Shewanella profunda sp. nov., isolated from deep marine sediment of the Nankai Trough

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A novel piezotolerant, mesophilic, facultatively anaerobic, organotrophic, polarly flagellated bacterium (strain LT13a^T) was isolated from a deep sediment layer in the Nankai Trough (Leg 190, Ocean Drilling Program) off the coast of Japan. This organism used a wide range of organic substrates as sole carbon and energy sources: pyruvate, glutamate, succinate, fumarate, lactate, citrate, peptone and tryptone. Oxygen, nitrate, fumarate, ferric iron and cystine were used as electron acceptors. Maximal growth rates were observed at a hydrostatic pressure of 10 MPa. Hydrostatic pressure for growth was in the range 0·1–50 MPa. Predominant cellular fatty acids were 16 : 1ω7*c*, 15 : 0 iso, 16 : 0 and 13 : 0 iso. The G+C content of the DNA was 44·9 mol%. On the basis of 16S rRNA gene sequences, strain LT13a^T was shown to belong to the γ-*Proteobacteria*, being closely related to *Shewanella putrefaciens* (98 %), *Shewanella oneidensis* (97 %) and *Shewanella baltica* (96 %). Levels of DNA homology between strain LT13a^T and *S. putrefaciens*, *S. oneidensis* and *S. baltica* were $<$20 %, indicating that strain LT13a^T represents a novel species. Genetic evidence and phenotypic characteristics showed that isolate LT13a^T constitutes a novel species of the genus *Shewanella*. Because of the deep origin of the strain, the name *Shewanella profunda* sp. nov. is proposed, with LT13a^T (=DSM 15900^T=JCM 12080^T) as the type strain.

Very few micro-organisms have been recovered from deep subseafloor sediments. *Desulfovibrio profundus* was the first piezophilic species to be isolated from deep marine deposits collected in the Japan Sea (Bale *et al.*, 1997). Recently, a novel methanogenic species, *Methanoculleus submarinus*, was isolated from Nankai Trough sediment (Mikucki *et al.*, 2003). The genus *Shewanella* comprises Gram-negative, facultative anaerobes that belong to the γ-*Proteobacteria* (MacDonell & Colwell, 1985) and is typically one of the deep-sea bacterial genera (DeLong *et al.*, 1997). This genus includes psychrophilic and mesophilic species originating mainly in freshwater and marine habitats (Jensen *et al.*, 1980; Semple & Westlake, 1987; Myers & Nealson, 1988; Brettar & Hölfe, 1993; Nogi *et al.*, 1998; Venkateswaran *et al.*, 1998; Ivanova *et al.*, 2003). Thermophilic *Shewanella*-like bacteria were recently cultivated (Ghosh *et al.*, 2003). The genus *Shewanella* was divided into two subgenera on the basis of phylogenetic structure, growth properties in relation to pressure, and polyunsaturated fatty acid production (Kato & Nogi, 2001).

The novel isolate was obtained from a sediment sample that was collected 4·15 m below the seafloor at a water depth of 4790·7 m in the Pacific Ocean at the Nankai Trough offshore from Japan (site 1173: 32° 14·7′ N 135° 1·5′ E) between 28 and 07 June 2000 on Leg 190 of the Ocean Drilling Program (site 1173). All details of the

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of *Shewanella profunda* LT13a^T is AY445591.

An electron micrograph and details of the growth behaviour and fatty acid and polar lipid compositions of strain LT13a^T are available as supplementary material in IJSEM Online.

environmental conditions, sampling and subsampling procedures have been reported previously (Cragg *et al.*, 1992a, b; Parkes *et al.*, 1995; Moore *et al.*, 2001).

