Shewanella electrodiphila sp. nov., a psychrotolerant bacterium isolated from Mid-Atlantic Ridge deep-sea sediments

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Abstract

Strains MAR441^T and MAR445 were isolated from Mid-Atlantic Ridge (MAR) sediments from a depth of 2,734 m, and found to belong to the genus *Shewanella*. The strains were rod shaped, pigmented, non-motile, and capable of anaerobic growth either by fermentation of carbohydrates or by anaerobic respiration. The strains utilized a variety of electron acceptors, including nitrate and ferric compounds and could utilize peptone when grown anaerobically in a two-chambered microbial fuel cell (MFC), which use carbon cloth electrodes and deliver a stable power output of ~150-200 mW/m^2. The major fatty acids were typical of the genus *Shewanella*, with major components of $C_{13:0}$, iso-$C_{13:0}$, iso-$C_{15:0}$, $C_{16:0}$, $C_{16:1}\omega7c$, $C_{18:1}\omega7c$ and $C_{20:5}\omega3$ fatty acids. The DNA G+C content of strains MAR441^T and MAR445 were 42.4 mol %. 16S rRNA gene sequence analysis indicated that strains MAR441^T and MAR445 were most closely related to *Shewanella olleyana* (sequence similarities 97.9 %). DNA-DNA hybridization demonstrated only 15.6-37.2 % relatedness between strain MAR441^T and the type strains of related *Shewanella* species. Phenotypic characteristics confirmed that these isolates constituted a novel species of the genus *Shewanella*. The type strain of *Shewanella electrodiphila* is MAR441^T (=ATCC BAA-2408^T =DSM24955^T).

Keywords *Shewanella*, Deep-sea psychrotolerant bacterium, Mid-Atlantic Ridge, non-vent sediment

The genus *Shewanella* was first described by MacDonell and Colwell in 1985, by separating it from the genus *Alteromonas* and *Pseudomonas*. At the time of writing, at least 62 members of the genus *Shewanella* have been reported, and they are mostly from aquatic/marine environments. Key features of the genus *Shewanella* are the ability to produce polyunsaturated fatty acids (PUFAs) particularly eicosapentaenoic acid (EPA), and to use various electron acceptors for anaerobic respiration (Nealson & Scott, 2006). High levels of bacterial EPA producers, such as *Shewanella marinintestina*, *S. schlegeliana*, *S. sairae*, *S. pealeana*, *S. benthica*, *S. baltica*, *S. pneumatohori* and *S. waksmanii* were isolated from the intestinal tract of various marine animals, with 15-37 % of their total fatty acids (TFA) as EPA (Amiri-Jami *et al.*, 2006; Hirota *et al.*, 2005; Leonardo *et al.*, 1999; Satomi *et al.*, 2003; Yazawa *et al.*, 1992). However, most of the deep-ocean *Shewanella* species, such as *S. benthica*, *S. abyssi*, *S. kaireitica*, *S. violacea*, *S. peizotolerans* and *S. psychrophila*, produced lower levels of EPA at 2-14 % of their total fatty acids (Delong *et al.*, 1997; Delong & Yayanos, 1986; Deming *et al.*, 1984; Miyazaki *et al.*, 2006; Nogi *et al.*, 1998; Xiao *et al.*, 2007). These deep-sea *Shewanella* species are characterized as high-pressure cold-adapted or mesophilic and pressure-sensitive, which may result in lower amounts of EPA being produced under atmospheric conditions.

Sediment samples were collected between 48° and 54°N using a megacore from a station on the Mid-Atlantic Ridge (MAR) South East of the Charlie-Gibb Fracture Zone (CGFZ) (49°05.40'N - 27°50.22'W) on board the R.R.S James Cook from 13th July to 18th August 2007 as described previously (Reid *et al.*, 2012). The sediments (1 g wet sediment) were subsequently diluted with 4 ml autoclaved seawater (Dove Marine Laboratory), which had been passed through a 0.2 μm-pore-size filter. After

vigorous shaking for 30 s, the sediments were allowed to settle for 5 min before 50 µl was inoculated onto marine agar 2216E (BD Difco™) plates. Plates were incubated both aerobically and anaerobically at 4 and 15 ºC for 15 and 30 days. Random colonies with differing morphologies were isolated and purified by the streak plate technique. Pure strains were stored at -80 ºC in marine broth 2216E (BD Difco™) supplemented with 15% (v/v) glycerol. The extent of PUFA production was ascertained by screening the fatty acid profiles of all isolates. Two bacteria with good EPA production were designated MAR441^T and MAR445.

