***Fundibacter jadensis* gen. nov., sp. nov., a new slightly halophilic bacterium, isolated from intertidal sediment**

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A moderately halophilic hydrocarbon-degrading bacterium was isolated from continuous cultures containing a suspension of intertidal sediment from the German North Sea coast with hexadecane as the sole carbon source. On the basis of phenotypic characteristics, fatty acid analysis and 16S rDNA sequence analysis, it was considered to be a new species belonging to a new genus. It is a Gram-negative, aerobic, rod-shaped bacterium, whose cell size varies. It grows at concentrations of 0.5–15% (w/v) NaCl and utilizes a restricted spectrum of carbon sources. The G+C content of the DNA is 63.6 mol%. Comparative 16S rDNA studies show a clear affiliation of this bacterium to the gamma subclass of the class *Proteobacteria*. Comparison of phylogenetic data indicate that it is most closely related to *Marinobacter hydrocarbonoclasticus* (88.9% similarity in 16S rRNA gene sequence). Since it is impossible to find a sufficiently closely related species, we propose the name *Fundibacter jadensis* gen. nov., sp. nov. for the bacteria. The type strain is T9^T (= DSM 12178^T).

Keywords: intertidal sediment, gamma-*Proteobacteria*, hydrocarbon degradation

INTRODUCTION

Studies were made to characterize the substrate-utilization pattern of the alkane-degrading bacterial population indigenous in the intertidal sediment of the Jadebusen Bay (German North Sea coast). For this, continuous-flow cultures containing mud-flat sediment and hexadecane as the sole carbon source were performed (Berthe-Corti *et al.*, 1997). These cultures led to the isolation of numerous hydrocarbon-degrading strains. A Gram-negative bacterium, named strain T9^T, able to grow both anaerobically and at oxygen saturation, showed a restricted spectrum of carbon sources that were metabolized, but was able to degrade various long-chain alkanes. The bacterium is characterized by a variable cell morphology and belongs to the gamma subclass of the class *Proteobacteria*. It was not possible to assign it to a known genus.

In this paper, we present the results of the phenotypic

and biochemical examinations of the strain. The phylogenetic relationships to other bacteria were analysed by 16S rDNA sequence analysis and the application of specific rRNA-directed oligonucleotide probes. The data suggest that the organism isolated belongs to a new genus and represents a new species, for which we propose the name *Fundibacter jadensis* gen. nov. sp. nov., according to the geographic site where the organism was isolated from.

METHODS

Isolation. Strain T9^T was isolated from an aerobic continuous culture with a suspension containing sediment of the intertidal zone of the German North Sea coast (Jadebusen). The suspension was supplemented with hexadecane as the sole carbon source (Berthe-Corti *et al.*, 1997). For isolation, 10 ml samples from the culture suspension were ultrasonically treated (3 × 30 s, 30 W, 4 °C; Branson sonifier), spread over a solid medium (for composition see modified nutrient agar) and incubated at 25 °C for 1–3 weeks. From plates containing 30–50 colonies, all colonies were picked and restreaked several times to obtain pure cultures. The isolated cultures were chosen to make detailed studies of their hydrocarbon-degrading activity.

Growth media. General laboratory cultivation was per-

Abbreviations: PHB, poly- β -hydroxybutyrate; SM, synthetic medium; SWM, seawater medium.

The EMBL accession number for the 16S rDNA sequence from *F. jadensis* reported in this paper is AJ001150.