Enrichment cultures were conducted anaerobically in medium containing the following (l^{-1}): 30 g sea salts (Sigma), 2 g yeast extract (Difco), 2 g peptone (Difco) and 0·5 mg resazurine. The medium was adjusted to pH 7·0 and autoclaved. Sterile additions were as follows: 10 ml 1 M $NaHCO_3$, 1 g $CH_3COONa.3H_2O$, 1 ml trace elements solution (Widdel & Bak, 1992), 1 ml vitamin solution (Widdel & Bak, 1992), 1 ml 0·01 % (w/v) thiamine (Widdel & Bak, 1992), 1 ml 0·005 % (w/v) vitamin B_{12} (Widdel & Bak, 1992), 1 ml growth factor (Pfennig *et al.*, 1981), 0·3 g KH_2PO_4, 0·5 mg sodium selenate and 0·5 mg sodium tungstate. The final pH of the medium was about 6·8. Medium was distributed into vials and initial vial preparation involved sequential evacuation and gassing with H_2/CO_2 (20 : 80, v/v) at 200 kPa. The medium was then reduced by using 0·5 g sodium sulphide l^{-1}. Sediment suspensions were inoculated (10 % final, v/v) into the medium and incubated at 25 °C. Growth of motile, rod-shaped micro-organisms was observed in enrichment cultures after 2 days incubation at 25 °C. Positive cultures were transferred at least three times at 10 % inoculum into fresh medium and spread on plates containing 1 % (w/v) agar (Difco). On agar plates, colonies were circular and transparent; they were 1–2 mm in diameter after 3–5 days incubation at 30 °C. Old cultures were slightly pinkish. One isolate was purified and designated LT13a^T (=DSM 15900^T=JCM 12080^T).

Isolate LT13a^T was grown routinely in the following medium (YP) containing the following (l^{-1}): 23 g NaCl, 3 g $MgCl_2$, 0·15 g $CaCl_2$, 0·5 g KCl, 4 g Na_2SO_4, 1 g yeast extract (Difco), 2 g peptone (Difco), 3·6 g PIPES buffer and 0·25 g KH_2PO_4. The pH of the medium was adjusted to 7·2 before autoclaving. Unless indicated otherwise, cultures were incubated aerobically at 30 °C and atmospheric pressure. Stock cultures of isolate LT13a^T were stored in culture medium at 4 °C. For long-term storage, pure cultures were stored at −80 °C in the same medium containing 20 % (v/v) glycerol.

The isolate was tested for Gram-staining, cell size, morphology [using phase-contrast microscopy (model BH2; Olympus) and electron microscopy (JEM 100 CX II; JEOL) after negative staining with 2 % (w/v) uranyl acetate] and for cytochrome oxidase and catalase (3 % H_2O_2).

Nankai Trough strain LT13a^T appeared as single rods that were approximately 0·5–0·7 × 2·5–3·5 μm (see Supplementary Fig. A in IJSEM Online) during the exponential phase of growth. Cells were motile by means of a single polar flagellum (see Supplementary Fig. A in IJSEM Online). They did not form endospores and spores were not produced in any phase of growth under any growth conditions tested. Cells stained Gram-negative and were oxidase-positive and catalase-negative.

To determine the optimum temperature, pH and NaCl concentration, cells were grown in YP medium. Growth at the following temperatures was tested: 4, 10, 15, 20, 25, 30, 37 and 40 °C. Growth at the following NaCl concentrations was tested: 0, 5, 10, 15, 20, 30, 40, 50, 60, 70, 80 and 90 g l^{-1}. Growth at the following pH values was tested: 3·0, 4·0, 5·0, 5·5, 6·0, 6·5, 7·0, 7·5, 8·0, 8·5, 9·0 and 10·0, as described by Widdel & Bak (1992). The effect of the following hydrostatic pressures on growth rates of strain LT13a^T was determined: 0·1, 10, 20, 30, 40 and 50 MPa at 30 °C, as previously described (Kato *et al.*, 1995).

