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JEB
Journal of Environmental Biology

ISSN: 0254-8704
CODEN: JEBIDP

Isolation and characterization of a novel bacterial strain *Shewanella marinisediminis* sp. nov. from deep-sea sediments

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Publication Info

Paper received:
01 February 2012

Revised received:
17 September 2012

Accepted:
22 October 2012

Abstract

A new bacterial strain designated DH39^T was isolated from marine sediment collected from the East Sea, Korea. Phylogenetic analysis using the 16S rRNA gene sequence revealed that strain DH39^T clustered with the genus *Shewanella* and is closely related to *Shewanella canadensis* HAW-EB2^T, *S. woodyi* MS32^T, and *S. sediminis* HAW-EB3^{T}with 98.1, 97.8, and 97.6% sequence similarities, respectively. The isolated bacterium was Gram-negative, rod-shaped, and aerobic. Its temperature range for growth was 4–30°C. The predominant fatty acids were 16:1ω7, 17:1ω8, 13:0-i, 16:0, and 15:0-i. The DNA G+C content was 45.0 mol%. DNA-DNA hybridization analysis showed that DNA-DNA relatedness values in the 16S rRNA phylogenetic tree of strain DH39^T and its nearest neighbors *S. hanedai* and *S. sediminis* were 52.9 and 58.7%. Phylogenetic evidence and phenotypic characteristics suggest strain DH39^T constitutes a novel *Shewanella* species. Therefore, we propose *Shewanella marinisediminis* sp. nov., with DH39^T (KCCM 42936^T = NCCB 100311^T) as the type strain.

Key words

New bacterial strain, *DH39^T*, *Shewanella marinisediminis*, Polyphasic taxonomy

Introduction

Shewanella species are widely distributed in nature throughout freshwater and marine environments (Skerratt *et al.,* 2002; Venkateswaran *et al.*, 1999). *Shewanella* are rod-shaped, Gram-negative, oxidase-positive, motile and chemo-organotrophic aquatic bacteria with 44–47 mol% G+C (MacDonell and Colwell, 1985). More than 30species have been reported so for (Kim *et al.*, 2007; Chang *et al.*, 2008; Bozal *et al.*, 2009). Kato and Nogi (2001) divided the genus into 2major branches, groups 1 and 2. Most species of group 1 are psychrophilic/psychrotolerant and piezophilic/ piezotolerant, and produce eicosapentaenoic acid (EPA); in contrast, species in group 2 are mesophilic, pressure-sensitive, and produce no or only trace amounts of EPA.

The metabolic capabilities of *Shewanella* species have received significant attention since the report of *Shewanella oneidensis* MR-1 utilization of diverse electron receptors (Myers and Nealson, 1988). With a respiratory system based on various organic and inorganic compounds including toxic elements and insoluble metals, *Shewanella* has demonstrated considerable potential for the remediation of contaminated environments and utility in microbial fuel cells (Martin-Gil *et al.*, 2004; Hau and Gralnick, 2007; Fredrickson *et al.,* 2008).

During an investigation of the microbial diversity in marine sediments collected from the East Sea, Korea, a novel species was identified and it was designated *Shewanella marinisediminis* DH39^T by its morphological and physiological characteristics.

Materials and Methods

Isolation of bacterial strain and growth conditions : Strain

DH39^T was isolated from marine sediment, collected from a depth of 1,500 m in the Ulleung Basin of the East Sea, Korea (Fig. 1). The strain was grown in marine 2216E agar medium (Difco) for 7 days and then cultured in 25 ml of TCG liquid medium in a 100 ml Erlenmeyer flask for 7 days at 25°C with shaking at 200 rpm. The TCG liquid medium consisted of 0.3% (w/v) tryptone (Difco), 0.5% (w/v) casitone (Difco), and 0.4% (w/v) glucose (Difco) in filtered seawater. The strain was stored at -80°C in TCG liquid medium containing 20% (v/v) glycerol. The sodium chloride (NaCl) requirement and tolerance for growth were examined in TCG liquid medium containing different concentrations (0, 1, 3, 5, 8, and 10% (w/w) of NaCl. The NaCl requirement and tolerance tests were performed in 100 ml Erlenmeyer flasks at 25°C with shaking (200 rpm). The temperature range for growth was determined in an incubator covering temperatures between 4 and 37°C using marine 2216E agar medium.

