Shewanella gaetbuli sp. nov., a slight halophile isolated from a tidal flat in Korea

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A Gram-negative, motile, non-spore-forming, rod-shaped strain, TF-27^T (=KCCM 41648^T=JCM 11814^T), was isolated from a tidal flat in Korea. This organism grew well at 25–35 °C, with optimum growth at 30 °C. Strain TF-27^T grew optimally in the presence of 2% NaCl; it did not grow without NaCl or in the presence of >8% NaCl. Strain TF-27^T simultaneously contained both menaquinones and ubiquinones as isoprenoid quinones. The predominant menaquinone was MK-7 and the predominant ubiquinones were Q-7 and Q-8. The major fatty acids in strain TF-27^T were iso-C_{15:0} (20.6%) and iso-C_{15:0} 2-OH and/or C_{16:1ω7c} (21.1%). The DNA G + C content of strain TF-27^T was 42 mol%. Phylogenetic analyses based on 16S rDNA sequences showed that strain TF-27^T falls within the radiation of the cluster that is encompassed by the genus *Shewanella*. Levels of 16S rDNA sequence similarity between strain TF-27^T and the type strains of *Shewanella* species were 93.2–96.8%. On the basis of phenotypic properties and phylogenetic data, strain TF-27^T should be placed in the genus *Shewanella* as a novel species, for which the name *Shewanella gaetbuli* sp. nov. is proposed.

The genus *Shewanella* was created by reclassification of two species that were assigned previously to the genus *Alteromonas*, *Alteromonas putrefaciens* and *Alteromonas hanedai* (MacDonell & Colwell, 1985). There are 22 *Shewanella* species with validly published names at the time of writing. The genus *Shewanella* accommodates Gram-negative, facultatively anaerobic, straight or curved rods that belong to the *Proteobacteria* (Stackebrandt *et al.*, 1988; Venkateswaran *et al.*, 1999; Boone *et al.*, 2001). This genus is also characterized by simultaneously having menaquinones and ubiquinones, although not all *Shewanella* species have been analysed (Venkateswaran *et al.*, 1999; Bozal *et al.*, 2002). The genus *Shewanella* is affiliated phylogenetically to the γ -subclass of the *Proteobacteria* (Anzai *et al.*, 2000). *Shewanella* species have been isolated from a variety of sources, including aquatic or marine

environments (Nealson *et al.*, 1991; Ivanova *et al.*, 2001; Bozal *et al.*, 2002), clinical samples (Nozue *et al.*, 1992; Brink *et al.*, 1995; Venkateswaran *et al.*, 1999), sediments (Myers & Nealson, 1988) and oilfield fluids (Semple & Westlake, 1987). *Shewanella* species have been strongly implicated as opportunistic pathogens of humans and aquatic animals (Aguirre *et al.*, 1994; Brink *et al.*, 1995) and as causal agents of the spoilage of proteinaceous foods (Jorgensen & Huß, 1989). *Shewanella* species have also been known to be important in the context of bioremediation, because of their considerable potential for co-metabolic bioremediation of halogenated organic pollutants (Petrovskis *et al.*, 1994), destructive souring of crude petroleum (Semple & Westlake, 1987) and dissimilatory reduction of manganese and iron oxides (Myers & Nealson, 1988), uranium (Lovley & Phillips, 1988) and other compounds (Perry *et al.*, 1993; Kostka *et al.*, 1996). In this study, we describe a Gram-negative, moderately halophilic, rod-shaped strain, TF-27^T, which was isolated from a tidal flat in Korea. From the results of 16S rDNA sequence comparison, this organism was considered to be related phylogenetically to the genus *Shewanella*. Accordingly, the aim of the present work was to establish the exact taxonomic position of strain TF-27^T by using a combination of polyphasic taxonomic data.

Abbreviations: FAME, fatty acid methyl ester; TEM, transmission electron microscopy.

The GenBank/EMBL/DDBJ accession number for the 16S rDNA sequence of strain TF-27^T is AY190533.

A full version of the phylogenetic tree shown in Fig. 1 is available as supplementary material in IJSEM Online.

