Shewanella xiamenensis sp. nov., isolated from coastal sea sediment

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A Gram-negative, motile, rod-shaped bacterium, strain S4^T, was isolated from coastal sediment collected off Xiamen, China. The physiological and biochemical features of strain S4^T, determined using the API 20NE, API ZYM and Biolog GN2 systems, were similar to those of members of the genus *Shewanella*. Phylogenetic analyses based on 16S rRNA and *gyrB* gene sequences placed strain S4^T in the genus *Shewanella*, and it was most closely related to *Shewanella oneidensis* and related species. DNA–DNA hybridization demonstrated only 11.9–30.4 % relatedness between S4^T and the type strains of related *Shewanella* species. On the basis of phylogenetic and phenotypic characteristics, strain S4^T is classified in the genus *Shewanella* as a representative of a distinct novel species, for which the name *Shewanella xiamenensis* sp. nov. is proposed. The type strain is S4^T (=CCTCC M 209017^T =JCM 16212^T).

Since the genus *Shewanella* was described (MacDonell & Colwell, 1985), numerous *Shewanella* species have been isolated from various environments, such as fresh water and the ocean (Hau & Gralnick, 2007). At the time of writing, at least 49 recognized distinct species belong to the genus *Shewanella*. Key features of the members of this genus include the ability to use a diverse range of electron acceptors for anaerobic respiration (Nealson & Scott, 2006) and the ability to grow at low temperatures (Bozal *et al.*, 2002). In this study, a novel Gram-negative, mesophilic, rod-shaped bacterium of the genus *Shewanella*, strain S4^T, is described.

Strain S4^T was obtained from a sediment sample that was collected from a coastal area of Xiamen, China. The novel strain was isolated by a standard dilution plating technique on Luria–Bertani (LB) agar supplemented with 40 mM sulfur and 30 mM lactate (LB/S^0/Lac; Moser & Nealson, 1996). The plates were first incubated aerobically at 30 °C for 24 h to obtain aerobic isolates. This collection of colonies was then screened for sulfur reduction using an anaerobic chamber (Forma Anaerobic System; model 1029) maintained with a $CO_2/H_2/N_2$ (5 : 10 : 85) atmosphere. Orange-coloured colonies showing clear zones were considered positive for sulfur reduction and were further subcultured by streaking on fresh plates for at least three rounds in order to obtain pure cultures. One isolate was purified and designated S4^T. For long-term storage, strain S4^T was stored at −80 °C in marine broth 2216 (Difco) supplemented with 15 % (v/v) glycerol.

Phenotypic properties of strain S4^T such as the utilization of various substrates as 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). Gram-staining, cell morphology and flagellum type were examined by light microscopy (Nikon E600) and transmission electron microscopy using exponentially growing cells. The pH and temperature ranges for growth were examined in marine broth 2216. Tests for salt tolerance were carried out in LB broth without NaCl. 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. Additional phenotypic characteristics were determined by using API ZYM and API 20NE test strips and Biolog GN2

Abbreviation: TMAO, trimethylamine *N*-oxide.

The GenBank/EMBL/DDBJ accession numbers for the nucleotide sequences reported in this study are respectively FJ589031 and FJ589040 (16S rRNA gene and partial *gyrB* gene sequence of strain S4^T) and FJ589036 and FJ589041 (partial *gyrB* gene sequences of *S. profunda* DSM 15900^T and *S. basaltis* JCM 14937^T).

Comparisons of fatty acid and isoprenoid quinone profiles and detailed DNA–DNA hybridization and 16S rRNA gene and *gyrB* sequence similarity results are available as supplementary material with the online version of this paper.

microplates (Makemson *et al.*, 1997). The DNA G+C content of strain S4^T was 46 mol%, which was determined using reversed-phase HPLC (Tamaoka & Komagata, 1984). Morphological, physiological and biochemical characteristics of strain S4^T are presented in Table 1 and in the species description.

The cellular fatty acid composition was analysed following a method reported previously (Svetashev *et al.*, 1995). Cell mass of strain S4^T was harvested from a loop of culture grown on marine broth 2216. The cellular fatty acid profile of strain S4^T contained large amounts of straight-chain (saturated and unsaturated), branched-chain and hydroxy fatty acids; the complete fatty acid composition is given in Supplementary Table S1, available in IJSEM Online. The major fatty acids of strain S4^T were iso-$C_{15:0}$, $C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH, $C_{16:0}$ and $C_{17:1}\omega8c$ (total of 66.9 %). This fatty acid profile was similar to those of related *Shewanella* species.

