Shewanella haliotis sp. nov., isolated from the gut microflora of abalone, *Haliotis discus hannai*

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A motile, rod-shaped, pink–orange pigmented bacterium, designated strain DW01^T, was isolated from the gut microflora of abalone collected from the South Sea (Republic of Korea). Cells were Gram-negative, facultatively anaerobic, catalase- and oxidase-positive. The major fatty acids were iso-C_{15:0} (17.7%), C_{16:0} (13.4%), iso-C_{15:0} 2-OH and/or C_{16:1}ω7c (12.5%) and C_{17:1}ω8c (10.7%). The DNA G + C content was 53.7 mol%. A phylogenetic tree based on the 16S rRNA gene sequences showed that strain DW01^T forms a lineage of the genus *Shewanella* and is closely related to *Shewanella algae* ATCC 51192^T (98.3% sequence similarity) and to other members of the genus *Shewanella* (91.0–94.9%). The phenotypic characteristics and DNA–DNA hybridization relatedness data indicate that strain DW01^T should be distinguished from *S. algae* ATCC 51192^T. On the basis of the data presented in this study, strain DW01^T represents a novel species, for which the name *Shewanella haliotis* sp. nov. is proposed. The type strain is DW01^T (=KCTC 12896^T=JCM 14758^T).

The genus *Shewanella* is a member of the class *Gammaproteobacteria* (Anzai *et al.*, 2000) and comprises a group of Gram-negative, motile, rod-shaped, oxidase-positive, non-fermentative and facultatively anaerobic aquatic and marine bacteria (Bowman, 2005; Gauthier *et al.*, 1995; MacDonell & Colwell, 1985; Venkateswaran *et al.*, 1999). At the time of writing, the genus *Shewanella* comprised 45 recognized species (<http://www.bacterio.-cict.fr/s/shewanella.html>). Strains of the genus *Shewanella* have been isolated from a variety of sources including marine environments (Venkateswaran *et al.*, 1998; Nealson *et al.*, 1991; Nogi *et al.*, 1998; Ivanova *et al.*, 2001, 2004a,b; Bozal *et al.*, 2002; Skerratt *et al.*, 2002; Yoon *et al.*, 2004b; Gram *et al.*, 1987; Stenstrom & Molin, 1990; Gram & Huss, 1996; Satomi *et al.*, 2006, 2007; Lee *et al.*, 2006; Satomi *et al.*, 2003; Simidu *et al.*, 1990; Yang *et al.*, 2006), sediments (Myers & Nealson, 1988; Venkateswaran *et al.*, 1999; Toffin *et al.*, 2004; Yoon *et al.*, 2004a; Gao *et al.*, 2006; Zhao *et al.*, 2005, 2006; Miyazaki *et al.*, 2006; Xiao *et al.*, 2007; Yang *et al.*, 2007), clinical samples (Brink *et al.*, 1995; Nozue *et al.*, 1992; Levin, 1972; Debois *et al.*, 1975;

Holmes *et al.*, 1975), oilfield fluids (Semple & Westlake, 1987) and activated sludge (Xu *et al.*, 2005). They have also been 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).

During the course of our study on gut microflora of abalone, a rod-shaped bacterial strain, designated DW01^T, was isolated and subjected to a taxonomic investigation. On the basis of the polyphasic evidence, strain DW01^T represents a novel species of the genus *Shewanella*, for which the name *Shewanella haliotis* sp. nov. is proposed.

Strain DW01^T was isolated from an abalone sample collected from the South Sea near Yeosu (34° 44' N, 127° 44' E) located in the Republic of Korea, during July 2006, using the standard dilution plating technique. Isolation was achieved using marine agar (MA; Difco) (Yang *et al.*, 2006) at 30 °C for 7 days. The isolate was routinely cultured on MA and maintained as a glycerol suspension (20%, w/v) at –80 °C.