Cell morphology, cell arrangement, cell size and motility were determined by phase contrast microscopy and electron microscopy using exponentially growing cells (Xiao, et al., 2007; Zhang & Zeng, 2008). The strains showed cellular and colonial morphologies and phenotypic profiles typical of *Shewanella* species. Cells were rod-shaped, Gram-negative, 1.5–4.5 µm in length, 0.4–0.76 µm in diameter, without flagella (Fig. 1). Colonies of strains MAR441^T and MAR445 on marine agar plates were slightly pinkish at the beginning, and then tan-pigmented, butyrous in consistency, smooth, and circular and convex in shape with an entire edge. Colonies of 2-4 mm in diameter were formed following a 2-day incubation at 15 °C. The agar beneath colonies on marine agar became softened, but not liquefied, however colonies became transparent and increasingly mucoid with prolonged incubation. Flooding the agar surface with Lugol's iodine solution revealed hydrolysis zones around the growth, suggesting that the strains have an agarolytic ability. No hydrolysis zone was formed in triacylglycerol agar plates, indicating that the strains are unable to produce lipase. MAR441^T and MAR445 cells were non motile when grown on plates of semi-solid motility test media containing 0.5 % triphenyltetrazolium chloride (TTC), whereas *S.*

japonica KMM 3299^T, *S. pacifica* KMM 3597^T and *S. olleyana* ACEM 9^T showed fuzzy growth (indicated by pink color) away from the line of inoculation, which denoted motility. Generally, flagella-based motility is typical of the genus *Shewanella* (Table 1), though this is not universal as *S. putrefaciens* has been reported as non-motile (Yilmaz *et al.*, 2007). MAR441^T and MAR445 showed no flagella, only fimbriae on the cell surface. Colonies of N-methyl-N′-nitro-N-nitrosoguanidine (NTG) mutants produced from MAR441^T and designated strains A4 and A13 were grown on marine agar and were 3-5 mm in diameter, tan-pigmented, opaque, dull, with dentate margins or undulate edges, with the central rough area loosely attached to the agar, making it easily moved along the agar surface (Supplementary Fig. S1). Treatment by NTG suggests that fimbriae may contribute to biofilm formation on a solid surface in the wild type cells, according to the two mutants which appeared to lack fimbriae.

Strains MAR441^T and MAR445 were psychrotolerant and euryhaline. Growth was observed between 4 and 30 °C with best growth at 15-20 °C (Supplementary Fig. S2). Growth on agar media at 4 °C was slower (2-4 d) and less prolific than that at 10-25 °C (1 d). No growth was observed for either strain above 30 °C. Weak growth was observed at 30 °C in marine broth. The effect of different concentrations of sodium chloride (supplemented to the synthetic ZoBell broth (Bacto peptone, 5 g; yeast extract, 1 g; ferric citrate, 0.1 g; distilled water to 1 l) with various concentrations [0, 1, 3, 5, 7, 9, 10 and 15% (w/v)] of NaCl (Sigma)) were investigated (ZoBell, 1946). The strains required Na^+ for growth and grew between 0.05 and 0.7 M NaCl (0.3-7 %), with best growth at 0.05-0.5 M NaCl (0.3-3 %). No growth was detected at 8 % NaCl. The strain could grow normally in medium supplemented with only K^+ or Fe^{3+}, but poorly in medium supplemented with only Zn^{2+} or Ca^{2+}.