Utilization of carbohydrates (listed in Table 1) as catabolic substrates was tested aerobically in a modified YP liquid medium from which carbon sources were omitted. The following carbon sources were tested: gelatin, maltose, glucose, fructose, galactose, arabinose, cellobiose, lactose, mannitol, mannose, rhamnose, sorbitol, sucrose, xylose, yeast extract, peptone and tryptone (each at 0·2 %, w/v); formate, pyruvate, propionate, butyrate, valerate,

Table 1. Differential phenotypic characterization of *S. profunda* strain LT13a^T in comparison with related *Shewanella* species

1, *S. profunda* LT13a^T; 2, *S. putrefaciens* (*n*=10); 3, *S. baltica* (*n*=5); 4, *S. oneidensis* (*n*=5). Data are from this study, Venkateswaran *et al.* (1999) and Brettar *et al.* (2002). +, Positive reaction; (+), weak reaction; −, no reaction. Numbers indicate percentages of strains that test positive. NA, No data available.

Characteristic	1	2	3	4
Optimal growth temp. (°C)	25–30	25–35	20–25	25–35
Growth at/in:				
4 °C	+	+	+	20
37 °C	+	+	−	+
40 °C	−	−	−	60
0 % NaCl	+	+	+	+
6 % NaCl	+	100	+	40
Production of:				
Amylase	+	0	−	0
Gelatinase	+	0	+	100
Acetoin	+	−	−	−
Ornithine decarboxylase	+	−	−	−
Utilization of:				
D-Galactose	−	100	−	80
Sucrose	−	40	100	0
Maltose	(+)	60	100	0
Lactose	−	30	−	0
Cellobiose	−	−	100	NA
D-Sorbitol	(+)	0	NA	0
Succinate	+	60	−	100
Fumarate	+	60	−	80
Citrate	+	0	100	0
DL-Malate	−	0	75	0
DL-Lactate	+	60	NA	100

isovalerate, glutamate, caproate, caprylate, malate, succinate, isobutyrate, heptanoate, 2-methylbutyrate, 3-methylbutyrate, monomethylamine, acetate, lactate, propanol, ethanol and methanol (each at 10 mM, v/v). The strain was also characterized by using the API 20E identification system (bioMérieux) at 30 °C, according to the manufacter's instructions.

Anaerobic growth tests were performed in a modified YP liquid medium prepared with 5 g NaCl l^{-1} and without Na_2SO_4, with 0·02 % (w/v) yeast extract to stimulate growth. L-Cystine (10 g l^{-1}), CO_2 (100 kPa), thiosulphate, tetrathionate, sulphate, sulphite, succinate, nitrate, nitrite, molybdate, glycine, fumarate, trimethylamine-*N*-oxide, manganese dioxide and ferric iron were tested as electron acceptors at final concentrations of 2 and 10 mM, with 10 mM lactate as the electron donor and carbon source. Sulphur reduction was also tested in overlay gel agar by using the modified techniques of Moser & Nealson (1996) and Lucas *et al.* (2002). Preparation of amorphous Fe(III) oxide and Fe(II) determination were performed as described earlier (Slobodkin *et al.*, 1999). Compounds were considered to be electron acceptors if they supported growth of strain LT13a^T on defined marine medium. Strain LT13a^T used oxygen, nitrate, ferric iron, fumarate and cystine as terminal electron acceptors with lactate as the electron donor, but did not use trimethylamine-*N*-oxide, DMSO, manganese dioxide, thiosulphate, sulphite, tetrathionate, sulphate, elemental sulphur, molybdate, nitrite, succinate, glycine or carbon dioxide.

Fermentation of various carbohydrates was tested by using modified YP medium from which carbon sources had been omitted. The following carbohydrates were tested: glucose, arabinose, cellobiose, fructose, galactose, lactose, maltose, mannitol, mannose, rhamnose, xylose, sucrose, sorbitol, Casamino acids, tryptone, peptone and yeast extract (each at 0·2 %, w/v), and pyruvate, lactate and malate (each at 10 and 30 mM).

Positive cultures were transferred twice (10 % inoculum) into the test media to confirm growth and were compared with the control cultures without the added carbon or electron acceptor. Growth observed within 48–72 h was considered as a positive result. The organic acid metabolic end products that were produced during fermentation of pyruvate were analysed by HPLC (Alliance 2690; Waters) as described by Wery *et al.* (2001).