formed on modified nutrient agar containing 2.5 g Bacto peptone (Difco), 1.5 g yeast extract, 2.0 ml hexadecane, 15.0 g agar-agar, 750 ml artificial seawater and 250 ml distilled water. The artificial seawater contained per litre distilled water (Levring, 1946): 23.6 g NaCl; 0.64 g KCl; 4.53 g $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$; 5.94 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$; 1.3 g $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$. To avoid precipitation, the $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ was sterilized separately. The pH of the medium was adjusted with NaHCO_3 to pH 7.2 and incubation was carried out at 25 °C. For some phenotypic characterizations, the organism was cultivated in a synthetic medium (SM) (Gauthier *et al.*, 1992) supplemented with 1% (w/v) acetate as the sole carbon source or in a seawater medium (SWM) containing (per litre artificial seawater): 0.1% (w/v) of the carbon sources listed in Table 1; 43 mg $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$; 220 mg NaNO_3 ; 650 mg NH_4Cl ; 46 mg NaHCO_3 ; 3 ml vitamin solution (Schlegel, 1992) and 1 ml mineral salts solution (medium no. 124; DSMZ catalogue of strains, 1993). Incubation was performed at 25 °C. To obtain biomass for the fatty acid analysis, cells were grown in a liquid medium containing: 4.16 g CH_3COONa ; 3 ml vitamin solution (as above); 1 ml mineral salts solution (as above); 1.46 g NaNO_3 ; 0.14 g Na_2HPO_4 ; 750 ml artificial seawater (Levring, 1946) and 250 ml distilled water; pH 7.2 (adjusted with NaOH). The incubation was performed at 25 °C.

Phenotypic characterization. Cell morphology was determined by examining cultures of different ages by phase-contrast microscopy. Flagella were examined by the staining method according to Drews (1976). Colony morphology was studied using a stereomicroscope. Transmission electron microscopy (transmission electron microscope 109, Zeiss) was carried out with negatively stained cells (Cole & Popkin, 1981) of 42 h and 4 d cultures grown in the liquid medium used for the fatty acid analysis. Catalase and oxidase tests were done as described by Smibert & Krieg (1994). The Gram reaction was studied with the aminopeptidase test (Merck) and by gram staining (Smibert & Krieg, 1994). The temperature range for growth was determined by incubating liquid cultures in SM between 4 and 50 °C. The pH range was determined in liquid SM with a final pH between 5.0 and 10.0. Salt tolerance was studied in liquid SM containing 0–30% (w/v) NaCl. Utilization of carbon sources was tested in liquid SM omitting peptone and acetate but supplementing with 0.1% (w/v) of the various carbon sources including the long-chain or aromatic hydrocarbons tetradecane, hexadecane, eicosane, heneicosane, pristane and phenanthrene (Table 1). Additionally, growth in a seawater medium supplemented with vitamins and trace elements, and with the same carbon sources (Table 1) except eicosane and heneicosane was examined. Growth was estimated after 1 week incubation at 25 °C from OD_{600} with a Perkin Elmer 550S UV/VIS spectrometer. Susceptibility to inhibitors was analysed on modified nutrient agar without hexadecane but supplemented with 0.3% (w/v) acetate by performing the disk diffusion test with the following antibiotics (Bio-disks; bioMérieux): ampicillin (10 µg), cefazolin (30 µg), cephalothin (30 µg), chloramphenicol (30 µg), erythromycin (15 µg), gentamicin (30 µg), lincomycin (15 µg), nalidixic acid (30 µg), novobiocin (5 µg), ofloxacin (5 µg), oxacillin (5 µg), penicillin (10 µg), tetracycline (30 µg) and tobramycin (30 µg).

For determining denitrification, cells were grown anaerobically in a modified liquid nutrient broth without hexadecane but supplemented with 0.3% (w/v) acetate and 0.1% (w/v) KNO_3 . After one week of incubation, the formation of nitrite was determined by a dot reaction described by Naveke

& Tepper (1979). Gaseous N_2 production was analysed gas chromatographically using a Shimadzu 8A chromatograph equipped with a thermal-conductivity detector. A Chrompack 13A column (80/100 mesh, 2 m × 3 mm; Macherey-Nagel) was used for the N_2 analysis and a Porapack QS column (100/120 mesh, 2 m × 3 mm; Macherey-Nagel) for the N_2O analysis. Helium was used as the carrier gas at a flow rate of 40 ml min⁻¹ and at a temperature of 50 °C. The injector and detector temperature was 140 °C.

To determine intracellular poly-β-hydroxybutyrate (PHB), heat-fixed cells were stained with 1% (w/v) Nile blue A solution for 10 min at 55 °C, rinsed with water and then with 8% (v/v) acetate in distilled water. Cells containing intracellular PHB showed an orange colour after excitation by light at 436 nm under an epifluorescence microscope.

