

Shewanella basaltis sp. nov., a marine bacterium isolated from black sand

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A Gram-negative, motile, rod-shaped bacterium was isolated from black sand collected at Soesoggak, Jeju island, Korea. The strain, designated J83^T, was able to grow in the presence of 5% NaCl, at temperatures of 4–45 °C and over the pH range 5.5–9.5. The isolate reduced nitrate to nitrite and was positive for oxidase, catalase, alkaline phosphatase and leucine arylamidase. Strain J83^T utilized malate, maltose, mannitol and glucose as sole sources of carbon. Phylogenetic analysis based on 16S rRNA gene sequences revealed that strain J83^T belongs to the class *Gammaproteobacteria* and is related to species of the genus *Shewanella*. Strain J83^T exhibited 97.8% 16S rRNA gene sequence similarity to the type strain of *Shewanella hafniensis*. Based on DNA–DNA hybridization, the level of relatedness between strain J83^T and *S. hafniensis* NBRC 100975^T was 39.3%. On the basis of phenotypic, genetic and phylogenetic data, strain J83^T is considered to represent a novel species of the genus *Shewanella*, for which the name *Shewanella basaltis* sp. nov. is proposed. The type strain is J83^T (=KCTC 22121^T =JCM 14937^T).

The genus *Shewanella* was first described by MacDonell & Colwell (1985) and, at the time of writing, comprises 47 recognized species (<http://www.bacterio.cict.fr/s/shewanella.html>). The genus belongs within the family *Shewanellaceae* (Ivanova *et al.*, 2004). Micro-organisms that belong to the genus *Shewanella* have a worldwide distribution in aquatic and marine environments (Bozal *et al.*, 2002; Yang *et al.*, 2006) and have been isolated from estuaries (Skerratt *et al.*, 2002; Venkateswaran *et al.*, 1998), deep-sea sediment (Xiao *et al.*, 2007; Yang *et al.*, 2007) and tidal flats (Yoon *et al.*, 2004). The majority of *Shewanella* species have been isolated from marine environments. A *Shewanella*-like, Gram-negative, rod-shaped bacterial strain, J83^T, was recently isolated from black sand collected from Soesoggak, Jeju island, Korea. Here we describe the phenotypic, genetic and chemotaxonomic characteristics as well as taxonomic position of strain J83^T.

Strain J83^T was routinely grown at 30 °C for 3 days on marine agar (MA; Difco) or marine salts basal medium (MB) (Baumann & Baumann, 1981) supplemented with various carbon sources. Cell morphology was examined by light microscopy (E600; Nikon) and transmission electron

microscopy. Cellular motility was determined by observing fresh wet-mounts of young bacterial MB cultures via the hanging drop method. The presence of flagella was investigated by transmission electron microscopy by using cells from an exponentially growing culture. The Gram reaction was determined by using a Gram stain kit (Difco) according to the manufacturer's instructions. Reference strain *Shewanella hafniensis* NBRC 100975^T was obtained from the NBRC, Japan, and was grown under the same conditions. Bacterial cultures of the novel isolate and reference strain were stored at –80 °C in MB containing 20% glycerol. For morphological and physiological characterization, strain J83^T was generally cultivated in MB and incubated by shaking at 30 °C. Growth at various NaCl concentrations, temperature and pH was measured in MB. Growth under anaerobic conditions was determined by incubation for 7 days in anaerobic Gaspak jars (BBL) containing an atmosphere of 80% N₂, 10% CO₂ and 10% H₂. Anaerobic growth was tested as described by Bowman *et al.* (1997) with various electron acceptors such as nitrate, iron oxide and sulfur and suitable donors such as lactate and pyruvate. Catalase activity was determined by bubble production in a 3% (v/v) H₂O₂ solution. Oxidase activity was determined by using an oxidase reagent (bioMérieux). API 20NE and API ZYM test strips (bioMérieux) were used

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain J83^T is EU143361.