For nitrite and ammonium analysis, cultures were grown anaerobically in solidified YP medium supplemented with 5 mM KNO_3 and incubated at 37 °C. Reduction of nitrate and nitrite was determined by using the indophenol blue method of Koroleff (1969) and Solorzano (1969). H_2S production was evaluated by adding 500 µl of a solution of $CuSO_4$ (5 mM) and HCl (50 mM) to a 250 µl culture grown at 30 °C. The dark-brown precipitate, demonstrating the presence of sulphide, was compared with uninoculated medium incubated under the same conditions.

Respiratory lipoquinones and polar lipids were extracted from 100 mg freeze-dried cell material by using the two-stage method described by Tindall (1990a, b). Respiratory quinones were extracted by using methanol/hexane (Tindall, 1990b) and polar lipids were extracted by adjusting the remaining methanol/0·3 % aqueous NaCl phase (containing the cell debris) to give a chloroform/methanol/0·3 % aqueous NaCl mixture (1 : 2 : 0·8, by vol.). The extraction solvent was stirred overnight and the cell debris was pelleted by centrifugation. Polar lipids were recovered into the chloroform phase by adjusting the chloroform/methanol/0·3 % aqueous NaCl mixture to a ratio of 1 : 1 : 0·9 (by vol.).

Respiratory lipoquinones were separated into their different classes (menaquinones and ubiquinones) by TLC on silica gel (Macherey-Nagel; part no. 805023), using hexane/tert-butylmethylether (9 : 1, v/v) as solvent. UV-absorbing bands corresponding to menaquinones or ubiquinones were removed from the plate and further analysed by HPLC. This step was carried out on LDC Analytical HPLC equipment (Thermo Separation Products) fitted with a reverse-phase column (Macherey-Nagel; 2 mm × 125 mm, 3 µm, RP_{18}) using methanol as the eluent. Respiratory lipoquinones were detected at 269 nm.

The major respiratory quinones present comprised both ubiquinones and menaquinones. The major ubiquinones present were ubiquinone 7 (Q_7) (21 %) and Q_8 (79 %). The major menaquinones present were menaquinone 7 (MK_7) (24 %), MK_8 (1·7 %), monomethylmenaquinone 7 (MMK_7) (70 %) and MMK_8 (4·3 %). The presence of ubiquinones, menaquinones and methylmenaquinones has been reported in members of the genus *Shewanella*. The presence of Q_7 and Q_8 as the dominant ubiquinones, but also MK_7 and MMK_7 among the naphthoquinones, has previously been reported only in members of the genus *Shewanella* (Akagawa-Matsushita *et al.*, 1992; Nogi *et al.*, 1998; Venkateswaran *et al.*, 1999; Bozal *et al.*, 2002; Satomi *et al.*, 2003).

Polar lipids were separated by two-dimentional silica-gel TLC (Macherey-Nagel; part no. 818135). The first direction was developed in chloroform/methanol/water (65 : 25 : 4, by vol.); the second was developed in chloroform/methanol/acetic acid/water (80 : 12 : 15 : 4, by vol.). Total lipid material and specific functional groups were detected by using dodecamolybdophosphoric acid (total lipids), Zinzadze reagent (phosphate), ninhydrin (free amino groups), periodic acid–Schiff stain (α-glycols), Dragendorff's reagent (quaternary nitrogen) and anisaldehyde/sulphuric acid (glycolipids).

Fatty acids were analysed as their methyl ester derivatives, which were prepared from 10 mg dry cell material. Cells were subjected to differential hydrolysis to detect ester-linked and non-ester-linked (amide-bound) fatty acids (B. J. Tindall, unpublished results). Fatty acid methyl esters were analysed by GC using a 0·2 µm × 25 m non-polar

capillary column and flame-ionization detection. The run conditions were as follows: injection and detector port temperature, 300 °C; inlet pressure, 60 kPa; split ratio, 50 : 1; injection volume, 1 μl; temperature program, 130–310 °C at a rate of 4 °C min^{-1}.