Morphological and microscopic analysis : Morphological examination of strain DH39^T was performed by scanning electron microscopy (SEM 515; Philips) and transmission electron microscopy (CM 20; Philips) according to previously described protocols (Beveridge *et al.*, 1994; Bozal *et al.*, 2002). Gram staining was performed as previously described (Murray *et al.*, 1994).

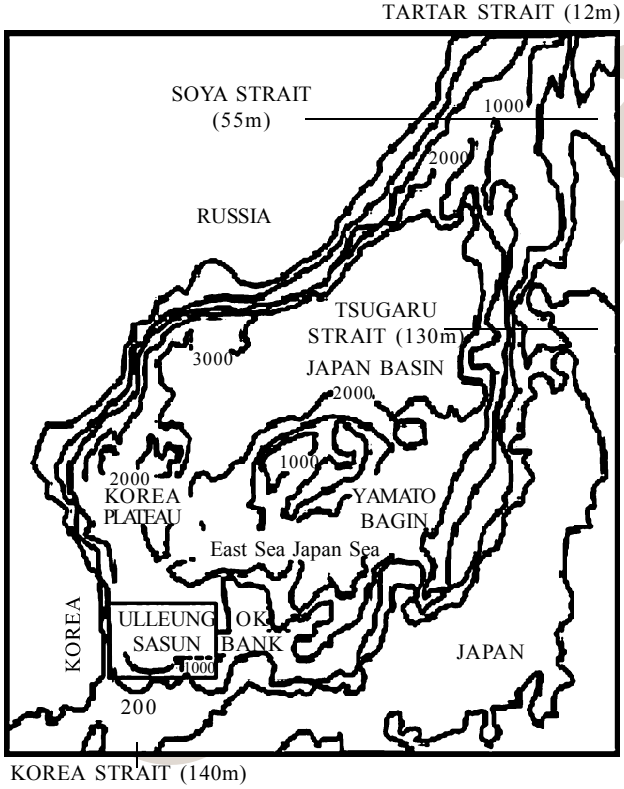

Fig. 1 : Physiographic map of the East Sea/Japan Sea and neighboring region

Biochemical and physiological studies : The biochemical and physiological characteristics of strain DH39^{T}were examined according to Bergey's Manual of Systematic Bacteriology. Additional biochemical analyses were performed using API 20E, API 20NE, API ZYM, and API 50CH test kits (BioMerieux). To analyze fatty acid methyl ester (FAME) composition, the cells were cultured for 2 days in TSB containing 3% NaCl at 25°C with gentle shaking. Fatty acids were extracted from dry cells, methylated, and analyzed by gas chromatography with a cross-linked 5% phenyl silicone capillary column. The FAME peaks were identified and quantified by comparison with the patterns of other microorganisms, using MIDI system software (MIDI/Hewlett Packard Microbial Identification System). The relative amount of each fatty acid was expressed as a percentage of the total.

Genetic analysis : Total genomic DNA preparation of strain DH39^T was performed with the G-spin^{TM} Genomic DNA Extraction Kit (iNtRON, Korea). PCR amplification was performed according to the procedures of Gurtler and Stanisich (1996) for 16S rDNA, and Yamamoto and Harayama (1995) for *gyrB*. The 16S rDNA and *gyrB* sequences were determined on a capillary DNA sequencer (Perkin-Elmer model ABI 3730XL, Applied Biosystems, Foster City, CA) and compared with published sequences using BLAST (www.ncbi.nlm.nih/blast).

