatty acids were i-13:0 (8.5%), 15:0 (4.2%), i-15:0 (23.2%), i-15:1 (7.9%), 16:0 (8.7%), 16:1 ω 7 (21.0%) and 17:1 ω 8 (6.4%) (see Supplementary Table S1 in IJSEM Online). The major respiratory quinone was Q-8, as determined by HPLC analysis (according to the method of Collins, 1985). The DNA G+C content was 52.8 mol%, as determined by HPLC using a symmetry reversed-phase C18 column (Waters) (Stackebrandt & Liesack, 1993).

Extraction of genomic DNA and amplification of the 16S rRNA gene were conducted according to Sohn *et al.* (2004) and a phylogenetic analysis using the 16S rRNA gene sequence was conducted according to the procedure

described by Kwon *et al.* (2005). The 16S rRNA gene sequence of strain HJ039^T comprised a continuous stretch of 1474 nt, and approximately 1350 bp corresponding to positions 94–1440 of the *Escherichia coli* numbering system (Weisburg *et al.*, 1991) was compared in considerations of sequence quality. Exceptions to this were for *Shewanella halifaxensis* (1279 bp) and *Shewanella sediminis* (1271 bp) because only short sequences were available from the GenBank database. *Aeromonas salmonicida* CIP 103209^T (GenBank accession no. X74681) and *Ferrimonas balearica* DSM 9799^T (GenBank accession no. X93021) served as outgroups for phylogenetic analysis. Sequence similarity after alignment indicated that the closest relatives of strain HJ039^T were *Shewanella gaetbuli* TF-27^T (95.2%), *Shewanella decolorationis* S12^T (94.9%), *Shewanella putrefaciens* LMG 26268^T (94.6%), *Shewanella hafniensis* P010^T (94.6%), *Shewanella algae* ATCC 51192^T (94.5%) and *Shewanella kaireitica* c931^T (94.5%).

The phylogenetic tree based on 16S rRNA gene sequences of members of the genus *Shewanella* has been divided into two

Table 1. Physiological characteristics differentiating strain HJ039^T and the type strains of related *Shewanella* species

Strains: 1, HJ039^T; 2, *S. gaetbuli* TF-27^T; 3, *S. decolorationis* S12^T; 4, *S. algae* ATCC 51192^T; 5, *S. hafniensis* P010^T; 6, *S. putrefaciens* LMG 26268^T; 7, *S. amazonensis* SB2B^T. Data for strain HJ039^T are from this study; data for the other taxa are from Bowman (2005), Satomi *et al.* (2006), Venkateswaran *et al.* (1999), Yoon *et al.* (2004) and Xu *et al.* (2005). +, Positive reaction; –, negative reaction; v, variable reaction; ND, not determined. All are straight, rod-shaped organisms, oxidase- and catalase-positive, and do not utilize citrate.

Characteristic	1	2	3	4	5	6	7
Optimal growth temperature (°C)	15	30	20–30	25–35	25	25–35	25–35
Optimal pH for growth	6–6.5	7–8	8	7–8	ND	7–8	7–8
Growth at:							
4 °C after 24 h	+	–	+	–	+	+	+
35 °C	–	+	+	+	–	v	+
40 °C	–	–	+	+	–	–	+
Reduction of:							
NO ₃ to NO ₂	–	–	+	+	+	+	+
NO ₂ to N ₂	–	–	+	–	ND	–	+
Haemolysis	ND	+	ND	v	ND	–	+
Growth in the presence of:							
0% NaCl	+	–	+	+	+	+	+
6% NaCl	+	+	–	+	+	+	–
10% NaCl	–	–	–	+	–	–	–
Production of:							
H ₂ S	–	–	+	+	+	+	+
Amylase	–	+	–	–	ND	–	–
Gelatinase	+	+	+	v	+	–	+
Lipase	–	+	ND	–	ND	–	–
Utilization of:							
D-Galactose	–	–	–	v	ND	+	+
D-Fructose	+	–	v	–	ND	–	–
Sucrose	+	–	+	–	–	v	–
Succinate	–	–	+	v	ND	v	+
DL-Lactate	–	–	+	+	+	v	+
DNA G+C content (mol%)	52.8	42	49.3	52–55	47	43–47	52

major groups, with *S. algae* and *Shewanella amazonensis* forming relatively distinct branches (Bowman, 2005). The first group (group I) includes psychrotolerant, non-halophilic strains and the second group (group II) includes psychrotolerant, Na⁺-requiring strains. According to this definition, the six closest relatives of strain HJ039^T could be affiliated into group II with the exception of *S. kaireitica*. However, strain HJ039^T shared a phyletic line with *S. algae* and *S. amazonensis* rather than with *S. gaetbuli*, *S. decolorationis*, etc., within group II (Fig. 1). This result matched well with the DNA G+C values (> 50 mol% for strain HJ039^T, *S. algae* and *S. amazonensis*), although these data cannot be used to specify the phylogenetic position of any particular strain.

Strain HJ039^T did not have an obligate Na⁺ requirement for growth, although the presence of Na⁺ ions enhanced growth, a trait observed for other members of *Shewanella* group II. Some characteristics, such as utilization of D-fructose and sucrose, lack of nitrate-reducing ability and high levels of the fatty acid i-15:1, can be used to differentiate strain HJ039^T from closely related members of the genus *Shewanella*. On the basis of this polyphasic

taxonomic evidence, strain HJ039^T is considered to represent a novel species of the genus *Shewanella*, for which the name *Shewanella spongiae* sp. nov. is proposed.

Description of *Shewanella spongiae* sp. nov.

Shewanella spongiae (spon.gi'ae. L. gen. n. *spongiae* of a sponge, the source of the type strain).

Cells are Gram-negative, rod-shaped (1.8–3.54 µm × 0.27–0.73 µm) and motile. Cells appear singly or in chains. Colonies formed after 2 days on MA at 15 °C are white, circular, opaque and convex with entire margins. Growth does not occur under anaerobic conditions on MA. Growth is observed at 5–26 °C (optimum 15 °C), pH 5.0–8.5 (optimum 6.0–6.5) and in the presence of 0–6% (w/v) NaCl (optimum 2.0%). Produces oxidase and catalase. When assayed with the API ZYM system, esterase (C4), esterase lipase (C8), alkaline phosphatase, leucine arylamidase, acid phosphatase and naphthol-AS-BI-phosphohydrolase are present and valine arylamidase activity is weakly positive. Negative for lipase (C14), cystine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-galactosi-

Fig. 1. Rooted neighbour-joining tree based on nearly complete 16S rRNA gene sequences showing the relationship between strain HJ039^T and members of the genus *Shewanella*. Approximately 1350 bp corresponding to positions 94–1440 of the *E. coli* numbering system was compared. Bootstrap values of 1000 resampled datasets at all nodes are shown. Bar, 0.01 substitutions per nucleotide position.

dase, β -glucuronidase, α -glucosidase, β -glucosidase, *N*-acetyl- β -glucosaminidase, α -mannosidase and α -fucosidase. Degrades sucrose, D-trehalose, dextrin, D-arabitol, D-fructose, α -D-glucose, D-mannose and L-serine and can utilize turanose (weakly) on Microlog GN2 plates. Major fatty acids are i-13:0, 15:0, i-15:0, i-15:1, 16:0, 16:1 ω 7 and 17:1 ω 8. The major respiratory quinone is Q-8. The DNA G+C content is 52.8 mol%.

The type strain, HJ039^T (= KCCM 42304^T = JCM 13830^T), was isolated from a marine sponge in the East Sea, Korea, at 20 m water depth.

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