The major polar lipids present were phosphatidylethanolamine and phosphatidylglycerol (see Supplementary Fig. C in IJSEM Online). Virtually no data are available on the polar lipid composition of *Shewanella* species, although unpublished work (G. Stöcklmair & B. J. Tindall) indicates that the presence of phosphatidylethanolamine and phosphatidylglycerol as the main phospholipids is not atypical of members of this genus. Furthermore, it would appear that both of these compounds resolve into subspots in the two-dimensional thin-layer chromatograms. This is in contrast to the polar lipids of *Escherichia coli*, for example, where the dominant compounds are also phosphatidylglycerol and phosphatidylethanolamine, but do not resolve into two spots (B. J. Tindall, unpublished results).

The fatty acids comprised straight-chain (saturated and unsaturated), branched-chain and hydroxy fatty acids (see Table 2 and Supplementary Table, available in IJSEM Online). These were predominantly 3-OH fatty acids, some of which were presumably amide-bound. Comparison with the fatty acid composition published previously indicates that the overall pattern is typical of members of this genus that have been examined to date. However, authors use different methods of analysing the fatty acids, so the results cannot be compared directly. On the basis of the fatty acid analyses reported previously by several authors, as well as the fact that much of the available literature is incomplete with respect to the presence of hydroxy fatty acids or 20 : 5ω3*c*, we can only state that the fatty acid composition of strain LT13a^T is based on all cellular components, including lipids and lipopolysaccharides and that mass spectrometry did not indicate that 20 : 5ω3*c* was present. This is, to our knowledge, the first report in which the presence of both hydroxy fatty acids and 20 : 5ω3*c* has been taken into consideration. The presence of hydroxy fatty acids, some of which appear to be amide-linked, is also consistent with the presence of a lipopolysaccharide and these components, though not always reported, may be of importance in differentiating members of the genus *Shewanella* (Makemson *et al.*, 1997; Leonardo *et al.*, 1999; Bozal *et al.*, 2002; Brettar *et al.*, 2002). Consistent with other reports on organisms similar to *Shewanella putrefaciens*, no polyunsaturated fatty acids (20 : 5ω3*c*) were detected.

Table 2. Fatty acid composition (%) of various *Shewanella* species

1, *S. profunda* LT13a^T; 2, *S. putrefaciens* ATCC 8071^T (Venkateswaran *et al.*, 1999); 3, *S. baltica* OS155^T, described as *S. baltica* NCTC 10735^T by Ziemke *et al.* (1998) and Brettar *et al.* (2002); 4, *S. oneidensis* ATCC 700550^T (Brettar *et al.*, 2002). ND, No data.

Fatty acid	1	2	3	4
Straight-chain fatty acids:				
14 : 0	4·0	2·3	2·2	2·6
15 : 0	4·3	3·2	7·8	4·7
16 : 0	13·3	19·1	4·3	14·8
17 : 0	0·8	1·5	0·6	2·8
18 : 0	ND	2·1	ND	1·1
Terminally branched fatty acids:				
13 : 0-iso	6·4	2·5	12·4	2·5
14 : 0-iso	ND	0·3	1·6	2·3
15 : 0-iso	15·1	21·1	14·3	25·4
16 : 0-iso	ND	0·1	0·2	1·4
17 : 0-iso	ND	1·7	0·5	1·7
Monounsaturated fatty acids:				
15 : 1ω6*c*	0·7	0·2	2·2	0·3
16 : 1ω7*c*	30·4	29·6	24·1	23·3
16 : 1ω9*c*	2·9	3·5	1·6	2·1
17 : 1ω6*c*	ND	0·9	1·4	1·5
17 : 1ω8*c*	5·9	6·7	11·0	8·0
18 : 1ω7*c*	1·8	6·0	0·8	5·7
18 : 1ω9*c*	1·5	3·8	0·8	2·9

Genomic DNA of strain LT13a^T was isolated by using the procedure described by Erauso *et al.* (1992). The G+C content of the genomic DNA was determined by HPLC according to the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ; Braunschweig, Germany). The genomic DNA G+C content of strain LT13a^T is 44·9 mol%, a value in accordance with that of *Shewanella* species studied to date (Venkateswaran *et al.*, 1999).