Strain TF-27^T was isolated from a tidal flat near the city of Mokpo, Korea, by the dilution-plating technique on marine agar 2216 (MA; Difco). Cell morphology was examined by light microscopy (Nikon E600) and transmission electron microscopy (TEM). Flagellum type was examined by TEM, using cells from exponentially growing cultures. Cells were stained negatively with 1% (w/v) phosphotungstic acid and grids were examined after air-drying by using a Philips CM-20 transmission electron microscope. Gram-reaction was determined by using a Gram Stain kit (bioMérieux) according to the manufacturer's instructions. The pH range for growth was determined in marine broth 2216 (MB; Difco) with pH adjusted to 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5 and 9.0. Growth at various NaCl concentrations was investigated in MB. Growth in the absence of NaCl was investigated in trypticase soy broth to which NaCl had not been added. Growth at various temperatures was measured on MA at 4–45 °C. Catalase activity was determined by bubble production in 3% (v/v) hydrogen peroxide solution. Oxidase activity was determined by oxidation of 1% (w/v) *p*-aminodimethylaniline oxalate. Hydrolysis of casein, starch and Tween 80 was determined as described by Cowan & Steel (1965). Hydrolysis of aesculin was determined according to the method of Lanyi (1987). Hydrolysis of hypoxanthine, tyrosine and xanthine was performed on MA by using substrate concentrations that were described by Cowan & Steel (1965). Hydrolysis of gelatin and nitrate reduction were determined as described by Lanyi (1987), with the modification that artificial sea water was used, which contained [(1 distilled water)⁻¹]: 23.6 g NaCl, 0.64 g KCl, 4.53 g MgCl₂·6H₂O, 5.94 g MgSO₄·7H₂O and 1.3 g CaCl₂·2H₂O (Levring, 1946). Hydrolysis of birch wood xylan (Sigma) was determined on solid marine salts basal medium (Baumann & Baumann, 1981) that contained 0.5% (w/v) xylan as the sole carbon source. H₂S production was tested as described by Bruns *et al.* (2001). Haemolytic activity was recorded on MA with 5% defibrinated sheep blood. The API ZYM system (bioMérieux) was used to determine enzyme activity. Acid production from carbohydrates was determined as described by Leifson (1963). Utilization of substrates as sole carbon and energy sources was tested as described by Baumann & Baumann (1981). Growth under anaerobic conditions was determined after incubation in an anaerobic chamber on anaerobically prepared MA.

Cell biomass of strain TF-27^T for respiratory lipoquinone analysis and for DNA extraction was obtained from MB cultures at 30 °C. For fatty acid methyl ester (FAME) analysis, cell mass of strain TF-27^T was obtained from agar plates after cultivation for 3 days at 30 °C on MA. Isoprenoid quinones were extracted and analysed as described by Komagata & Suzuki (1987), using reverse-phase HPLC. For quantitative analysis of cellular fatty acid compositions, a loop of cell mass was harvested and FAMES were prepared and identified by following the instructions of the Microbial Identification system (MIDI). Chromosomal DNA was isolated and purified according to a

method described previously (Yoon *et al.*, 1996), except that ribonuclease T1 was used with ribonuclease A. DNA G + C content was determined by the method of Tamaoka & Komagata (1984). DNA was hydrolysed and the resultant nucleotides were analysed by reverse-phase HPLC. 16S rDNA was amplified by PCR using two universal primers, as described previously (Yoon *et al.*, 1998). Sequencing of the amplified 16S rDNA and phylogenetic analysis were performed as described by Yoon *et al.* (2003).

Morphological, physiological and biochemical characteristics of strain TF-27^T are given in the species description below and in Table 1, together with those of other *Shewanella* species. Strain TF-27^T simultaneously contained both menaquinones and ubiquinones as isoprenoid quinones. This profile has been also observed in other *Shewanella* species (Nogi *et al.*, 1998; Venkateswaran *et al.*, 1999; Bozal *et al.*, 2002). Strain TF-27^T contained MK-7 (approx. 90%) as the predominant menaquinone. The predominant ubiquinones detected in strain TF-27^T were Q-7 and Q-8, at peak area ratios of approximately 45 and 52%, respectively. Strain TF-27^T had a cellular fatty acid profile that contained large amounts of straight-chain, branched, unsaturated and hydroxy fatty acids. It contained the following major fatty acids: iso-C_{15:0}, 20.6%; iso-C_{15:0} 2-OH and/or C_{16:1}ω7c, 21.1%; iso-C_{13:0}, 9.4%; C_{16:0}, 8.4%; C_{17:1}ω8c, 6.4%; iso-C_{13:0} 3-OH, 6.1%; C_{15:0}, 3.8%; C_{12:0}, 3.1%; C_{18:1}ω7c, 2.5%; iso-C_{14:0}, 2.0%. The DNA G + C content of strain TF-27^T was 42 mol%, which is in the range for known *Shewanella* species (Table 1).