Fatty acid analysis. Cells were cultivated in the above-mentioned medium for fatty acid analysis, harvested by centrifugation (30 min, 25 900 g) and washed twice in artificial seawater (Grasshoff, 1983). The extraction of fatty acids was done according to the MIDI system (Sasser, 1990). This system is based on the methylation of fatty acids. The extracted fatty acid methyl esters were separated by chromatography on a fused-silica capillary column [50 m × 0.25 mm (internal diameter)] with an HP 5980A chromatograph (Hewlett Packard) equipped with a flame-ionization detector and a HP-3396 integrator (Hewlett Packard), using H_2 as the carrier gas at a flow rate of 15 ml min⁻¹ and at temperatures ranging from 150 to 250 °C, ramp rate 4 °C min⁻¹. A standard mixture (Matreya) containing defined fatty acids (C_{11} – C_{20} straight-chain, saturated, unsaturated, hydroxy, cyclopropane, iso and anteiso fatty acids) was used as a reference for comparison.

DNA base composition. The G + C content of the DNA of strain T9^T was determined from DNA extracted with the QIAmp Tissue Kit (Qiagen). The procedure recommended by the manufacturer was modified: the enzymes and buffers were used as described in the Large Sample Protocol, but the actual extraction was done by the Tissue Protocol. Before loading the columns, SDS was added to the suspension at a final concentration of 2% (w/v) and incubated for 10 min at 65 °C. To gain a higher DNA purity, an additional washing step with buffer AW1 (Qiagen) was carried out and the solution was extracted twice with chloroform/isoamylalcohol (24:1) (Hill, 1968). The dialysed DNA (in 0.1 × SSC) was examined using the thermal denaturation method (Mandel & Marmur, 1968) with a Gilford 2600 spectrophotometer.

16S rDNA sequence determination. This analysis was done by the DSMZ. Genomic DNA was extracted, and PCR amplification of the 16S rRNA genes was performed as described by Rainey *et al.* (1996). Purified PCR products were sequenced using a PRISM Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems) as recommended by the manufacturer. The purified sequence reaction mixtures were electrophoresed using an Applied Biosystems model 373A DNA sequencer. The determined sequence was manually aligned with the alignment editor ae2 (Maidak *et al.*, 1996) and compared with sequences of reference micro-organisms which belong to the gamma subclass of the *Proteobacteria* (EMBL and RDP databases). Evolutionary distances calculated by the method of Jukes & Cantor (1969) were used to construct a phylogenetic tree by the neighbour-joining method (Saitou & Nei, 1987).

RESULTS

Morphological and cultural characteristics

F. jadensis strain T9^T is a Gram-negative, rod-shaped, non-motile organism. Cells grown in the liquid medium used for fatty acid analysis were 0.8–1.8 µm long and 0.3–0.7 µm wide. The cell sizes were quite variable (Fig. 1a). Since an infection by other bacteria can be excluded, we have to assume that the variability of the cell size is a feature of this strain. Cells of older cultures (4 d) were characterized by peritrichous pili which could be seen by transmission electron microscopy of negatively stained cells (Fig. 1b). No flagella were observed (Fig. 1b, c). Cells divided by binary fission (Fig. 1c).

On modified nutrient agar, the colonies of strain T9^T were 0.25–1.25 mm in diameter, smoothly circular, slightly raised, with regular edges and ivory pigmentation.

Growth properties

Strain T9^T is a slightly halophilic bacterium. It grows in liquid media containing 0.5–15% (w/v) NaCl, with optimal growth at 3% (w/v) NaCl. The pH tolerance ranged between pH 6.0 and 8.5, the optimum for growth was pH 7.0. The strain was able to grow at temperatures from 10 to 40 °C, with optimal growth at 30 °C.

Physiological characteristics

Strain T9^T showed anaerobic growth with nitrate as an electron acceptor and acetate as a carbon source. It is an organism which anaerobically reduces nitrate to nitrite, but is not able to produce N₂. Using a reference colour scale (max. 1 g NaNO₂ l⁻¹) in the dot reaction we concluded that almost all of the nitrate supplementation in the medium was reduced to nitrite. However, no production of N₂ or N₂O during growth in the medium supplemented with nitrate was detected by GC.