to analyse the biochemical and physiological characteristics of the bacterial strains, and additional biochemical tests were performed with the methods and media described by Gordon *et al.* (1973). The ability to grow on various carbon sources was tested as described by Gonzalez *et al.* (1997). Bacteria grown on MA for 3 days at 30 °C were used for analysis of fatty acid methyl esters. Fatty acids were extracted and prepared according to standard protocols provided by the MIDI/Hewlett Packard Microbial Identification System (Sasser, 1990). Chromosomal DNA was extracted and purified according to the method of Sambrook *et al.* (1989). The 16S rRNA gene was amplified by PCR by using two universal primers as described by Stackebrandt *et al.* (1993). Sequencing of the amplified 16S rRNA gene and phylogenetic analysis were performed according to Yoon *et al.* (1998). DNA–DNA hybridization was performed fluorometrically according to the method of Ezaki *et al.* (1989) by using photobiotin-labelled DNA probes and microwell plates. The 16S rRNA gene sequence of strain J83^T was aligned with 13 reference sequences from the RDP database (Fig. 1) by using the multiple sequence alignment program CLUSTAL_X (v1.8) (Thompson *et al.*, 1997). Phylogenetic relationships between representatives of the genus *Shewanella* were determined by using MEGA version 2.1 software. Distance matrices were determined following the assumptions described by Kimura (1980). These matrices were used to elaborate dendrograms according to the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Kluge & Farris, 1969) methods. A bootstrap analysis to investigate the stability of the trees was performed by obtaining a consensus tree based on 1000 randomly generated trees.

The morphological, cultural, physiological and biochemical characteristics of strain J83^T and of related *Shewanella* species are given in Table 1 and in the species description. Cells of strain J83^T were Gram-negative, motile rods. Growth was observed at 4–45 °C but not above 50 °C. Cells grew in the pH range 5.5–9.5 but not at below pH 4.5 or above pH 11. Growth was observed in the presence of 5 %

NaCl but no growth was detected at concentrations above 6 % NaCl. Colonies were circular to slightly irregular, opaque, smooth, convex, slightly ivory-coloured and 2–3.5 mm in diameter after incubation for 3 days on MA. Growth did not occur under anaerobic conditions. Cells were oxidase-, catalase- and β -galactosidase-positive and urease- and arginine dihydrolase-negative. Nitrate was reduced to nitrite. Strain J83^T was able to grow on glucose, mannitol, maltose and malate as carbon sources. No growth was observed on arabinose, mannose, *N*-acetylglucosamine, gluconate, caprate, adipate, citrate or phenylacetate. The dominant cellular fatty acids were C_{12:0} (14.11 % of the total), C_{12:0} 3-OH (12.04 %), C_{16:0} (11.80 %) and C_{16:1} ω 7*c*/iso-C_{15:0} 2-OH (9.82 %).

The 16S rRNA gene sequence of strain J83^T (1268 bp) was compared with those of the type strains of reference species in the family *Shewanellaceae*. Strain J83^T fell within a cluster comprising *Shewanella* species (Fig. 1), and exhibited 96.7–97.8 % similarity in its 16S rRNA gene sequence to the type strains of other *Shewanella* species. Phylogenetic analysis revealed that strain J83^T comprises a relatively long subline of descent and occupies a phylogenetically distinct position on the main *Shewanella* branch. DNA–DNA hybridization experiments were performed to determine the genetic relationship between strain J83^T and *S. hafniensis* NBRC 100975^T, its closest relative based on 16S rRNA gene sequence analysis. The level of DNA–DNA relatedness between the two strains was 39.3 %. Taken together, the phylogenetic and DNA–DNA hybridization results suggest that strain J83^T represents a novel species of the genus *Shewanella*, for which the name *Shewanella basaltis* sp. nov. is proposed.

Description of *Shewanella basaltis* sp. nov.

Shewanella basaltis (ba.sal'tis. N.L. masc. gen. n. *basaltis* of basalt, pertaining to the source of isolation).