Sequencing reactions were performed by using a Thermo Sequenase primer cycle sequencing kit (Amersham Biosciences) and an automatic DNA analysis system (LI-COR 4000; Scientec) according to the manufacturers' protocols. A total of 1334 nt from the 16S rRNA gene was sequenced as described previously (Moore *et al.*, 1995). Phylogenetic analysis was performed by using the software package ARB (Ludwig *et al.*, 2004). Distance analysis [using the correction factor of Jukes & Cantor (1969)] and clustering with neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony analysis (Lake, 1987) revealed that strain LT13a^T affiliated within the genus *Shewanella* within the *γ-Proteobacteria* (Fig. 1). Strain LT13a^T was related more closely to *S. putrefaciens* (98 % similarity), an environmental bacterium isolated from aquatic environments (Brettar & Höfle, 1993), sediments (Myers & Nealson, 1990) and oilfields (Semple & Westlake, 1987). Strain LT13a^T was also related closely to *Shewanella oneidensis* (97 %) and *Shewanella baltica* (96 %). Strain LT13a^T and *S. putrefaciens* clustered together in 98 and 70 % of bootstrap trees (by distance and parsimony, respectively) (Fig. 1).

Strains included in the DNA–DNA hybridization experiments were *S. putrefaciens* DSM 6067^T, *S. baltica* DSM

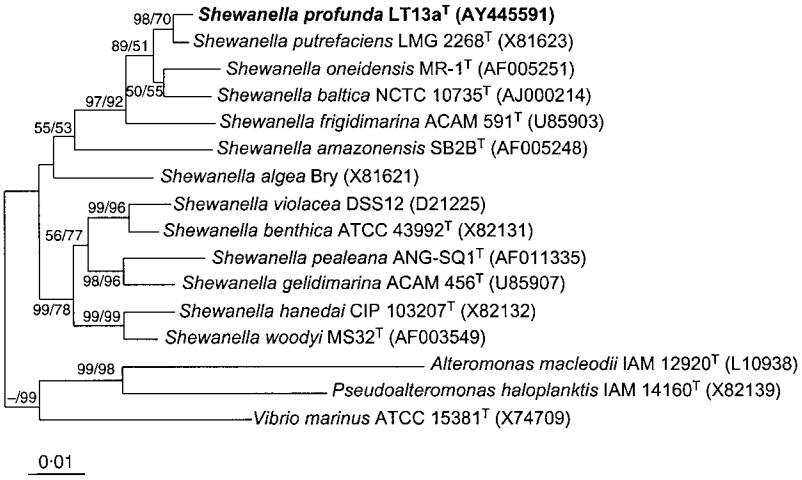

Fig. 1. Phylogenetic relationships of *S. profunda* (strain LT13a^T) and other *Shewanella* species produced by neighbour-joining with the Jukes–Cantor correction factor (Jukes & Cantor, 1969). The topologies of the trees were evaluated by maximum-parsimony (Lake, 1987) by using the program DNAPARS. The 16S rRNA gene sequence of strain LT13a^T was aligned with other 16S rRNA gene sequences from the Ribosomal Database Project (Maidak *et al.*, 2001) and GenBank. Numbers at branch nodes are bootstrap values based on 1000 replicates (distance and parsimony; Felsenstein, 1985) and are shown for branches with >50 % bootstrap support. The tree was generated with *Alteromonas macleodii* IAM 12920^T, *Pseudoalteromonas haloplanktis* IAM 14160^T and *Vibrio marinus* ATCC 15381^T as the outgroup. Bar, 0·01 expected changes per sequence position.