Analysis of respiratory quinones was carried out by the Identification Service and Dr Brian Tindall, Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (Braunschweig, Germany). Strain S4^T contained both menaquinones and ubiquinones as isoprenoid quinones. The major menaquinone was MK-7 (58 %) and the predominant ubiquinones were Q-7 and Q-8, at peak area ratios of 54 and 43 %, respectively. The ubiquinone composition of strain S4^T strongly resembled those of *Shewanella oneidensis* and *Shewanella putrefaciens* (Supplementary Table S2). However, strain S4^T contained more MMK-7 (35 %) than did *S. oneidensis* (6 %) or *S. putrefaciens* (7 %) (Venkateswaran *et al.*, 1999).

Genomic DNA was extracted using an E.Z.N.A. Bacterial DNA kit (Omega) following the manufacturer's instructions for DNA G+C content determination, 16S rRNA and *gyrB* gene amplification and DNA–DNA hybridization. The nearly complete 16S rRNA gene was amplified with primers 8F and 1525R, as described previously (Coates *et al.*, 2001). Almost entire *gyrB* genes of strain S4^T, *Shewanella profunda* DSM 15900^T and *Shewanella basaltis* JCM 14937^T were amplified and sequenced using degenerate primers UP-1 and UP-2r (Yamamoto & Harayama, 1995). These gene sequences were aligned and compared to the GenBank nucleotide database using an online BLAST search. Phylogenetic trees were constructed with the neighbour-joining method using the MEGA software (Tamura *et al.*, 2007). DNA–DNA hybridization was performed as described by Ezaki *et al.* (1989). 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.

A 1535 bp fragment representing almost the entire 16S rRNA gene of strain S4^T was amplified and sequenced in this study. This sequence was compared with those of the

Table 1. Phenotypic characteristics of strain S4^T (*Shewanella xiamenensis* sp. nov.) and type strains of related *Shewanella* species

Strains: 1, *S. xiamenensis* sp. nov. S4^T; 2, *S. oneidensis* ATCC 700550^T; 3, *S. putrefaciens* ATCC 8071^T; 4, *S. profunda* DSM 15900^T; 5, *S. decolorationis* JCM 21555^T; 6, *S. baltica* DSM 9439^T; 7, *S. hafniensis* NBRC 100975^T; 8, *S. morhuae* NBRC 100978^T; 9, *S. glacialipiscicola* NBRC 102030^T. All strains are positive for oxidase and catalase, reduction of nitrate to nitrite and growth at 4 °C and in the absence of NaCl. All strains are negative for production of urease and arginine dihydrolase, growth in the presence of 10 % NaCl and growth on adipate. +, Positive reaction; (+), weakly positive reaction; −, negative reaction; ND, no data available. Data were obtained in this study or taken from Venkateswaran *et al.* (1999), Nozue *et al.* (1992), Satomi *et al.* (2003, 2006, 2007), Toffin *et al.* (2004), Xu *et al.* (2005), Ziemke *et al.* (1998) and Fonnesbech Vogel *et al.* (2005).

Characteristic	1	2	3	4	5	6	7	8	9
DNA G+C content (mol%)	46	45	45	45	49	46	47	44	44
Growth at/in:									
37 °C	+	+	+	+	+	−	−	−	−
40 °C	−	+	−	−	+	−	−	−	−
6 % NaCl	+	−	−	+	−	+	+	−	−
Production of:									
Gelatinase	+	+	+/−	+	+	+	+	+	+
Amylase	−	−	−	+	−	−	ND	ND	ND
β-Glucosidase	+	−	(+)	−	(+)	ND	+	+	−
Fermentation of D-glucose	+	−	−	+	+	−	−	−	−
Utilization of:									
α-Cyclodextrin	+	ND	−	ND	−	+	ND	ND	ND
Dextrin	+	ND	−	ND	−	+	ND	ND	ND
Glycogen	(+)	ND	−	ND	−	+	ND	ND	ND
Tween 80	+	ND	+	ND	−	+	ND	ND	ND
Gentiobiose	−	ND	−	ND	−	+	ND	ND	ND
Cellobiose	−	ND	−	−	+	+	ND	ND	ND
N-Acetyl-glucosamine	+	+	+	−	+	+	+	+	+
α-Hydroxybutyrate	+	ND	−	ND	−	−	ND	ND	ND
Caprate	+	−	−	+	+	−	ND	−	ND
Glutamate	−	ND	ND	+	+	ND	ND	ND	ND
Gluconate	−	−	−	−	−	+	+	+	+
D-Galactose	−	+	+	−	−	ND	ND	ND	ND
D-Glucose	+	−	−	−	+	+	+	−	−
Citrate	−	−	−	+	−	+	+	−	−
Sucrose	+	−	−	−	+	+	−	−	−
Malate	+	−	+	−	+	+	+	+	+
Maltose	+	−	−	+	+	+	+	−	−
Lactate	+	+	+	+	+	+	+	−	+
Arabinose	+	−	−	+	−	−	−	−	ND
Reduction of:									
Nitrite	+	+	+	−	+	+	ND	ND	ND
Thiosulfate	+	+	+	−	+	ND	+	+	+
TMAO	+	+	−	−	+	ND	+	+	+

