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 ϒ-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-27T, 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-27T by using a combination of polyphasic taxonomic data.

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

Jung-Hoon Yoon,1 Kook Hee Kang,2 Tae-Kwang Oh1 and Yong-Ha Park1,3

1Korea Research Institute of Bioscience and Biotechnology (KIRI), PO Box 115, Yusong, Taejon, Korea
2Department of Food and Life Science, Sungkyunkwan University, Chunchun-dong 300, Jangan-gu, Suwon, Korea
3National Research Laboratory of Molecular Ecosystematics, Institute of Probionic, Probionic Corporation, Bio-venture Center, Korea Research Institute of Bioscience and Biotechnology (KIRI), PO Box 115, Yusong, Taejon, Korea

A Gram-negative, motile, non-spore-forming, rod-shaped strain, TF-27T (=KCCM 41648T = JCM 11814T), was isolated from a tidal flat in Korea. This organism grew well at 25–35 °C, with optimum growth at 30 °C. Strain TF-27T grew optimally in the presence of 2 % NaCl; it did not grow without NaCl or in the presence of > 8 % NaCl. Strain TF-27T 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-27T were iso-C15:0 (20–6 %) and iso-C16:0 2-OH and/or C16:1ω7c (21–1 %). The DNA G+C content of strain TF-27T was 42 mol%. Phylogenetic analyses based on 16S rDNA sequences showed that strain TF-27T falls within the radiation of the cluster that is encompassed by the genus *Shewanella*. Levels of 16S rDNA sequence similarity between strain TF-27T and the type strains of *Shewanella* species were 93–2–96 %.

On the basis of phenotypic properties and phylogenetic data, strain TF-27T should be placed in the genus *Shewanella* as a novel species, for which the name *Shewanella gaetbuli* sp. nov. is proposed.
Strain TF-27<sup>T</sup> 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 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 hyphoxanthine, 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 MgCl<sub>2</sub>·6H<sub>2</sub>O, 5-94 g MgSO<sub>4</sub>·7H<sub>2</sub>O and 1-3 g CaCl<sub>2</sub>·2H<sub>2</sub>O (Leving, 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. H<sub>2</sub>S 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<sup>T</sup> 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<sup>T</sup> 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<sup>T</sup> are given in the species description below and in Table 1, together with those of other Shewanella species. Strain TF-27<sup>T</sup> 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<sup>T</sup> contained MK-7 (approx. 90%) as the predominant menaquinone. The predominant ubiquinones detected in strain TF-27<sup>T</sup> were Q-7 and Q-8, at peak area ratios of approximately 45 and 52 %, respectively. Strain TF-27<sup>T</sup> 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<sub>15</sub>:0, 20-6 %; iso-C<sub>15</sub>:0 2-OH and/or C<sub>16</sub>:1<sub>ω7c</sub>, 21-1 %; iso-C<sub>13</sub>:0, 9-4 %; C<sub>16</sub>:0, 8-4 %; C<sub>17</sub>:1<sub>ω9c</sub>, 6-4 %; iso-C<sub>15</sub>:0 3-OH, 6-1 %; C<sub>15</sub>:0, 3-8 %; C<sub>12</sub>:0, 3-1 %; C<sub>18</sub>:1<sub>ω7c</sub>, 2-5 %; iso-C<sub>14</sub>:0, 2-0 %. The DNA G+C content of strain TF-27<sup>T</sup> was 42 mol%, which is in the range for known Shewanella species (Table 1).

The almost-complete 16S rDNA sequence of strain TF-27<sup>T</sup>, 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<sup>T</sup> was within the radiation of the cluster that comprised Shewanella species. In a phylogenetic tree based on the neighbour-joining algorithm, strain TF-27<sup>T</sup> 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 oleyana 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<sup>T</sup> 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<sup>T</sup> 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<sup>T</sup> falls within the evolutionary clade that comprises Shewanella species (Fig. 1).
Table 1. Phenotypic characteristics of strain TF-27T and related Shewanella species

Taxa: 1, strain TF-27T; 2, Shewanella algera; 3, Shewanella amazonensis; 4, S. baltica; 5, S. frigidimarina; 6, S. japonica; 7, S. livingstonensis; 8, S. oniedensis; 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-27T 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.

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<td>46</td>
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<td>41</td>
<td>45</td>
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Chemotaxonomic analysis supports the result of the monothetic classification, based on 16S rDNA sequence analysis. The cellular fatty acid profile of strain TF-27T 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-27T was found to have an isoprenoid quinone pattern similar to those of Shewanella species: these species, as well as strain TF-27T, 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-27T can be classified as a member of the genus Shewanella. Strain TF-27T 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-27T and the type strains of other Shewanella species are low enough (93.2–96.8%) to categorize strain...
TF-27T as a species that is distinct from previously described *Shewanella* species (Stackebrandt & Goebel, 1994). Therefore, on the basis of these data, strain TF-27T 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* (ga-et'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. Growth is observed at pH 5–0, but not above 40 °C. 

Acknowledgements

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