Bacterial DNA preparation, PCR amplification and sequencing of the 16S rRNA gene were carried out as described previously (Chun & Goodfellow, 1995). The

The GenBank/EMBL/DDJB accession number for the 16S rRNA gene sequence of strain DW01^T is EF178282.

Fig. 1. Neighbour-joining tree based on the almost-complete 16S rRNA gene sequences showing the relationship between strain DW01^T and members of the genus *Shewanella*. The percentage numbers at the nodes are the levels of bootstrap support based on neighbour-joining analyses of 1000 resampled datasets. The sequence of *Vibrio cholerae* ATCC 14035^T (Z21856) was used as an outgroup (not shown). Bar, 0.01 nucleotide substitutions per position.

resultant sequence of strain DW01^T was aligned manually against sequences obtained from the GenBank database. Phylogenetic trees were inferred from the regions available for all sequences (positions 38–1450; *Escherichia coli* numbering system) using the Fitch–Margoliash (Fitch & Margoliash, 1967) and neighbour-joining (Saitou & Nei, 1987) methods. Evolutionary distance matrices were generated according to Jukes & Cantor (1969). The resultant neighbour-joining tree topology was evaluated by bootstrap analyses (Felsenstein, 1985) based on 1000 resamplings. Alignment and phylogenetic analyses were carried out using the jPHYDIT program (available at [\[chunlab.snu.ac.kr/jphydit/\]\(http://chunlab.snu.ac.kr/jphydit/\)\) and PAUP 4.0 \(Swofford, 1998\) as described previously \(Chun *et al.*, 2000\).](http://</p>
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Preliminary sequence comparison with the 16S rRNA gene sequences held in GenBank indicated that our isolate was related closely to members of the genus *Shewanella*. The newly determined sequence was then aligned manually against representatives of the genus *Shewanella*. Strain DW01^T showed the highest 16S rRNA gene sequence similarity to *Shewanella algae* ATCC 51192^T (98.3%), followed by *Shewanella profunda* DSM 15900^T (94.9%), *Shewanella kaireitica* DSM 17170^T (94.4%) and *Shewanella*

schlegeliana JCM 11561^T (94.1%). To elucidate the phylogenetic relationship between the novel isolate and other species of the genus *Shewanella*, phylogenetic trees were constructed using two different tree-making algorithms. The neighbour-joining tree (Fig. 1) showed that strain DW01^T formed a monophyletic clade with *S. algae* ATCC 51192^T with 100 % bootstrap support.

Growth on various standard bacteriological media was tested by using nutrient agar (NA; Difco), tryptic soy agar (TSA; Difco) and R2A agar (Difco) according to the manufacturer's instructions. Cells of strain DW01^T grown on TSA at 30 °C for 3–7 days were used for the physiological and biochemical tests. Motility was examined by observing the cells grown in wet mounts using phase-contrast microscope (Nikon TMS-F). The pH range (pH 3–12), growth temperature (4–42 °C), NaCl tolerance [0, 1, 2, 3, 5, 10 % (w/v)] and growth in an anaerobic chamber (CO₂/H₂/N₂, 10:10:80; Sheldon Manufacturing) were checked using TSA, using up to 1 week incubation periods. Catalase and oxidase activities were determined using 3 % (v/v) hydrogen peroxide and Kovac's reagent (Kovacs, 1956), respectively. Other physiological and biochemical tests were performed using API 20E, API 20NE and API 50CH (bioMérieux). Enzymic activities were tested using API ZYM kit (bioMérieux) following the manufacturer's instructions. Antibiotic resistance was determined with the disc diffusion method. The results were interpreted according to the guidelines set forth by the National Committee for Clinical Laboratory Standards.