Anaerobic respiration was examined in a defined medium (Myers & Nealson, 1990) containing an electron acceptor [ferric citrate, fumarate, amorphic ferric oxide, trimethylamine N-oxide (TMAO), nitrite, thiosulfate or selenite] at appropriate concentrations using lactate as an electron donor. Anaerobic growth was carried out at 20 °C in a 2.5 l anaerobic container system (GasPak™ EZ, BD, Maryland). Media controls prepared without an electron acceptor were also used according to published methods (Coates *et al.*, 1999; Skerratt *et al.*, 2002). Strains MAR441^T and MAR445 were facultatively anaerobic. Respiratory anaerobic growth was supported on a variety of electron acceptors when sodium lactate or sodium acetate was used as the electron donor. Electron acceptors used included Fe(III) (50 mM ferric citrate or 10 mM amorphous ferric oxides), 10 mM sodium nitrate, 25 mM sodium fumarate and sodium nitrite (5 mM) and sodium sulfite (10 mM) also supported growth.

Microbial fuel cells (MFCs) were constructed and electrochemical measurements were carried out as described previously (Zhang *et al.*, 2012). Strains could grow well anaerobically in peptone-fed two-chamber MFCs when they were cultivated in a fresh anaerobic growth medium with 10 mM peptone as the electron donor. Generally, the microbial fuel cells exhibited a lag phase (about 2 days) before voltage started to increase. As shown in Supplementary Fig. S3, the voltage output produced by one of the microbial fuel cells inoculated with MAR441^T and MAR445, delivered a stable power output of ~150-200 mW/m^2 for 6 days, and then decreased gradually, probably due to peptone depletion. The control microbial fuel cell remained sterile and did not show voltage increase. The power density produced by strains MAR441^T was higher than MAR445, both of which were competitive compared to *S. oneidensis* MR-1

(Watson & Logan, 2010). Appendages of *S. oneidensis* have been implicated in electricity conduction (El-Naggar *et al.*, 2010; Gorby & Beveridge, 2005; Reguera *et al.*, 2005). *Shewanella putrefaciens* was also reported to transfer electrons directly to an electrode, through outer membrane cytochromes or through the reduction of redox mediators (quinones and quinolines) secreted by the bacteria (Kim *et al.*, 2002; Lovley, 2006; von Canstein *et al.*, 2008). The electron transfer mechanisms that strains MAR441^T and MAR445 employ would presumably similar to those used by *S. putrefaciens* due to the lack of flagella.

Phenotypic properties of strains MAR441^T and MAR445 such as the utilization of carbon and energy sources, nitrate reduction, catalase and oxidase activities, gelatin liquefaction, lysine decarboxylase activity and the ability to hydrolyse starch, alginate and casein were characterized using standard procedures (Smibert & Krieg, 1994). Additional phenotypic characteristics were performed with API-NE and API 20E test strips (bioMérieux, UK) (Makemson & Hastings, 1979). The strains are able to utilize D-glucose, D-gluconate and maltose as carbon sources. The strains are oxidase- and catalase-positive, haemolytic, produce esterase (Tween 20, 40, 80) and proteinase. Arginine dihydrolase and lysine decarboxylase are not observed. H_2S is formed from thiosulfate anaerobically. Indole is not formed from L-tryptophan. The Voges–Proskauer test is negative. The strains do not utilize D-galactose, D-fructose, N-acetylglucosamine, succinate, D-mannose, lactose, propionic acid, fumarate or L-tyrosine, triacylglycerol, cellulose, chitin, dextran, casein, elastin, DNA or uric acid.

The DNA G+C content of strains MAR441^T and MAR445 were 42.4 mol %, which was determined by thermal denaturation (Sly *et al.*, 1986), using a Lambda Bio 20

UV/Visible spectrophotometer (Applied Biosystems, Cheshire UK). Morphological, physiological and biochemical characteristics of strains MAR441^T and MAR445 are presented in Table 1 and in the species description.