Tests for aerobic growth on liquid SM (medium without vitamins and mineral salts) supplemented with different carbon sources and ammonium as a nitrogen source, as performed by Gauthier *et al.* (1992), revealed a very restricted spectrum of growth substrates. In SM, only acetate and pyruvate caused detectable cell growth within an incubation time of 14 d (Table 1). Growth on the same carbon sources was also tested using SWM (containing vitamins and trace elements). Besides pyruvate and acetate, strain T9^T also utilized pristane, tetradecane and hexadecane for growth. Using SWM and omitting vitamins and trace elements, we were able to detect the requirement of strain T9^T for both vitamins and trace elements to utilize the hydrocarbons.

Without an added carbon source there was no growth of strain T9^T in SWM. In addition to the growth with

Fig. 1. Transmission electron micrographs of negatively stained strain T9^T cells grown at 25 °C on modified nutrient agar. (a) A 42 h culture showing varying cell sizes; bar, 1.42 µm. (b) A cell from a 4 d culture showing pili; bar, 0.39 µm. (c) A 42 h culture showing binary cell division; bar, 0.21 µm.

Table 1. Phenotypic properties that differentiate *F. jadensis* from other related genera and species of Gram-negative bacteria

+, Positive reaction or growth; d, variable reaction; —, negative reaction or no growth; DR, different results; ND, no data available.

Character	<i>Marinomonas</i> spp.*	<i>Oceanospirillum</i> spp.†	<i>Marinobacter</i> <i>hydrocarbono-</i> <i>clasticus</i> ‡	<i>Fundibacter</i> <i>jadensis</i>
Typical Gram-negative cell wall profile	+	+	+	+
Morphology	Rods	Helical cells	Rods	Rods
No. and arrangement of flagella	1, polar	Bipolar tufts	1, polar, or none	None
Optimal temp (°C)	20–25	25–32	32	30
Growth at 4 °C	—	DR	—	—
Growth at 45 °C	+	—	+	—
Optimal NaCl concn (%)	0.7–3.5	0.5–8	3–6	3
Maximal NaCl concn (%)	ND	9.75	20	15
Nitrate reduced to nitrite	—	—	+	+
Nitrite reduced to nitrogen	—	—	+	—
Oxidase test	—	+	+	+
Catalase reaction	ND	ND	+	+
Utilization of:				
D-Glucose	+	—	—	—
D-Fructose	ND	—	—	—
D-Mannose	+	—	—	—
Sucrose	—	ND	—	—
Cellobiose	DR	—	—	—
D-Mannitol	+	—	—	—
Glycerol	+	—	—	—
D-Gluconate	+	ND	—	—
Succinate	+	+	+	—
L-Glutamate	+	DR	+	—
Aspartate	ND	—	—	—
DL-Alanine	+	DR	—	—
L-Arginine	DR	—	—	—
L-Serine	DR	—	+	—
Pyruvate	ND	d	ND	+
Acetate	ND	d	ND	+
Tetradecane	ND	ND	+	+
Hexadecane	+	ND	+	+
Pristane	ND	ND	+	+
Accumulation of PHB	—	+	—	+
G + C content (mol%)	44–48	42–51	52.7	63.6

* Data from Baumann *et al.* (1984) and Gauthier & Breittmayer (1992).

† Data from Krieg (1984).

‡ Data from Gauthier *et al.* (1992).

different carbon sources, Table 1 summarizes the morphological and the nutritional profiles of strain T9^T and its nearest relatives. All strains listed need NaCl for growth. They were all motile with the exception of strain T9^T. Comparing the four strains, only *Marinomonas* was able to use a variety of sugars as carbon sources. *Oceanospirillum*, *Marinobacter* as well as strain T9^T failed to use most of the carbon sources tested. Strain T9^T accumulated PHB as well as *Oceanospirillum* (Krieg, 1984).