9439^T (DSMZ) and *S. oneidensis* strain MR-1^T (=CIP 106686^T) [Collection de l'Institut Pasteur (CIP), Paris, France]. All strains were cultured aerobically at 30 °C with agitation, according to the instructions of DSMZ and CIP. Levels of genetic relatedness were determined by performing DNA–DNA dot-blot hybridization experiments as reported previously, with modifications (Marteinsson *et al.*, 1995). DNA probes were labelled by using an enhanced chemifluorescence random-prime labelling kit (Amersham Biosciences). The level of hybridization is the mean level of binding for at least four replicates. The results of DNA–DNA hybridization experiments revealed that similarity for strain LT13a^T was <20 % with the three closest reference strains (*S. putrefaciens* DSM 6067^T, *S. baltica* DSM 9439^T and *S. oneidensis* strain MR-1^T), indicating that strain LT13a^T is a novel species of *Shewanella* (Stackebrandt & Goebel, 1994).

When different taxonomic parameters were compared, strain LT13a^T was found to differ from the *Shewanella* species that have been described (Table 1). Strain LT13a^T differs from its nearest phylogenetic neighbours as follows: (i) strain LT13a^T was able to produce acetoin, amylase and ornithine decarboxylase, unlike *S. putrefaciens*, *S. baltica* or *S. oneidensis*; (ii) strain LT13a^T can be distinguished from *S. putrefaciens* by means of gelatinase activity; (iii) strain LT13a^T was able to use citrate as sole carbon source; (iv) strain LT13a^T did not produce H_2S from thiosulphate and elemental sulphur and did not reduce trimethylamine-*N*-oxide, but reduced cystine to H_2S; (v) the optimal hydrostatic pressure for growth was 10 MPa; and (vi) levels of DNA similarity between strain LT13a^T and the three closest neighbours were <20 %. On the basis of physiological properties, in combination with DNA–DNA hybridization with the closest described relatives, strain LT13a^T represents a separate species within the genus *Shewanella*. Consequently, we propose to name this novel species *Shewanella profunda* sp. nov., with strain LT13a^T as the type strain.

Description of *Shewanella profunda* sp. nov.

Shewanella profunda (L. fem. adj. *profunda* from the deep).

Cells are Gram-negative, rod-shaped and motile by means of a single polar flagellum. Facultatively anaerobic heterotroph. Oxidase-, amylase-, gelatinase-, ornithine decarboxylase- and acetoin-positive. No endospores or spores are formed. Colonies on agar are circular. Temperature range for growth is 4–37 °C (optimum growth occurs at 25–30 °C); pH range for growth is 6·5–8·0 (optimum, approx. pH 7·0); NaCl concentrations for growth are in the range 0–60 g l^{-1} (optimum, 5 g NaCl l^{-1}). Hydrostatic pressure for growth is in the range 0·1–50 MPa at 30 °C (optimum, 10 MPa). Under optimal growth conditions, the doubling time of strain LT13a^T is 45 min with agitation for a maximum cell density of 1×10^8 cells ml^{-1}. Maltose, mannose, arabinose, sorbitol, succinate, glutamate, fumarate, citrate, lactate, pyruvate, yeast extract, peptone and tryptone are used as sole carbon and energy sources. Anaerobic growth occurs by fermentation of tryptone, peptone, yeast extract and pyruvate. Acids formed by fermentation of pyruvate are acetate, lactate and succinate. Strain LT13a^T uses oxygen, nitrate, ferric iron, fumarate and cystine as terminal electron acceptors with lactate as electron donor. On the basis of 16S rRNA gene sequence analysis, the strain belongs to the γ-*Proteobacteria* and is a member of the genus *Shewanella*. Q_7, Q_8, MK_7, MMK_7 and MMK_8 are present. Major polar lipids present are phosphatidylethanolamine and phosphatidylglycerol. Major fatty acids are 16 : 1ω7*c*, 15 : 0 iso, 16 : 0 and 13 : 0 iso. Polyunsaturated fatty acids

(e.g. 20 : 5ω3*c*) are not present. The G+C content of the DNA is 44·9 mol%.

The type strain is LT13a^T (=DSM 15900^T=JCM 12080^T) and was isolated from deep marine sediment at the Nankai Trough, off the coast of Japan in the Pacific Ocean (Ocean Drilling Program, Leg 190).

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