The sequences were aligned with CLUSTAL X software (Thompson *et al.*, 1997). A phylogenetic tree was constructed using MEGA 4.0 (Tamura *et al.*, 2007) with the neighbor-joining (Saitou and Nei, 1987), maximum parsimony (Eck and Dayhoff, 1966), and minimum evolution algorithms (Rzhetsky and Nei, 1992). An evolutionary distance matrix for the neighbor-joining method was generated according to Kimura's two-parameter (Kimura, 1980). Bootstrap analysis (500 re-samplings) was used to evaluate the tree topology (Felsenstein *et al.*, 1985). Sequence data for *Shewanella* sp. DH39^{T}were deposited in GenBank (accession number GQ869534 for the 16S rDNA and GU182405 for the *gyrB* gene).

G+C content was determined by high performance liquid chromatography (HPLC) (Mesbah and Whitman 1989). DNA-DNA hybridization was carried out between strain DH39^T and its closest relatives, *Shewanella sediminis* DSM 17055^T and *S. hanedai* ATCC 33224^T using the fluorometric micro-well method (Ezaki *et al.*, 1989)

Results and Discussion

Morphological and phenotypic characteristics : Novel species DH39^T, a Gram-negative bacterium, grew well on marine agar or in TCG liquid medium containing 3% NaCl at 25°C. The growth of strain DH39 was observed at pH 6-12.

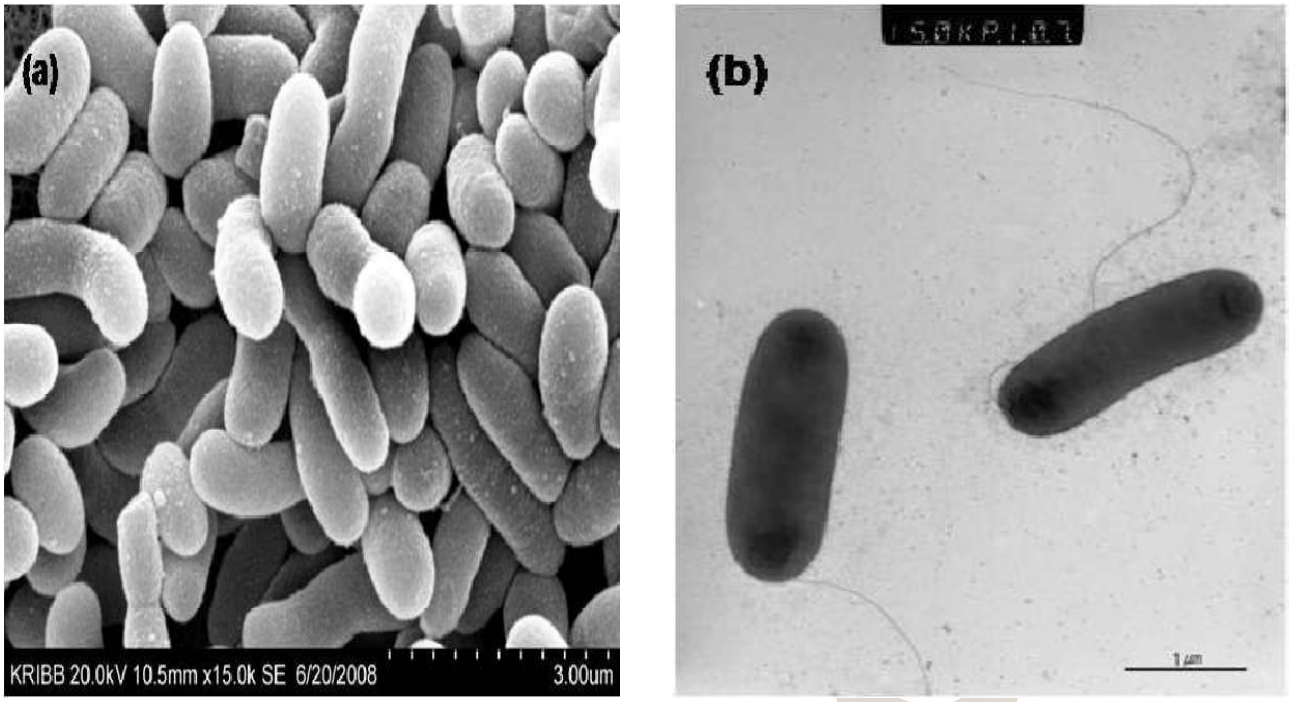

Fig. 2 : Morphology of strain DH39^{T}by (a) Scanning electron microscopy; (b) Transmission electron micrograph of a negatively stained cell showing a single polar flagellum