The almost-complete 16S rDNA sequence of strain TF-27^T, comprising 1497 nt (approx. 96% of the *Escherichia coli* 16S rRNA gene sequence), was determined directly after PCR amplification. Phylogenetic analysis based on 16S rDNA sequences showed that strain TF-27^T was within the radiation of the cluster that comprised *Shewanella* species. In a phylogenetic tree based on the neighbour-joining algorithm, strain TF-27^T occupied an independent lineage within the evolutionary radiation that was encompassed by the genus *Shewanella*, particularly within the clade that comprised *Shewanella oneidensis*, *Shewanella putrefaciens*, *Shewanella baltica*, *Shewanella livingstonensis*, *Shewanella frigidimarina*, *Shewanella denitrificans*, *Shewanella olleyana* and *Shewanella japonica* (Fig. 1). The relationship between this clade and the cluster that comprised other *Shewanella* species was supported by a high bootstrap resampling value (Fig. 1). Strain TF-27^T exhibited 16S rDNA similarity levels of 93.2–96.8% to the type strains of *Shewanella* species with validly published names, and levels of <91.6% to other species used in the phylogenetic analysis.

The result of 16S rDNA sequence analysis indicated that strain TF-27^T had closest phylogenetic affinity with the *γ-Proteobacteria*, and particularly to the genus *Shewanella*. Phylogenetic inference based on 16S rDNA sequences revealed that strain TF-27^T falls within the evolutionary clade that comprises *Shewanella* species (Fig. 1).

Table 1. Phenotypic characteristics of strain TF-27^T and related *Shewanella* species

Taxa: 1, strain TF-27^T; 2, *Shewanella algae*; 3, *Shewanella amazonensis*; 4, *S. baltica*; 5, *S. frigidimarina*; 6, *S. japonica*; 7, *S. livingstonensis*; 8, *S. oneidensis*; 9, *S. putrefaciens*. All species have a single polar flagellum, are positive for growth at 3 % NaCl, catalase and oxidase and are Gram-negative. Data for strain TF-27^T are from this study; data for other taxa are from Nogi *et al.* (1998), Venkateswaran *et al.* (1999) and Ivanova *et al.* (2001). +, Positive reaction; –, negative reaction; v, variable reaction; ND, not determined; n, no. strains.

Characteristic	1 (n=1)	2 (n=4)	3 (n=1)	4 (n=1)	5 (n=1)	6 (n=2)	7 (n=1)	8 (n=5)	9 (n=10)
Cell shape	Straight rod	Straight rod	Straight rod	Straight rod	Straight or curved rod	Straight rod	Straight rod	Straight rod	Straight rod
Optimal temperature for growth (°C)	30	25–35	25–35	ND	20–22	20–25	15	25–35	25–35
Optimal pH for growth	7–8	7–8	7–8	ND	ND	7.5	ND	7–8	7–8
Growth at:									
4 °C after 24 h	–	–	+	+	+	–	ND	v	+
35 °C	+	+	+	ND	–	+	–	+	v
40 °C	–	+	+	–	ND	–	–	v	–
Reduction of:									
NO ₃ to NO ₂	–	+	+	+	+	+	+	v	+
NO ₂ to N ₂	–	–	+	ND	ND	ND	–	–	–
Haemolysis	+	v	+	ND	ND	+	ND	–	–
Production of:									
H ₂ S	–	+	+	+	+	ND	+	+	+
Amylase	+	–	–	ND	–	+	ND	–	–
Gelatinase	+	v	+	ND	+	+	+	+	–
Lipase	+	–	–	+	+	+	+	–	–
Chitinase	ND	ND	ND	ND	–	–	ND	ND	ND
Growth at:									
0 % NaCl	–	+	+	ND	+	ND	ND	+	+
6 % NaCl	+	+	–	ND	+	ND	–	v	+
10 % NaCl	–	+	–	ND	–	ND	–	–	–
Utilization of:									
D-Galactose	–	v	+	ND	–	+	+	v	+
D-Fructose	–	–	–	ND	–	+	ND	–	–
Sucrose	–	–	–	+	+	–	–	–	v
Maltose	–	v	–	+	+	+	+	–	v
Lactose	–	–	–	ND	ND	–	ND	–	v
Succinate	–	v	+	ND	+	+	+	+	v
Fumarate	–	v	+	ND	+	–	ND	v	v
Citrate	–	–	–	+	ND	–	ND	–	–
D-Mannitol	–	–	–	ND	+	–	+	–	–
Glycerol	–	–	–	ND	ND	–	ND	–	–
D-Sorbitol	–	–	–	ND	ND	–	ND	–	–
DL-Malate	–	–	–	+	ND	–	ND	–	–
DL-Lactate	–	+	+	ND	ND	ND	+	+	v
DNA G+C content (mol%)	42	52–55	52	46	40–43	43–44	41	45	43–47