Strain DW01^T was facultatively anaerobic, Gram-negative, motile and rod-shaped. Colonies grown on TSA plates for 5 days at 30 °C were circular, convex, entire margin, smooth, opaque, pink–orange coloured and approximately 5.0 mm in diameter. The novel strain grew well on MA, TSA, plate count agar (PCA; Difco) and NA. On TSA medium, strain DW01^T was able to grow at 10–42 °C. The detailed results of physiological and biochemical analyses are given in Table 1 and the species description. It is evident from Table 1 that there are several phenotypic characters that readily separate strain DW01^T from phylogenetically related species, namely *S. algae* and *Shewanella amazonensis*.

Cellular fatty acids of strain DW01^T were analysed as methyl esters by GLC according to the instructions of the Microbial Identification System (MIDI). The cellular fatty acid profile of strain DW01^T is described in Table 2. The G+C content of the DNA was determined by using the thermal denaturation method of Marmur & Doty (1962). The DNA G+C content of DW01^T was 53.7 mol%.

The taxonomic relationship between strain DW01^T and *S. algae* ATCC 51192^T was further examined using DNA–DNA hybridization. Genomic relatedness was determined using a membrane filter technique (Seldin & Dubnau, 1985) with a DIG High Prime DNA Labelling and Detection Starter kit II (Roche). When DNAs were used

individually as labelled DNA probes for reciprocal hybridization experiments and conducted in duplicate, the DNA–DNA relatedness value between strain DW01^T and *S. algae* ATCC 51192^T was 35.8 %. It is clear from the 16S rRNA gene sequence and DNA–DNA hybridization data that strain DW01^T represents a novel species of the genus *Shewanella* (Wayne *et al.*, 1987).

In addition, a number of physiological and chemotaxonomic characters clearly distinguished our isolate from other phylogenetically related species (Tables 1 and 2). Therefore, strain DW01^T should be classified as a novel species of the genus *Shewanella*, for which the name *Shewanella haliotis* sp. nov. is proposed.

Description of *Shewanella haliotis* sp. nov.

Shewanella haliotis (ha.li.o'tis. N.L. gen. n. *haliotis* of *Haliotis*, the scientific name of abalones).

Cells are rod-shaped, Gram-negative and facultatively anaerobic bacterium. Cells grow best on media such as

Table 1. Phenotypic characteristics that differentiate strain DW01^T from its phylogenetic neighbours of *Shewanella* species

Strains: 1, DW01^T; 2, *S. algae* ATCC 51192^T; 3, *S. amazonensis* ATCC 700329^T. Data from Khashe & Janda (1998), Nozue *et al.* (1992), Venkateswaran *et al.* (1998) and this study. +, Positive; –, negative; w, weakly positive; ND, no data available. All strains are Gram-negative, rod-shaped, motile and positive for catalase, oxidase, H₂S production, gelatinase and reduction of nitrates to nitrites. All strains are negative for the utilization of D-glucose, adipate, glycerol, mannitol, sorbitol, maltose, lactose and sucrose as carbon sources. Two species (1 and 2) are resistant to vancomycin (30 µg per disc), ampicillin (10 µg per disc) and penicillin (10 U per disc).

Characteristic	1	2	3
Habitat	Abalone	Salt marshes	Mud
Colony colour	Pink–orange	Pink	Pink
Growth at 4 °C	–	–	+
Growth at 42 °C	+	+	–
Tolerance to 6 % NaCl	+	+	–
Ornithine decarboxylase	+	+	–
Citrate utilization	–	–	+
Reduction of nitrates to nitrites	+	+	–
Acidification of glucose	–	w	–
Assimilation of:			
Mannose	–	+	+
Caprate	+	+	–
Malate	+	–	–
Fructose	–	+	+
Antibiotic resistance (µg per disc):			
Polymyxin B (300)	R	S*	ND
DNA G+C content (mol%)	53.7	54.0	51.7

*Data from this study.

Table 2. Cellular fatty acid composition (%) of strain DW01^T and its phylogenetic neighbours of *Shewanella* species

Strains: 1, DW01^T; 2, *S. algae* ATCC 51192^T; 3, *S. amazonensis* ATCC 700329^T. Data from Venkateswaran *et al.* (1998) and this study. Values are percentages of total fatty acids. —, Fatty acid not present; tr, trace amount (<1 %); ND, no data available.

