Shewanella loihica sp. nov., isolated from iron-rich microbial mats in the Pacific Ocean

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A novel marine bacterial strain, PV-4^T, isolated from a microbial mat located at a hydrothermal vent of Loihi Seamount in the Pacific Ocean, has been characterized. This micro-organism is orangey in colour, Gram-negative, polarly flagellated, facultatively anaerobic and psychrotolerant (temperature range, 0–42 °C). No growth was observed with nitrate, nitrite, DMSO or thiosulfate as the electron acceptor and lactate as the electron donor. The major fatty acid detected in strain PV-4^T was iso-$C_{15:0}$. Strain PV-4^T had ubiquinones consisting mainly of Q-7 and Q-8, and possessed menaquinone MK-7. The DNA G+C content of the strain was 53·8 mol% and the genome size was about 4·5 Mbp. Phylogenetic analyses based on 16S rRNA gene sequences placed PV-4^T within the genus *Shewanella*. PV-4^T exhibited 16S rRNA gene sequence similarity levels of 99·6 and 97·5 %, respectively, with respect to the type strains of *Shewanella aquimarina* and *Shewanella marisflavi*. DNA from strain PV-4^T showed low mean levels of relatedness to the DNAs of *S. aquimarina* (50·5 %) and *S. marisflavi* (8·5 %). On the basis of phylogenetic and phenotypic characteristics, the bacterium was classified in the genus *Shewanella* within a distinct novel species, for which the name *Shewanella loihica* sp. nov. is proposed. The type strain is PV-4^T (=ATCC BAA-1088^T=DSM 17748^T).

The genus *Shewanella* consists of rod-shaped, Gram-negative, facultatively anaerobic, readily cultivated gammaproteobacteria (Gauthier *et al.*, 1995; MacDonell & Colwell, 1985; Venkateswaran *et al.*, 1999). While many *Shewanella* strains remain uncharacterized, there are 32 recognized *Shewanella* species: the latter were isolated from a variety of sources, primarily aquatic environments and sediments (Bowman *et al.*, 1997; Bozal *et al.*, 2002; Brettar *et al.*, 2002; Coyne *et al.*, 1989; Ivanova *et al.*, 2001, 2003a, b, 2004a, b, c; Leonardo *et al.*, 1999; Makemson *et al.*, 1997; Nogi *et al.*, 1998; Nozue *et al.*, 1992; Satomi *et al.*, 2003, 2006; Skerratt *et al.*, 2002; Toffin *et al.*, 2004; Venkateswaran *et al.*, 1998, 1999; Xu *et al.*, 2005; Yoon *et al.*, 2004a, b; Zhao *et al.*, 2005, 2006; Ziemke *et al.*, 1998). The bacteria of this genus have attracted great attention because of their diverse respiratory capacities, illustrated by their ability to utilize a wide range of terminal electron acceptors, including oxygen, nitrate, metals and sulfur compounds (Kostka *et al.*, 1996; Myers & Nealson, 1988; Venkateswaran *et al.*, 1999; http://www.shewanella.org). Some *Shewanella* strains are also able to degrade pollutants such as chlorinated solvents (Petrovskis *et al.*, 1994), petroleum (Semple & Westlake, 1987) and RDX (1,3,5-trinitroperhydro-1,3,5-triazine) (Zhao *et al.*, 2004), some can produce polyunsaturated fatty acids (Bowman *et al.*, 1997; Russell & Nichols, 1999; Satomi *et al.*, 2003) and some are able to grow under extreme conditions (Bozal *et al.*, 2002; Kato *et al.*, 1998; Nogi *et al.*, 1998; Stapleton *et al.*, 2005).

In a previous study, several *Shewanella* strains were isolated from marine-sediment samples at a variety of locations in the Pacific Ocean (Stapleton *et al.*, 2005). Among these

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain PV-4^T is DQ286387.