Table 1 : Physiological characteristics that differentiate strain DH39^T from related species within the genus *Shewanella*

Characteristic	**1**	**2**	**3**	**4**	**5**	**6**
Optimal temperature for growth (°C)	20–25	10	25	10	15–20	20–25
Pigmentation of biomass	pinkish	dark orange/ pinkish	pinkish	pinkish	pinkish	pinkish
DNA G+C content (mol%)	45	46	39	45	45	45
Growth in the presence of						
0% NaCl	-	-	ND	-	ND	+
3% NaCl	+	+	+	+	+	+
6% NaCl	+	-	+	-	-	+
Production of H_2S	-	w (-)	+	+	+	+
Gelatinase	+	+	+	-	+	+
Amylase	-	-	+	ND	ND	-
Lipase	+	-	-	+	+	v
Utilization of:						
Glucose	+	w	-	-	+	+
Cellobiose	+	-	+	-	-	-
N-Acetylglucosamine	+	+	-	+	+	-
Ribose	-	ND	-	-	+	ND
Galactose	+	-	+	-	+	-
Malate	+	+	-	+	-	ND
Reference	This study	Zhao, 2007	Makemson,1997	Zhao, 2005	Bowman, 1997	Ivanova, 2003

Taxa: **1**, strain DH39^T; **2**, *S. canadensis*; **3**, *S. woodyi*; **4**, *S. sediminis*;**5**, *S. hanedai*; **6**, *S. fidelis*; +, Positive; -, negative; w, weak; ND, no data; v, Variable reaction depending on the strain