For lipid analysis, cells were harvested in late exponential phase at various temperatures, centrifuged immediately and lyophilized before use. Extraction of total lipids, fractionation, and fatty acid composition by GC-MS followed previous published methods (Komagata & Suzuki, 1987; Christie, 1989). Analysis of the lipid components were conducted on thin-layer chromatography (TLC) with silica gel plates (Silica gel 60 F254, Merck) developed in $CHCl_3$/MeOH/CH_3COOH/H_2O (85:15:10:3.5, v/v/v/v) as published previously (Nichols *et al.*, 1997). Good yield of total lipid was obtained from the dry cell mass (10.3 %), with 82% phospholipids and 18% neutral lipids respectively. Phosphatidyl ethanolamine (PE) was the dominant lipid class in phospholipids (50 %) followed by phosphatidyl glycerol (PG) (40 %). About 5 % of diphosphoglyceride (DPG) and 3 % of lysophosphatidylethanolamine (LPE) were also detected with some unidentified phospholipids (2 %) (Supplementary Fig. S4). The cellular fatty acid profile of strain MAR441^T contained large amounts of straight-chain (saturated and unsaturated), branched-chain and hydroxyl fatty acids; the complete fatty acid composition is given in Supplementary Table S1. The major fatty acids of strains MAR441^T and MAR445 were $C_{13:0}$, iso-$C_{13:0}$, iso-$C_{15:0}$, $C_{16:0}$, $C_{16:1}\omega7c$, $C_{18:1}\omega7c$ and $C_{20:5}\omega3$ acids (15 % of TFA) at 10 ºC. MAR441^T produced 25-30% higher EPA than MAR445 at lower temperatures. The fatty acid profiles of both strains was similar to those of related *Shewanella* species, such as *S. pacifica, S. olleyana, S. japonica and S. frigidimarina* (Bowman *et al.*, 1997b; Ivanova *et al.*, 2001; Skerratt, et al., 2002; Ivanova *et al.*, 2004).

The fatty acid composition of MAR441^T exhibited changes in response to growth temperature. Growth at temperatures within or below the optimum resulted in an increased percentage of PUFAs (27.8 % at 4 °C versus 2 % at 25 °C), and a decreased proportion of short-chain saturated components (Supplementary Table S1). Both the percentage and the quantitative level of EPA decreased markedly at growth temperatures above the growth optimum (21.4 % at 4 °C versus 0.6 % at 25 °C), indicating that PUFA may play a critical role in the modulation of membrane fluidity and the homeostatic maintenance of membrane viscosity (Russell & Nichols, 1999). In addition, as the growth temperature increased, MAR441^T also demonstrated a novel response with regard to fatty acid composition, resulting in an increase in the percentage of $C_{13:0}$ and iso-$C_{15:0}$ with a corresponding decrease of $C_{16:1}\omega 7c$ and $C_{18:1}\omega 7c$. The values of average chain length (ACL) (from 16.29-14.67) and quantitative level of EPA decreased with increasing growth temperature (24–0.2 mg g^{-1} cells dry weight) at all growth temperatures from 4-25 °C. When strain MAR441^T was cultured anaerobically in marine broth at 15 °C, the MUFA content increased to 56 % of the TFAs, mainly through accumulation of $C_{16:1}\omega 7c$ and $C_{18:1}\omega 7c$, and by decreasing the content of SCFAs, BCFAs and PUFAs, and with only 1.4 % EPA present.

The presence of EPA is an important physiological and descriptive component that allows differentiation between *Shewanella* species (Skerratt, et al., 2002). Strain MAR441^T is one of the highest bacterial producers of EPA by proportion and/or concentration compared to other high EPA-producing *Shewanella* species isolated from polar, deep sea and estuarine environments (Bowman *et al.*, 1997a; Bowman, et

al., 1997b; Nichols, et al., 1997; Kato & Nogi, 2001; Skerratt, et al., 2002). The proportion of unsaturated fatty acids, such as $C_{16:1}\omega 7c$, $C_{18:1}\omega 7c$ and $C_{20:5}\omega 3$, varied inversely with temperature due to changes in production of other saturated fatty acids, e.g. $C_{13:0}$ and iso-$C_{15:0}$, for strain MAR441^T (Supplementary Table S1), indicating that temperature remains the primary controlling factor in PUFA synthesis in these bacterial isolates. Production of EPA by some bacteria increases as temperature decreases, leading to the hypothesis that these molecules may be important for growth at low temperatures (Amiri-Jami, et al., 2006; Delong & Yayanos, 1986; Valentine & Valentine, 2004). Cells must cope with decreases in temperature by modulating the composition of their lipid membrane, which can crystallize or enter nonbilayer phases at low temperatures (Russell & Nichols, 1999). High content of unsaturated fatty acids was observed in MAR441^T anaerobic cultures, probably due to the activation of an oxygen-independent (anaerobic) pathway catalysed by a fatty acid synthetase (Yano *et al.*, 1998). A shortage of oxygen can often occur in deep-sea environments where reduced sulfur compounds or other metals are supplied constantly as final electron acceptor for microbes in their respiratory pathways (Woulds *et al.*, 2007).