The fatty acid compositions of strain PV-4^T and *S. aquimarina* JCM 12193^T are detailed in a supplementary table in IJSEM Online.

strains was *Shewanella* sp. PV-4^T, which was isolated from iron-rich microbial mats at the active, deep-sea, hydrothermal Naha Vent (1325 m below sea level) located on the South Rift of Loihi Seamount, Hawaii (http://www.soest.hawaii.edu/GG/HCV/loihivents.html). Although the draft genome sequence of strain PV-4^T was released recently (by the Joint Genome Institute; see http://www.jgi.doe.gov) and its morphology, metal-reduction capacity and biomineralization ability have been explored, its taxonomic status has remained undefined (Roh *et al.*, 2006). The objective of the present study was to establish the taxonomic position of strain PV-4^T, by using a combination of polyphasic taxonomic data.

Standard protocols, including those for determining the Gram reaction, catalase and oxidase activities and spore formation (Smibert & Krieg, 1994), were employed to establish the physiological and biochemical properties of strain PV-4^T. Enzymic hydrolysis of various substrates, including casein, starch, gelatin, Tweens 20, 40 and 80 and DNA, and a determination of the production of H_2S from thiosulfate were conducted, using marine broth 2216, as described elsewhere (Smibert & Krieg, 1994; Bowman *et al.*, 1997). Other phenotypic and enzymic characterizations of strain PV-4^T were conducted using API 20E, API ID 32A and API ZYM test kits (bioMérieux) and Biolog PM plates (Biolog), according to the instructions of the manufacturers. The pH and temperature ranges for growth were determined on marine 2216 medium (Difco). The requirement for Na^+ ions was studied using a medium described elsewhere (Ivanova *et al.*, 2003b). Salt-tolerance tests were performed on marine 2216 medium with NaCl concentrations of 0·5–8·0 % (w/v). The reduction of electron acceptors was assessed using M1 defined medium supplemented with lactate (10 mM) as the electron donor and one of the electron acceptors as described previously (Roh *et al.*, 2006). The reduction of electron acceptors with *N*-acetylglucosamine (10 mM) as the electron donor was examined in this study by using the same procedure. The electron acceptors tested include MnO_2 (5 mM), ferric citrate (20 mM), ferric EDTA (10 mM), akaganeite (β-FeOOH; 70 mM), cobalt [Co(III)] EDTA (1·5 mM), potassium chromate [Cr(VI); 0·5 mM], uranyl [U(VI)] carbonate (5 mM), hydrous ferric oxides (40 mM), DMSO (10 mM), sodium nitrate (3 mM), sodium nitrite (0·5 mM), sulfur (40 mM), sodium thiosulfate (5 mM), sodium sulfate (5 mM), sodium sulfite (5 mM) and trimethylamine *N*-oxide (10 mM).

The morphological, physiological and biochemical characteristics of strain PV-4^T are given in Table 1. Consistent with species of the genus *Shewanella*, strain PV-4^T is a rod-shaped bacterium with a single polar flagellum (Roh *et al.*, 2006). Biomass of strain PV-4^T exhibited an orangey colour under aerobic conditions. In general, the physiological and biochemical characteristics of strain PV-4^T are typical of species of the genus *Shewanella* (Venkateswaran *et al.*, 1999). However, strain PV-4^T exhibits some unique features. Strain PV-4^T was found to be psychrotolerant and able to grow over unusually wide ranges of temperature (0–42 °C), pH (4·5–10) and salt (0·5–5 %). The optimal temperature, pH and salt concentration for growth were 18 °C, pH 6–8 and 2 %, respectively. In contrast to most *Shewanella* species, strain PV-4^T was able to utilize alanine. However, unlike most *Shewanella* species, PV-4^T was unable to utilize acetate, propionate or Tween 40. Unlike some *Shewanella* species, strain PV-4^T does not show any growth with nitrate, nitrite, thiosulfate, sulfur, sulfate, sulfite or DMSO as the electron acceptor and lactate as the electron donor.