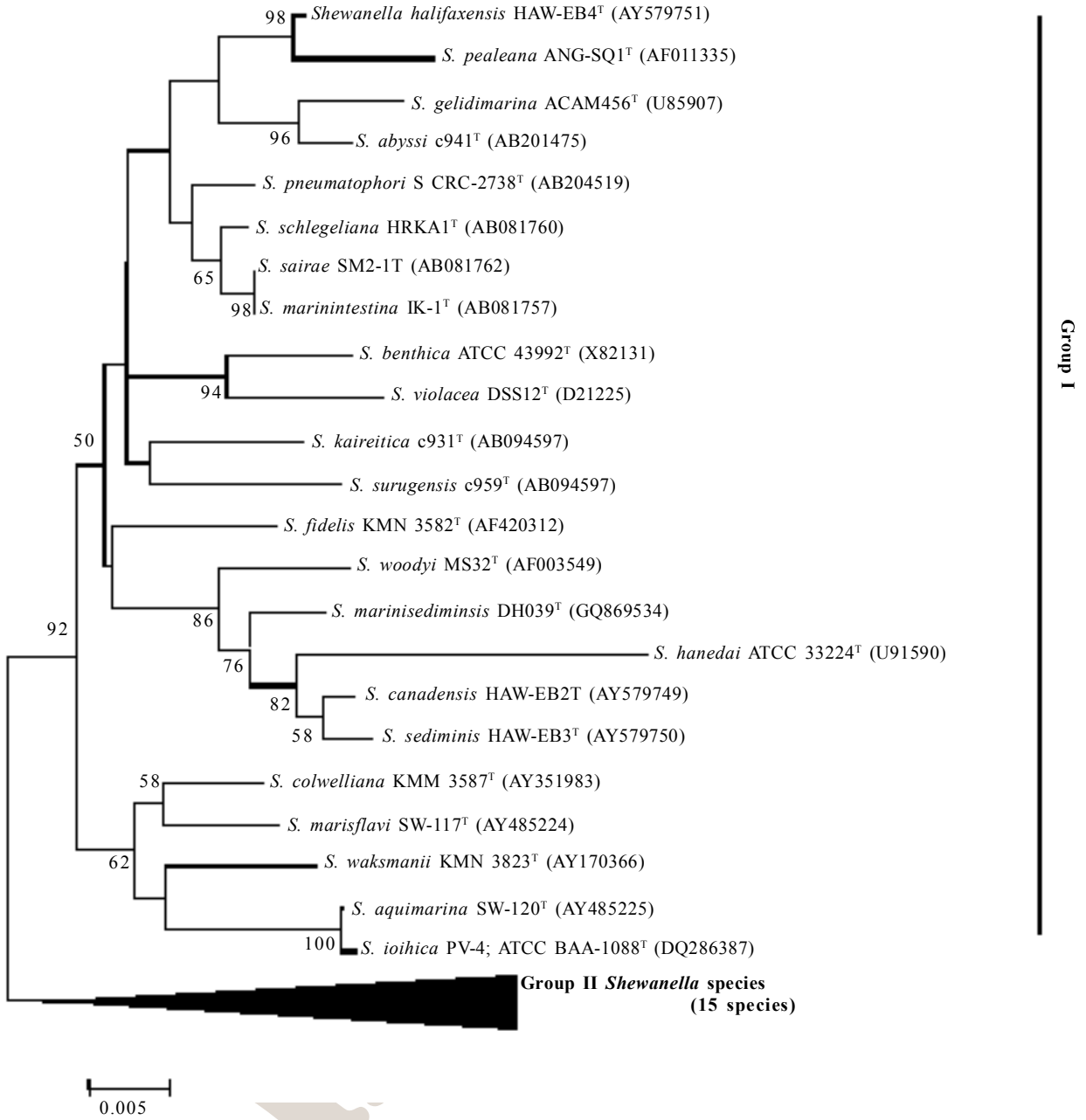

Fig. 3 : Neighbor-joining phylogenetic tree showing the relationship of DH39^{T}to other related species of the genus *Shewanella* based on 16S rRNA gene sequences. Numbers at nodes are percentage bootstrap values based on 500 trials. Bar, 0.005% sequence divergence

The biomass of strain DH39^T exhibited a pinkish color, typical of *Shewanella* species (Venkateswaran *et al.*, 1999). Scanning electron micrographs revealed a rod-shaped bacterium (0.5–1 m in diameter and 1.5–2.0 µm in length) (Fig. 2a). Negatively stained transmission electron micrographs showed the presence of a single polar flagellum (Fig. 2b) consistent with other species of *Shewanella*.

Phylogenetic analysis : Homology of the DH39^{T}16S rRNA gene sequence to previously known sequences was determined by performing BLAST searches in the GenBank/EMBL/DDBJ database. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain DH39^{T}is a member of the genus *Shewanella* with 95.0–98.1% sequence similarity to all 37 recognized species. In the phylogenetic tree constructed using the neighbor-joining method (Fig. 3), strain DH39^T was positioned within *Shewanella* group 1 and formed a monophyletic lineage with *S. canadensis* HAW-EB2^T, *S. sediminis* HAW-EB3^T, and *S. hanedai* ATCC 33224^T (98.1, 97.8% and 96.8 16S rRNA sequence similarity).