Genomic DNA was extracted and purified according to published methods (DiLella & Woo, 1987), and used as a template for PCR amplification of the 16S rRNA gene fragments with primers 27F (5`-AGAGTTTGATCMTGGCTCAG-3`) and 1492R (5`-TACGGYTACCTTGTTACGACTT-3`), as described previously (DeLong, 1992; Rainey *et al.*, 1996). The PCR products were fully, bidirectionally sequenced by Eurofins MWG Operon using the PCR amplification primer sequences after purification with PureLink™ PCR Purification Kit (Invitrogen Ltd, Paisley, U.K) following the manufacturer's protocols. These gene sequences were obtained using

DNAMAN (Version 5.1) and compiled with 16S rRNA gene sequences of related taxa obtained from GenBank. Multiple alignment was performed using CLUSTAL_X (Thompson *et al.*, 1997). Evolutionary distances were calculated using the Kimura two-parameter model (Kimura, 1980). A phylogenetic tree was constructed using the neighbour-joining method (Saitou & Nei, 1987) using MEGA4 software (Tamura *et al.*, 2007) and maximum likelihood with PHYLIP (Felsenstein, 1981). Bootstrap values were calculated using 1000 replications (Felsenstein, 1985). DNA–DNA hybridization studies were conducted by the non-radioactive method described by (Ziemke *et al.*, 1998). DIG-11-dUTP and biotin-16-dUTP were used for double-labelling DNA using the Boehringer Mannheim nick-translation kit (Boehringer Mannheim). Hybridization of each sample was examined with five replications; the highest and lowest values for each sample were excluded, and the mean of the three remaining values was reported as the result.

The nearly complete sequence of the 16S rRNA gene sequences generated for MAR441^T and MAR445 (1480 bp) were determined, and deposited in GenBank under accession numbers FR744784 and FR744787 respectively. 16S rRNA gene sequence analysis showed that strains MAR441^T and MAR445 exhibited a sequence similarity of 92.6-97.9 % to the type strains of the 61 other *Shewanella* species. Among these, four type strains showed 16S rRNA gene sequence similarity of more than 97 % to strains MAR441^T. Strain MAR441^T and MAR445 were most closely related (97.9 %) to *S. pacifica* KMM 3597^T (AF500075), which was isolated from Sea of Japan, Pacific Ocean with production of EPA (Ivanova, et al., 2004) (Fig. 2); 97.9 % to *S. olleyana* strain ACEM 9^T (NR_025123) isolated from a temperate estuary with high levels of EPA production (Skerratt, et al., 2002); 97.9 % to *S. japonica* KMM 3299^T (NR_025012) (Ivanova, et al., 2001) and 97.5 % to *S. donghaensis* strain LT17^T

(AY326275) isolated from deep-sea sediments with high production of PUFAs (Yang *et al.*, 2007), and 95.5 % to other type strains *S. arctica* 40-3T (AJ877256) (unpublished), *S. baltica* OS185 (AJ000216) (Ziemke, et al., 1998), *S. massilia* (AJ006084) (Dos Santos *et al.*, 1998), *S. gaetbuli* isolate UL19 (AM180742) (Yoon *et al.*, 2004). Strains MAR441^T and MAR445 were phylogenetically close and affiliated with strain ACEM 9^T (NR_025123) in the same clade on the phylogenetic tree, although they only shared a 16S rRNA gene sequence similarity of 97.9 %. Therefore we placed these strains in the genus *Shewanella*, and they may be considered as a separate species due to the low similarity and phylogenetic differences to other *Shewanella* species. However, an important criterion remains DNA–DNA relatedness. DNA–DNA hybridization indicated that the relatedness of DNA from strain MAR441^T and the nine close relatives was only 11.2-37.2 %, well below the 70 % threshold for the definition within a species (Stackebrandt & Goebel, 1994). 16S rRNA gene and DNA–DNA relatedness results are summarized in Supplementary Table S2.