Fatty acid	1	2	3
Straight-chain saturated			
C _{12:0}	2.21	1.93	ND
C _{14:0}	1.91	1.19	1.43
C _{15:0}	2.75	3.72	9.17
C _{16:0}	13.43	11.56	6.11
C _{17:0}	2.49	2.99	3.95
C _{18:0}	6.54	3.31	tr
C _{12:0} 3-OH	1.96	—	—
Branched saturated			
iso-C _{13:0}	4.10	3.28	4.70
iso-C _{14:0}	1.11	tr	1.55
iso-C _{15:0}	17.72	20.72	26.69
iso-C _{16:0}	tr	tr	1.41
iso-C _{17:0}	1.62	1.79	1.80
iso-C _{13:0} 3-OH	—	2.87	—
Monounsaturated			
C _{16:1} ω7c	—	—	14.65
C _{16:1} ω9c	1.11	1.29	tr
C _{17:1} ω6c	tr	tr	2.43
C _{17:1} ω8c	10.70	11.81	23.45
C _{18:1} ω7c	5.44	4.81	4.46
C _{18:1} ω9c	4.52	4.09	1.39
Summed feature 1*	1.50	1.85	ND
Summed feature 2*	1.10	1.16	ND
Summed feature 3*	12.45	13.49	ND

*Summed features represent groups of two fatty acids that could not be separated by GLC with the MIDI system. Summed feature 1 contained C_{13:0} 3-OH and iso-C_{15:1} I/H. Summed feature 2 contained C_{14:0} 3-OH and iso-C_{16:1} I. Summed feature 3 contained C_{16:1}ω7c and iso-C_{15:0} 2-OH.

MA, TSA, PCA and NA, but weakly on R2A. Colonies on TSA are circular, low-convex, entire margin, smooth, opaque, pink–orange coloured and approximately 5.0 mm in diameter after 5 days at 30 °C (pH 7). Cells are motile rods and 0.5–0.7 × 2.0–4.3 µm in size. Growth occurs in 0–10 % (w/v) NaCl (optimum 4 %). Growth occurs in pH 5–11 (optimum pH 7) and at 10–42 °C (optimum 37 °C). Oxidase-positive, catalase-positive. Reduces nitrate to nitrite. Negative for glucose fermentation. Does not produce arginine dihydrolase, urease, lysine decarboxylase, β-galactosidase or tryptophan deaminase. Produces H₂S but not acetoin or indole. Produces gelatinase, α-chymotrypsin, alkaline phosphatase and ornithine decarboxylase, but not lipase (C14). Utilizes the following substrates as sole carbon and energy sources: *N*-acetylglucosamine, caprate and malate. Does not utilize the following substrates: D-glucose, galactose, fructose, mannose,

melibiose, D-arabinose, L-arabinose, D-xylose, aesculin, salicin, glycerol, ribose, adonitol, sorbose, dulcitol, inositol, mannitol, sorbitol or inulin. Cells are sensitive to (µg per disc, unless otherwise indicated): amikacin (30), gentamicin (10), streptomycin (10), tetracycline (30), erythromycin (15), chloramphenicol (30), kanamycin (30) and nalidixic acid (30) but resistant to ampicillin (10), vancomycin (30), polymyxin B (300) and penicillin (10 U). Other physiological and biochemical characteristics are given in Table 1. Major fatty acids are iso-C_{15:0} (17.7 %), C_{16:0} (13.4 %), iso-C_{15:0} 2-OH and/or C_{16:1}ω7c (12.5 %) and C_{17:1}ω8c (10.7 %), and complete fatty acid composition is given in Table 2. The DNA G+C content is 53.7 mol%.

The type strain, DW01^T (=KCTC 12896^T=JCM 14758^T), was isolated from the gut microflora of abalone collected from the South Sea, Republic of Korea.

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