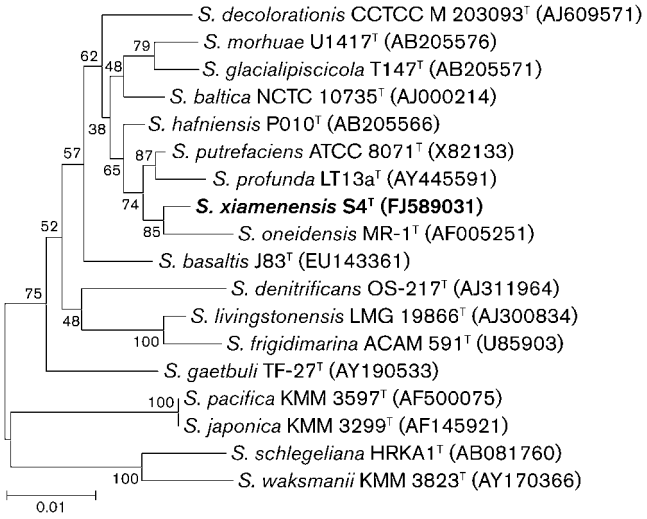

Fig. 1. Phylogenetic placement of strain S4^T according to 16S rRNA gene sequence analysis. The tree was constructed using the neighbour-joining method, and genetic distances were computed from Kimura's two-parameter model. Bootstrap values, expressed as percentages of 1000 replications, are given at branching points. Bar, 0.01 substitutions per nucleotide position.

type strains of recognized *Shewanella* species. The results showed that strain S4^T exhibited 16S rRNA gene sequence similarity of 91.2–98.8 % to the type strains of the other 49 *Shewanella* species. Among these, nine type strains showed 16S rRNA gene sequence similarity of more than 97 % (97.4–98.8 %) to strain S4^T (Fig. 1). Though the 16S rRNA gene sequence is accepted as defining phylogenetic relationships between bacterial species, it sometimes lacks sufficient specificity for the differentiation of close relatives (Venkateswaran *et al.*, 1999; Yamamoto & Harayama, 1995). Therefore, the more rapidly evolving *gyrB* gene was employed to distinguish closely related strains. The *gyrB* gene sequence of strain S4^T exhibited 82.2–91.9 % similarity to those of the type strains of nine closely related *Shewanella* species (Fig. 2). Only one type strain, *Shewanella decolorationis* JCM 21555^T, exhibited *gyrB* sequence similarity (91.9 %) above the suggested species cut-off value of 90 % for *gyrB* sequences within the genus *Shewanella* (Venkateswaran *et al.*, 1999). However, the ultimate criterion remains DNA–DNA relatedness. DNA–DNA hybridization indicated that the relatedness of DNA from strain S4^T and the nine close relatives was only 11.9–30.4 %, well below the 70 % threshold for the definition within a species (Stackebrandt & Goebel, 1994). 16S rRNA gene and *gyrB* sequence similarity and DNA–DNA relatedness results are summarized in Supplementary Table S3.

Strain S4^T can be distinguished easily from *S. oneidensis* and *S. putrefaciens* by its capacity to utilize sucrose, maltose, arabinose, D-glucose and caprate. Strain S4^T cannot grow in the presence of 6 % NaCl, unlike *S. decolorationis* and *Shewanella hafniensis* (Satomi *et al.*, 2006). Production of gelatinase but not amylase also clearly differentiated strain S4^T from *S. profunda*. Strain S4^T could grow at 37 °C and ferment D-glucose, whereas *Shewanella baltica*, *S. hafniensis*, *S. morhuae* and *S. glacialipiscicola* could not. Therefore, on the basis of phenotypic, chemotaxonomic and phylogenetic data, combined with DNA–DNA relatedness, strain S4^T represents a distinct species within the genus *Shewanella*, for which the name *Shewanella xiamenensis* sp. nov. is proposed.