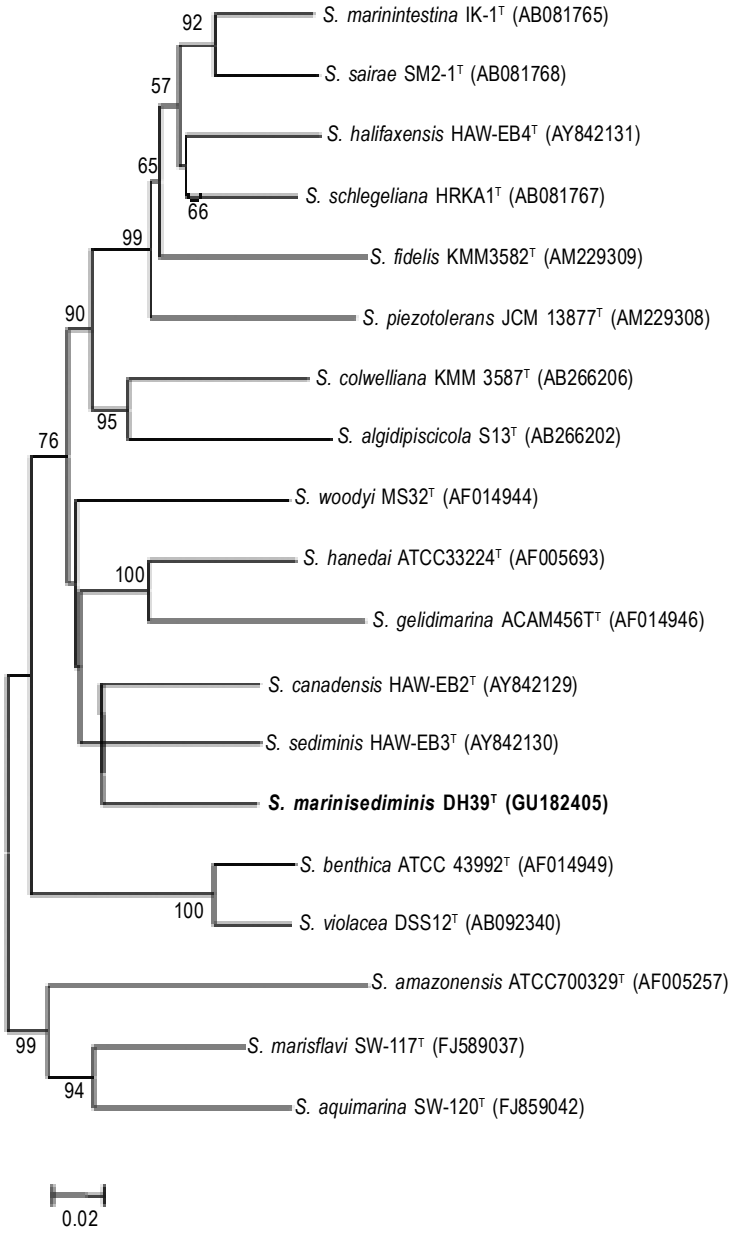

Fig. 4 : Neighbor-joining phylogenetic tree showing the relationship of DH39Tto other related *Shewanella* species based on *gyrB* sequences: Numbers at nodes are percentage bootstrap values based on 500 trials. Bar, 0.02 % sequence divergence

Similar phylogenetic relationships were observed in trees constructed with maximum parsimony and minimum evolution algorithms.

Strain DH39T showed highest *gyrB* sequence similarity with the type strain of *S. sediminis* HAW-EB3T (88.8%). This is lower than the 90% cut-off value proposed for *Shewanella* within species by Venkateswaran *et al.* (1999), providing further evidence that strain DH39T is an independent species. The phylogenetic relationship of strain DH39T with other *Shewanella* species based on *gyrB* gene sequences is shown in Fig. 4.

DNA G+C content : The DNA G+C content of strain DH39T was found to be 45 mol%, consistent with the overall DNA G+C composition of 40 to 54 mol% for known *Shewanella* species (Vogel *et al.,* 1997). Based on DNA-DNA hybridization analysis, strain DH39T exhibited a low level of DNA relatedness with *S. hanedai* (52.9%) and *S. sediminis* (58.7%). Strain DH39T could be described as a new species of *Shewanella* because the hybridization values are below 70%, the species cut-off value recommended for bacteria.

Table 2 : Fatty acid content (%) of strain DH39^T and related species of the genus *Shewanella*