Table 1. Characteristics that differentiate strain PV-4^T from phylogenetically related species

Taxa: 1, strain PV-4^T; 2, *Shewanella affinis*; 3, *S. aquimarina*; 4, *Shewanella colwelliana*; 5, *S. marisflavi*; 6, *Shewanella waksmanii*. Data are from this study, Ivanova *et al.* (2003b, 2004b), Weiner *et al.* (1988) and Yoon *et al.* (2004b). Cells of all species are straight, rod-shaped, Gram-negative and polarly flagellated. All species are oxidase-, catalase- and gelatinase-positive and negative for the utilization of sucrose, D-fructose and glycerol. Symbols: +, positive; −, negative; V, variable (depending on the strain); ND, no data available.

Characteristic	1	2	3	4	5	6
DNA G+C content (mol%)	54	45	54	46	51	43
Growth in/at:						
4 °C	+	−	−	+	+	+
42 °C	+	−	+	−	+	−
0 % NaCl	−	−	−	−	+	−
8 % NaCl	−	V	+	−	+	−
pH 4·5	+	ND	−	ND	−	−
pH 10	+	ND	−	ND	−	+
Reduction of nitrate to nitrite	−	+	+	+	+	+
H_2S from thiosulfate	−	+	+	ND	+	ND
α-Glucosidase	+	ND	−	ND	−	ND
β-Glucosidase	+	ND	−	ND	−	ND
Utilization of:						
Fumarate	+	−	−	+	−	−
Galactose	+	−	+	−	−	−
Glucose	+	+	−	ND	+	+
Citrate	+	+	−	−	−	−
Lactate	+	+	+	−	+	−
Malate	+	ND	+	−	+	ND
Maltose	+	ND	+	−	+	ND
N-Acetylglucosamine	+	ND	+	ND	+	−
Succinate	+	−	+	−	+	−

For quantitative analysis of cellular fatty acid compositions, cell mass of strains PV-4^T and *Shewanella aquimarina* JCM 12193^T was obtained from Luria–Bertani agar plates after cultivation for 2 days at room temperature and fatty acid profiles were determined using the Sherlock System (MIDI) at the University of Florida, Gainesville, FL, USA (http://plantpath.ifas.ufl.edu/fame/). Isoprenoid quinones were extracted and analysed as described by

Ivanova *et al.* (2003b). The cellular fatty acids observed in PV-4^T were similar to those of *S. aquimarina* JCM 12193^T, ranging from C_{12} to C_{18}, and included saturated, monoenoic, straight-chain and iso-branched components (see Supplementary Table S1, available in IJSEM Online). Major fatty acids included $C_{i13:0}$, $C_{i13:0}$ 3-OH, $C_{i15:0}$, $C_{16:0}$, $C_{i17:0}$, $C_{17:1}\omega8$, $C_{18:1}\omega7$. Strain PV-4^T contained ubiquinones, consisting mainly of Q-7 and Q-8, and menaquinone MK-7. However, methylmenaquinones were not detected.

The DNA G+C content of strain PV-4^T is 53·8 mol% and the genome size is 4·5 Mbp (on the basis of the draft genome sequence). The almost-complete 16S rRNA gene sequence for strain PV-4^T was also amplified and sequenced as described elsewhere (Roh *et al.*, 2006). Other *Shewanella* 16S rRNA gene sequences were obtained from GenBank or the Ribosomal Database Project (http://rdp.cme.msu.edu/index.jsp). Sequence alignment and phylogenetic relationships were established with the neighbour-joining DNA distance program in the MEGA3 package (Kumar *et al.*, 2004) (Fig. 1). The phylogenetic analysis clearly showed that strain PV-4^T belonged to the genus *Shewanella*. The 16S rRNA gene sequence of strain PV-4^T showed 99·6 and 97·5 % similarity, respectively, to those of the type strains of its nearest phylogenetic relatives, *S. aquimarina* and *Shewanella marisflavi*. The levels of 16S rRNA gene sequence similarity between strain PV-4^T and the type strains of other recognized *Shewanella* species were below 96·5 %. As reported by Stackebrandt & Goebel (1994), species definition in general requires 16S rRNA sequence similarities greater than 97·5 %. Thus, strain PV-4^T could be a strain belonging to the species *S. aquimarina* or *S. marisflavi*.