Chemotaxonomic analysis supports the result of the monothetic classification, based on 16S rDNA sequence analysis. The cellular fatty acid profile of strain TF-27^T was found to be similar to those that have been described previously for *Shewanella* species (Bowman *et al.*, 1997; Venkateswaran *et al.*, 1999; Ivanova *et al.*, 2001; Bozal *et al.*, 2002). Strain TF-27^T was found to have an isoprenoid quinone pattern similar to those of *Shewanella* species: these species, as well as strain TF-27^T, simultaneously contain both menaquinones and ubiquinones, although their compositions are variable, depending on strains or

species (Nogi *et al.*, 1998; Venkateswaran *et al.*, 1999; Bozal *et al.*, 2002). Accordingly, in view of combined morphological, phenotypic, chemotaxonomic and phylogenetic data, strain TF-27^T can be classified as a member of the genus *Shewanella*. Strain TF-27^T can be differentiated from phylogenetically related *Shewanella* species by some physiological and biochemical characteristics, such as growth temperature, NaCl tolerance and the ability to utilize certain substrates (Table 1). 16S rDNA similarity levels between strain TF-27^T and the type strains of other *Shewanella* species are low enough (93.2–96.8 %) to categorize strain

Fig. 1. Neighbour-joining tree showing the phylogenetic position of strain TF-27^T and representatives of other related taxa, based on 16S rDNA sequences. Bootstrap values (expressed as percentages of 1000 replications) of >50% are shown at branch-points. The full tree from which Fig. 1 was taken is available as supplementary material in IJSEM Online. Bar, 0.01 substitutions per nucleotide position.

TF-27^T as a species that is distinct from previously described *Shewanella* species (Stackebrandt & Goebel, 1994). Therefore, on the basis of these data, strain TF-27^T should be placed in the genus *Shewanella* as a novel species, for which we propose the name *Shewanella gaetbuli* sp. nov.

Description of *Shewanella gaetbuli* sp. nov.

Shewanella gaetbuli (gaet.bu'li. N.L. gen. n. *gaetbuli* of gaetbul, the Korean name for a tidal flat).

Cells are straight rods, 0.5–0.7 × 1.5–3.0 µm on MA. Gram-stain reaction is negative. Non-spore-forming. Motile by means of a single polar flagellum. Colonies are smooth, glistening, circular to slightly irregular, flat to raised, light brown in colour and 2.0–4.0 mm in diameter after 3 days incubation at 30 °C on MA. Growth occurs at 4 and 39 °C, but not above 40 °C. Growth is observed at pH 5.0, but not at pH 4.5. Optimal growth occurs in the presence of 2% NaCl; no growth occurs in the presence of >8% NaCl. Growth occurs under anaerobic conditions on MA. Aesculin, casein, gelatin, starch and Tween 80 are hydrolysed. Hypoxanthine, tyrosine, xanthine and xylan (birch wood) are not hydrolysed. When assayed with the API ZYM system, alkaline phosphatase, esterase (C4), esterase lipase (C8), α-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase and *N*-acetyl-β-glucosaminidase are present and leucine arylamidase is weakly present, but lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, α-mannosidase and α-fucosidase are absent. Acid is produced from *myo*-inositol, D-ribose and D-trehalose. Acid is not produced from L-arabinose, D-cellobiose, D-fructose, D-galactose, D-glucose, lactose, maltose, D-mannose, D-melezitose, melibiose, D-raffinose, L-rhamnose, stachyose, sucrose, D-xylose, adonitol, D-mannitol or D-sorbitol. Both menaquinones and ubiquinones are present; predominant menaquinone is MK-7 and predominant ubiquinones are Q-7 and Q-8. Major

fatty acids are iso-C_{15:0} and iso-C_{15:0} 2-OH and/or C_{16:1}ω7c. DNA G + C content is 42 mol% (HPLC). Other characteristics are given in Table 1.

The type strain is TF-27^T (=KCCM 41648^T =JCM 11814^T). Isolated from a tidal flat near Mokpo, Korea.

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