Cells are Gram-negative, motile rods, 0.5–0.8 μ m wide by 1–1.5 μ m long in 3-day cultures growing at 30 °C on MA

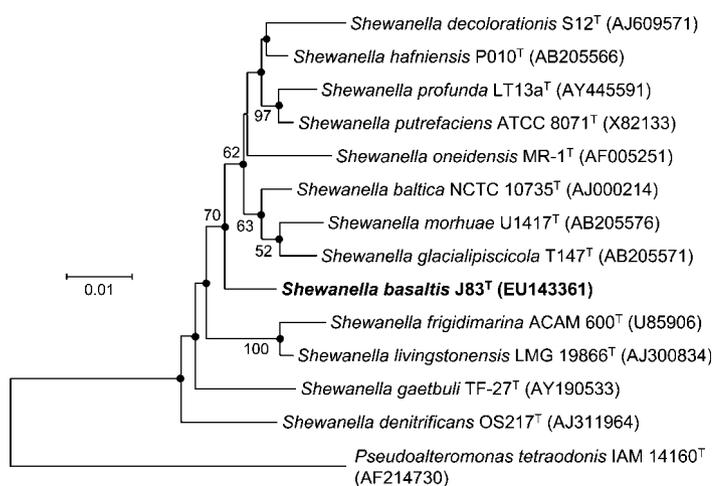


Fig. 1. Consensus phylogenetic tree based on 16S rRNA gene sequences showing the relationship between strain J83^T and the type strains of the most closely related *Shewanella* species. The tree was constructed based on the neighbour-joining method and p-distances. Filled circles indicate generic branches that were also recovered by using the maximum-parsimony algorithm. Bootstrap analyses were performed with 1000 repetitions; only values >50 % are shown. GenBank accession numbers are given in parentheses. Bar, 0.01 % sequence divergence.

Table 1. Differential characteristics between strain J83^T and related species of the genus *Shewanella*

Taxa: 1, J83^T; 2, *S. hafniensis* (data from Satomi *et al.*, 2006); 3, *S. morhuae* (Satomi *et al.*, 2006); 4, *S. glacialipiscicola* (Satomi *et al.*, 2007); 5, *S. baltica* (Ziemke *et al.*, 1998); 6, *S. oneidensis* (Venkateswaran *et al.*, 1999); 7, *S. putrefaciens* (Venkateswaran *et al.*, 1999). +, Positive; -, negative; ND, no data available; w, weakly positive. All strains grew at 4 °C, reduced nitrate to nitrite and were positive for cytochrome oxidase and catalase.

Characteristic	1	2	3	4	5	6	7
Growth at/in:							
37 °C	+	-	-	-	-	+	+
6% NaCl	-	+	-	-	+	-	-
Gelatin hydrolysis	-	+	+	+	+	+	-
Assimilation of:							
Sucrose	-	-	w	-	+	-	-
Glucose	+	+	-	-	+	-	-
Arabinose	-	w	+	+	-	-	+
Mannose	-	-	-	-	ND	ND	ND
Mannitol	+	-	-	-	ND	-	ND
N-Acetylglucosamine	-	+	+	+	+	+	+
Maltose	+	+	-	-	+	-	-
Gluconate	-	+	+	+	+	-	-
Malate	+	+	+	+	+	-	+
Citrate	-	+	-	-	+	-	-

plates. Colonies are circular to slightly irregular, opaque, smooth, convex, slightly ivory-coloured and 2–3.5 mm in diameter after incubation for 3 days on MA. Cells grow in the presence of 5% NaCl but not in 6% NaCl. Growth occurs at 4–45 °C, and at pH 5.5–9.5. No growth is detected below pH 4.5 or above pH 11. Cells are catalase- and oxidase-positive and urease-, indole- and arginine dihydrolyase-negative, hydrolyse gelatin and reduce nitrate to nitrite. Glucose, mannitol, maltose and malate can be utilized as sole carbon and energy sources, but not arabinose, mannose, N-acetylglucosamine, gluconate, caprate, adipate, citrate or phenylacetate. Positive for alkaline phosphatase, esterase lipase (C₈), valine arylamidase, naphthol-AS-BI-phosphohydrolase and N-acetyl-β-glucosaminidase activities (API ZYM). Negative for esterase (C₄), lipase (C₁₄), leucine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, α-mannosidase and α-fucosidase activities. The predominant fatty acids are C_{12:0} (14.11% of the total), C_{12:0} 3-OH (12.04%), C_{16:0} (11.80%) and C_{16:1}ω7cliso-C_{15:0} 2-OH (9.82%).

The type strain, J83^T (=KCTC 22121^T =JCM 14937^T), was isolated from black sand from Soesoggak, Jeju island, Korea.

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