To determine whether PV-4^T is a strain within *S. aquimarina* or *S. marisflavi*, DNA–DNA hybridizations were performed between PV-4^T and *S. aquimarina* JCM 12193^T and between PV-4^T and *S. marisflavi* JCM 12192^T. Genomic DNA was extracted from these two strains for DNA–DNA hybridization, as described previously (Zhou *et al.*, 1996). DNA hybridizations were carried out using the microplate procedure, as described elsewhere (Goris *et al.*, 1998). Strain PV-4^T displayed mean DNA–DNA relatedness values of 50·5 and 8·5 % with respect to *S. aquimarina* JCM 12193^T and *S. marisflavi* JCM 12192^T, respectively. As these values are below the 70 % similarity threshold specified by Wayne *et al.* (1987), strain PV-4^T should be considered as representing a different species within the genus *Shewanella*.

In summary, on the basis of phenotypic, physiological, chemotaxonomic, phylogenetic and genetic data, we propose that strain PV-4^T represents a novel species of

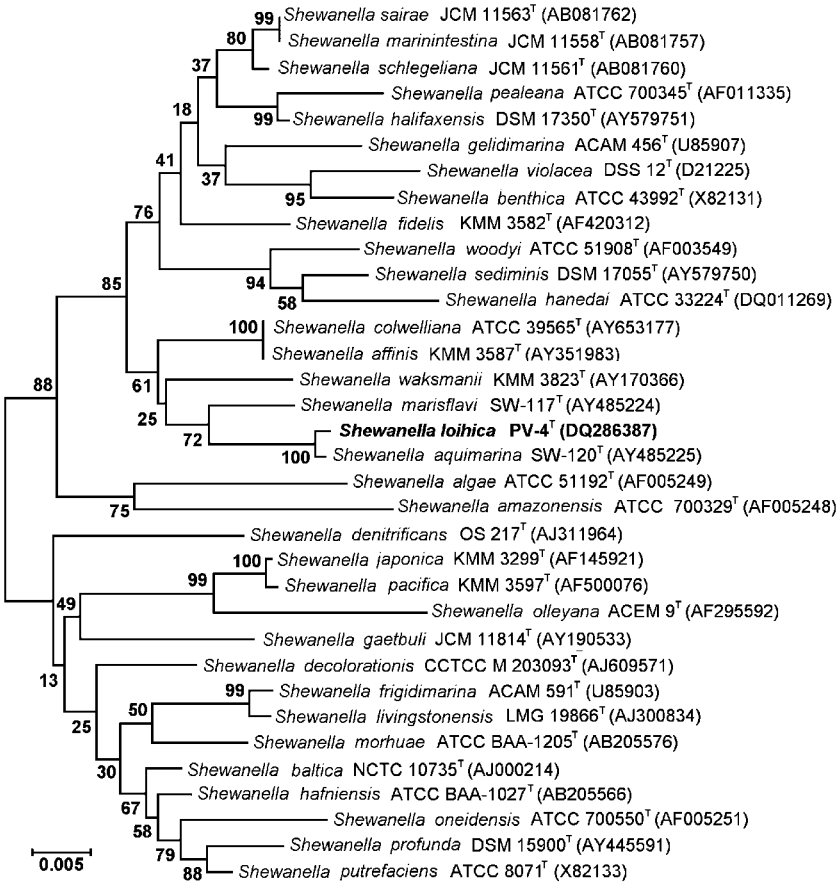

Fig. 1. Phylogenetic tree, based on 16S rRNA gene sequences, showing the taxonomic position of strain PV-4^T within the genus *Shewanella*. The tree was created using the neighbour-joining method included in MEGA3. Numbers at nodes indicate bootstrap percentages. Bar, 5 nucleotide differences per 1000 positions.

Shewanella, for which we propose the name *Shewanella loihica* sp. nov.

Description of *Shewanella loihica* sp. nov.

Shewanella loihica (lo.i.hi′ca. N.L. fem. adj. *loihica* of Loihi Seamount, where the type strain was isolated).