Among the most phylogenetically related *Shewanella* type species, strain MAR441^T and MAR445 (=DSM24934) can be distinguished easily from *S. pacifica* by its capacity to utilize D-gluconate, its inability to secrete DNase, lipase and grow at 30 °C; from *S. olleyana* by utilizing D-Glucose but not sucrose, secreting gelatinase and not growing at 30 °C; from *S. japonica* by metabolizing D-Gluconate but not N-Acetylglucosamine, not secreting DNase and growing at 0 % NaCl or 35 °C. A lack of motility and the inability to produce lipase also clearly differentiated strain MAR441^T and MAR445 from other *Shewanella* species. Therefore, on the basis of phenotypic, chemotaxonomic and phylogenetic data, combined with DNA–DNA

relatedness, strains MAR441^T and MAR445 represent a distinct species within the genus *Shewanella*, for which the name *Shewanella electrodiphila* sp. nov. is proposed.

Description of *Shewanella electrodiphila* sp. nov.

Shewanella electrodiphila (N.L. n. *electrodum* electrode; N.L. adj. *philus* –a -um (from Gr. adj. philos -ê -on), friend, loving; N.L. fem. adj. *electrodiphila*, loving electrodes)

Cells are rod-shaped, Gram-negative, 1.5-4.5 µm in length, 0.4-0.8 µm in diameter and have no flagella. Temperature range for growth is 0-30 °C. Optimum temperature for growth was 15 °C. Requires Na^+ ions for growth (minimum 0.05 M, optimal 0.1-0.5 M, maximum 1.2 M). Oxidase- and catalase positive, haemolytic. Facultatively anaerobic chemoheterotroph. Can grow anaerobically by respiration using ferric citrate, fumarate, amorphic ferric oxide, nitrate, thiosulfate, trimethylamine *N*-oxide (TMAO) and anthraquinone-2,6-disulfonate (AQDS) as electron acceptors and lactate as an electron donor. Carbohydates are fermented with the production of electricity. Nitrate is reduced to nitrite in the presence of oxygen. Cells are able to secrete gelatinase. Ornithine decarboxylase, arginine dihydrolase and lysine decarboxylase are not observed. Hydrolyses dextrin, starch, cellulose, agarose, Tween 40, 60 and 80, L-arabinose, D-cellobiose, D-glucose, D-gluconate, maltose, c-hydroxybutyric acid, α-ketobutyric acid, aketoglutaric acid, α-ketovaleric acid, D-saccharic acid, succinic acid, L-alanine, L-alanyl-glycine, L-aspartic acid, L-glutamic acid, glycyl-L-aspartic acid, glycyl-L-glutamic acid, L-ornithine, L-proline, L-pyroglutamic acid, L-serine, L-threonine, L-leucine, DL-carnitine, c-aminobutyric acid, urocanic acid, putrescine, pyruvate, 2-aminoethanol, 2,3-butanediol, glycerol, DL-α-glycerol phosphate, α-D-

glucose 1-phosphate and D-glucose 6-phosphate, but not D-galactose, D-fructose, N-acetylglucosamine, succinate, D-mannose, lactose, propionic acid, fumarate or L-tyrosine, triacylglycerol, cellulose, chitin, dextran, casein, elastin, DNA or uric acid. Agarolytic activity is positive. Production of H_2S from L-cysteine is negative (from API 20E test strip results) but H_2S was formed from thiosulfate anaerobically. Indole is not formed from L-tryptophan. Voges-Proskauer test is negative. Forms tan-pigmented, butyrous in consistency, smooth, and circular and convex in shape with an entire edge colonies 2-4 mm in diameter following 2 d incubation at 15 °C. Major fatty acids are $C_{13:0}$, iso-$C_{13:0}$, iso-$C_{15:0}$, $C_{16:0}$, $C_{16:1}\omega7c$, $C_{18:1}\omega7c$ and $C_{20:5}\omega3$ (Supplementary Table S1). The DNA G+C content of the type strain is 42.4 mol %. Based on 16S rDNA nucleotide sequence analysis, the species belongs to the family *Alteromonadaceae*, order *Alteromonadales* and class *Gammaproteobacteria*. The type strain is MAR441^T (ATCC BAA-2408^T =DSM24955^T) isolated from Mid-Atlantic Ridge (MAR) "non-vent" sediments at a depth of 2,734 m. The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain MAR441^T is FR744784.