Fatty acid	Fatty acid composition (%)					
	1	**2**	**3**	**4**	**5**	**6**
12:0	3 ω 4	ND	4 ω 64	ND	6 ω 04	1 ω 2
13:0	1 ω 4	ND	1 ω 09	ND	0 ω 94	1 ω 5
14:0	2 ω 4	12	6 ω 42	4	10 ω 66	2 ω 3
15:0	5 ω 0	1	4 ω 80	1	3 ω 35	8 ω 7
16:0	9 ω 4	19	26 ω 07	17	17 ω 85	12 ω 9
17:0	2 ω 1	ND	1 ω 41	ND	ND	1 ω 5
16:1ω9	0 ω 8	ND	ND	ND	1 ω 55	ND
16:1ω7	28 ω 1	39	16 ω 19	33	23 ω 40	20
17:1ω8	9 ω 6	ND	1 ω 55	ND	1 ω 37	6 ω 3
17:1ω6	1 ω 1	ND	ND	ND	ND	1 ω 9
18:1ω9	2 ω 3	ND	1 ω 03	ND	0 ω 77	0 ω 4
18:1ω7	4 ω 0	4	ND	7	ND	5 ω 6
20:5ω3	-	4	ND	3	19-23	-
12:0 3-OH	1 ω 2	1	2 ω 06	7	3 ω 94	ND
13:0 3-OH	3 ω 2	ND	5 ω 35	8	6 ω 14	ND
13:0-i	9 ω 9	5	10 ω 5	3	14 ω 07	10 ω 7
15:0-i	8 ω 9	8	14 ω 32	8	7 ω 19	2 ω 3
Reference	This study	Zhao 2007	Makemson 1997	Zhao 2005	Makem [a]Bowman	Ivanova 2003

Taxa: **1**, strain DH39^T; **2**, *S. canadensis* HAW-EB2^T; **3**, *S. woodyi* MS32^T; **4**, *S. sediminis* HAW-EB3^T; **5**, *S. hanedai* ATCC 33224^T; **6**, *S. fidelis* KMM 3582^T; -, not detected; ND, no data.

Biochemical characteristics : In general, the physiological and biochemical properties of strain DH39^T were consistent with *Shewanella* species, most of which are positive for catalase, oxidase, and lipase activities, and utilize glucose and N-acetyl-D-glucosamine as carbon sources. In addition, they grow at temperatures ranging from 4–30°C in the presence of 1–4% NaCl. DH39^T grew over a temperature range of 4–30°C with optimal growth temperature of 20–25°C; no growth was observed at 37°C. The strain was saline tolerant at 1–8% NaCl. Growth of strain DH39^T was optimal at 3% NaCl.

Strain DH39^T was catalase-, oxidase-, gelatinase-, and lipase-positive; it was unable to hydrolyze starch and did not produce indole. In addition, strain DH39^T exhibited β-glucosidase and β-galactosidase activity. Strain DH39^T was able to utilize glucose, galactose, fructose, mannitol, N-acetyl-glucosamine, maltose, caprate, malate, esculin, cellobiose, and melibiose (Table 1). In contrast, *Shewanella sediminis*, *S. hanedai* and *S. canadensis* could not utilize cellobiose and *S. woodyi* was unable to utilize glucose and N-acetylglucosamine. The cellular fatty acids (C12-C18) were composed of saturated, monoenoic, straight-chain, and iso-branched components (Table 2). The dominant cellular fatty acids were 16:1ω7, 13:0-i, 16:0 and 15:0-i. This fatty acid profile was similar to those of *S. canadensis*, *S. woodyi, S. sediminis, S. hanedai* and *S. fidelis* (Makemson *et al.*, 1997; Zhao *et al.*, 2005; Bowman *et al.*, 1997; Ivanova *et al.*, 2003, Zhao *et al.*, 2007). In addition, strain DH39^T lacked the polyunsaturated fatty acid, eicosapentaenoic acid (20:5ω3), while *S. canadensis*, *S. hanedai* and *S. sediminis*, which are the closest relatives of strain DH39^T, contained 4%, 19–23% and 3% eicosapentaenoic acid (20:5ω3) in the whole-cell fatty acid profiles, respectively. This further supports the designation of strain DH39^T as a new species of *Shewanella*.

In conclusion, the phenotypic characteristics and phylogenetic analysis of strain DH39^{T}indicate a novel species of the genus *Shewanella*, and the name *Shewanella marinisediminis* sp. nov. was proposed.

Acknowledgments

This study was funded by a grant from the National Fisheries Research and Development Institute (RP-2012-Me-046), Korea, and in part by the Korea Institute of Science and Technology institutional program, under Grant# 2Z3550.

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