Gram-negative, non-spore-forming, straight rod with a mean length of 1·8 μm and a mean width of 0·7 μm. Motile by means of a single polar flagellum. Facultative psychrotolerant anaerobe. Colonies are smooth, glistening, circular, flat to slightly raised, orange in colour and 2·0–4·0 mm in diameter after 2 days incubation in air at room temperature on Luria–Bertani agar plates. Grows at temperatures ranging from 0 to 42 °C, with 18 °C as the optimum. Does not grow at temperatures above 43 °C. pH range for growth is 4·5–10·0 (optimum, pH 6·0–8·0). Na^+ is required for growth. Grows at 0·5–5 % NaCl, with an optimum at 2 % NaCl; does not grow in the presence of more than 6 % NaCl. Grows under anaerobic conditions. With lactate as the substrate, fumarate, MnO_2, ferric citrate, akaganeite, cobalt [Co(III)] EDTA, potassium chromate, uranyl carbonate, hydrous ferric oxides and trimethylamine *N*-oxide are reduced, but ferric EDTA, nitrate, nitrite, thiosulfate, sulfur, sulfate, sulfite and DMSO are not. Susceptible to chloramphenicol, erythromycin, gentamicin, kanamycin, rifampicin and tetracycline. Slightly susceptible to spectinomycin and streptomycin. Resistant to ampicillin. Casein, gelatin, Tween 20 and Tween 80 are hydrolysed. Positive for oxidase, ornithine decarboxylase, urease, citrate utilization, *p*-phenylalanine deaminase, arginine dihydrolase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase and malonate utilization. Negative for α-galactosidase, β-galactosidase, β-phospho-6-galactosidase, α-arabinosidase, α-fucosidase, β-glucuronidase, alkaline phosphatase, arginine arylamidase, proline arylamidase, leucyl glycine arylamidase, phenylalanine arylamidase, leucine arylamidase, pyroglutamate arylamidase, tyrosine arylamidase, alanine arylamidase, glycine arylamidase, histidine arylamidase, glutamyl glutamate arylamidase, serine arylamidase, lysine decarboxylase, mannose fermentation, raffinose fermentation, indole formation and acetoin production. Positive for utilization of *N*-acetylglucosamine, succinate, DL-malate, L-malate, α-ketoglutarate, alanine, threonine, isoleucine, leucine, glycyl aspartate, glycyl glutamate, glycyl proline, alanyl glycine, gelatin, α-ketobutyrate, monomethyl succinate, pyruvate, butyrate, caproate, β-hydroxypyruvate, α-D-glucose, dextrin, D-galactose, maltose, α-cyclodextrin, β-cyclodextrin, γ-cyclodextrin, maltotriose, adenosine, inosine, Tween 20, Tween 80, chondroitin sulfate, acetamide, putrescine and 2,3-butanediol; negative for utilization of acetate, propionate and Tween 40. Acid is produced from *N*-acetyl-D-glucosamine. Acid is not produced from L-arabinose, D-xylose, D-adonitol, L-rhamnose, D-cellobiose, D-melibiose, sucrose, D-trehalose, D-raffinose or D-glucose. Fatty acids $C_{i13:0}$ (10 %), iso-$C_{13:0}$ 3-OH (6 %), iso-$C_{15:0}$ (36 %), $C_{16:0}$ (5 %), iso-$C_{17:0}$ (3 %), $C_{17:1}\omega8$ (13 %) and $C_{18:1}\omega7$ (3 %) are present. Quinone composition is Q-7 (53 %), Q-8 (28 %) and MK-7 (74 %). The DNA G+C content is 53·8 mol% (http://www.jgi.doe.gov) and the genome size is about 4·5 Mbp.

The type strain, PV-4^T (=ATCC BAA-1088^T=DSM 17748^T), was isolated from iron-rich microbial mats at the active, deep-sea, hydrothermal Naha Vent located on the South Rift of Loihi Seamount, Hawaii, in the Pacific Ocean.

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