Description of *Shewanella xiamenensis* sp. nov.

Shewanella xiamenensis (xia.me.nen′sis. N.L. fem. adj. *xiamenensis* from Xiamen, China, where the type strain was isolated).

Cells are Gram-negative rods, 0.7–0.8 × 2.5–4.0 μm, motile by means of a single polar flagellum. Facultatively anaerobic chemoheterotroph. Endospores are not formed. Colonies on marine agar 2216 are smooth, circular, slightly brown in colour and convex with clear edges. Growth

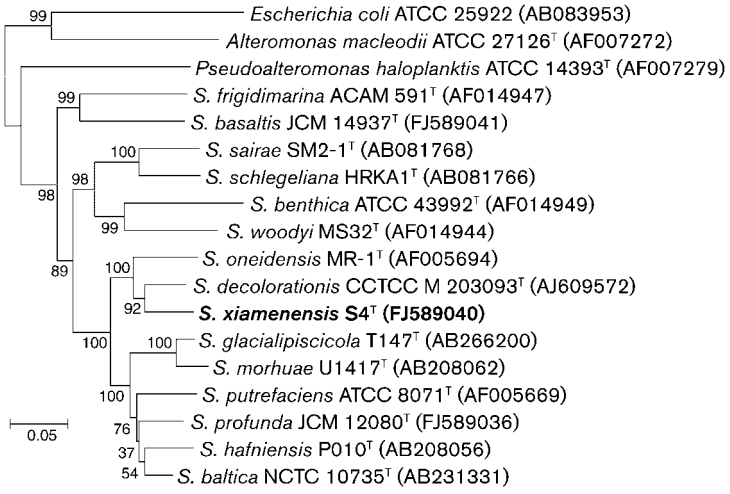

Fig. 2. Phylogenetic placement of strain S4^T according to *gyrB* gene sequence analysis. The tree was constructed using the neighbour-joining method, and genetic distances were computed from Kimura's two-parameter model. Bootstrap values, expressed as percentages of 1000 replications, are given at branching points. Bar, 0.05 substitutions per nucleotide position.

occurs at 4 and 37 °C, but not above 37 °C. The pH range for growth is 6.0–9.0, with optimum growth around pH 7.0. Sodium ions are not required for growth; growth occurs in the presence of 0–4 % NaCl and optimal growth occurs in 1–2 % NaCl. Cells are positive for oxidase, catalase, *N*-acetyl-*β*-glucosaminidase, α-glucosidase, *β*-glucosidase, acid and alkaline phosphatase, leucine aminopeptidase, valine aminopeptidase and naphthol-AS-BI-phosphoamidase activities. Cells are able to reduce nitrate, nitrite, TMAO, selenite, fumarate, ferric citrate and ferric oxide with lactate as electron donor. Hydrolysis of gelatin, DNA and Tween 80 is positive; negative for starch hydrolysis. H_2S is formed anaerobically from thiosulfate. Fermentation is observed on D-glucose. Utilizes a limited range of carbon sources according to Biolog: α-cyclodextrin, dextrin, Tweens 40 and 80, L-arabinose, α-D-glucose, maltose, sucrose, acetic acid, formic acid, α-hydroxybutyric acid, α-ketobutyric acid, DL-lactic acid, succinic acid, bromosuccinic acid, glycyl L-aspartic acid, glycyl L-glutamic acid, L-serine, inosine, uridine and thymidine. Glycogen, *N*-acetyl-D-glucosamine, monomethyl succinate, *β*-hydroxybutyric acid, *γ*-hydroxybutyric acid, α-ketovaleric acid, propionic acid, succinamic acid, L-alanine, L-alanyl glycine, L-leucine and L-threonine are utilized weakly. Both menaquinones and ubiquinones are present, with Q-7 and Q-8 as the predominant ubiquinones and MK-7 as the predominant menaquinone. The main fatty acids are iso-$C_{15:0}$, $C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH, $C_{16:0}$ and $C_{17:1}\omega8c$. The DNA G+C content of the type strain is 46 mol%.

The type strain, S4^T (=CCTCC M 209017^T =JCM 16212^T), was isolated from coastal sediment collected off Xiamen, China.

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