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Figure legends

Fig. 1 Transmission electron microscopy (Bar represents 500 nm) of negatively-stained cells of *Shewanella electrodiphila* strain MAR441^T.

Fig. 2 Phylogenetic tree based on 16S rRNA gene sequences of strains MAR441^T and closely related *Shewanella* type species constructed from maximum-likelihood distances clustered by neighbour-joining method using the MEGA software package. 1000 trials of bootstrap analysis were used to provide confidence estimates for phylogenetic tree topologies. Bars, 0.005 nucleotide substitution per site.

Table 1 Characteristics that differentiate *Shewanella electrodiphila* from phylogenetically related species.

Strains: 1, *S. electrodiphila* sp. nov. MAR441^T and MAR445; 2, *S. pacifica* KMM 3597^T (Ivanova, et al., 2004); 3, *S. olleyana* ACEM 9^T (Skerratt, et al., 2002); 4, S. *japonica* KMM 3299^T (Ivanova, et al., 2001); 5, *S. frigidimarina* ACAM 591^T (Bowman, et al., 1997b); 6, *S. baltica* NCTC 10735^T (Brettar *et al.*, 2003); 7, *S. donghaensis* LT17^T (Yang, et al., 2007); 8, *S. gaetbuli* TF-27^T (Yoon, et al., 2004); 9, *S. livingstonensis* LMG 19866^T (Bozal *et al.*, 2002) and *S. putrefaciens* (Venkateswaran *et al.*, 1999). -, test is positive; +, test is negative; ND, data not available. None of the strains produced indole or acetoin. Data of strains from columns 1- 6 were achieved in this study in parallel, whereas data of strains from columns 7-10 were obtained from indicated references; in column 10, 40-90% of strains giving a positive reaction indicated as '+'.

Phenotypic characteristic	1	2	3	4	5	6	7	8	9	10
Requires Na^+ ions for growth	+	+	+	-	-	+	+	+	+	-
Growth in NaCl at:										
0 %	-	-	-	+	+	+	-	-	+	+
3 %	+	+	+	+	+	+	+	+	+	+
6 %	+	+	+	-	+	+	-	-	-	+
Growth at 4 °C	+	+	+	-	+	+	+	-	+	+
Growth at 30 °C	-	+	+	+	-	-	-	+	-	
Growth at 35 °C	-	-	-	+	-	-	-	+	-	+
Ornithine decarboxylase	-	-	-	-	-	+	-	-	+	-
NO3 → NO2 (+O2)	+	+	+	+	+	+	+	-	+	+
Production of										
DNase	-	+	-	+	+	+	ND	ND	ND	ND
Amylase	+	+	+	+	-	-	-	+	-	-
Lipase	-	+	+	+	+	+	+	+	+	-
Gelatinase	+	+	-	+	+	+	+	+	+	-
Chitinase	-	-	-	-	-	-	-	ND	-	-
Utilization of:										
D-Glucose	+	+	-	+	+	+	-	-	+	-
N-Acetylglucosamine	-	-	-	+	+	+	-	-	-	ND
Maltose	+	+	-	+	-	+	-	-	-	+
Sucrose	-	-	+	-	+	+	-	-	-	+
D-Gluconate	+	-	+	-	-	+	-	-	-	+
DL-Lactate	-	-	-	-	+	-	-	-	+	+
Succinate	-	-	-	-	+	+	-	-	ND	+
Citrate	-	-	-	-	+	+	-	-	-	-
EPA synthesis	+	+	+	+	+	-	+	-	-	-
Electricity production	+	ND	ND	ND	ND	ND	ND	ND	ND	+
Motile	-	+	+	+	+	+	+	+	+	+
Mol % GC	42.4	43-44	44	43-44	45	46	38.8	42	41	43-47