Fatty acid composition

The analysed fatty acids of strain T9^T were as follows: 5.24% dodecanoic acid (C_{12:0}), 1.06% 2-hydroxy-dodecanoic acid (2-OH C_{12:0}), 4.91% 3-hydroxy-dodecanoic acid (3-OH C_{12:0}), 1.73% tetradecanoic acid (C_{14:0}), 0.23% 12-methyl-tetradecanoic acid (a-C_{15:0}), 13.50% *cis*Δ⁹-hexadecenoic acid (Δ⁹-C_{16:1}), 23.40% hexadecanoic acid (C_{16:0}), 20.70% *cis*Δ¹¹-octadecenoic acid (Δ¹¹-C_{18:1}) and 29.23% unidentified

Table 2. Levels (%) of 16S rDNA sequence similarity between strain T9^T and related organisms

Strain	1	2	3	4	5	6	7	8	9	10	11	12	13
1 <i>Fundibacter jadensis</i> strain T9 ^T	—												
2 <i>Marinobacter hydrocarbonoclasticus</i> ATCC 49840 ^T	88.9	—											
3 <i>Oceanospirillum beijerinckii</i> ATCC 12754 ^T	88.5	89.9	—										
4 <i>Oceanospirillum kriegii</i> ATCC 27133 ^T	88.5	87.3	90.4	—									
5 <i>Marinomonas vaga</i> ATCC 27119 ^T	88.4	87.9	89.0	90.9	—								
6 <i>Oceanospirillum multiglobuliferum</i> ATCC 33336	87.8	88.5	95.7	90.4	89.6	—							
7 <i>Oceanospirillum maris</i> ATCC 27649	87.3	88.1	95.8	89.8	89.0	95.6	—						
8 <i>Oceanospirillum japonicum</i> ATCC 19191 ^T	86.7	87.9	89.6	88.1	88.8	89.0	89.7	—					
9 env. Agg53	86.5	86.9	86.9	87.5	88.4	87.5	86.6	85.9	—				
10 <i>Piscirickettsia salmonis</i>	85.0	83.5	84.7	84.9	86.3	84.9	83.9	85.0	83.5	—			
11 <i>Thiomicrospira thyasirae</i> DSM 5322 ^T	84.5	83.0	84.3	84.9	84.7	85.2	84.5	84.0	85.0	83.9	—		
12 <i>Thiomicrospira crunogena</i> ATCC 35932 ^T	83.7	82.1	82.1	84.7	86.5	83.3	82.8	83.0	84.5	85.3	91.2	—	
13 env. SAR92	83.6	83.6	84.9	83.7	84.0	83.8	83.4	82.7	83.0	80.4	80.4	79.5	—

fatty acids. Strain T9^T had a fatty acid profile which featured high proportions of palmitic (C_{16:0}), palmitoleic (Δ⁹-C_{16:1}) and *cis*-vacenic (Δ¹¹-C_{18:1}) acid.

Susceptibility to inhibitors

Strain T9^T is sensitive to chloramphenicol, gentamicin and tobramycin. It is resistant to ampicillin, cefazolin, cephalotin, erythromycin, linomycin, nalidixic acid, novobiocin, ofloxacin, oxacillin, penicillin and tetracycline.

DNA base composition

The G+C content of the DNA is 63.6 mol%, as determined by thermal denaturation.

Phylogenetic analysis

The almost complete 16S rDNA sequence of strain T9^T was determined by the DSMZ. Phylogenetic analysis of the 16S rDNA demonstrated that this bacterium belongs to the class *Proteobacteria*, gamma subclass. This was confirmed by labelling the strain with a 23S rRNA-directed oligonucleotide probe (target position 1027–1043, 5' GCCTTCCCACAT-CGTTT 3').

Construction of a dendrogram (Fig. 2) revealed that strain T9^T is most closely related to species of the *Colwellia* subclass of the gamma-*Proteobacteria* (Maidak *et al.*, 1996). The greatest extent (88.9%) of similarity exists between strain T9^T and *Marinobacter hydrocarbonoclasticus*. Calculating the relationship between strain T9^T and strains of the genera *Marinobacter*, *Oceanospirillum*, *Marinomonas*, *Piscirickettsia* and *Thiomicrospira* as well as between strain T9^T and the isolates env. agg53 (DeLong *et al.*, 1993) and env. SAR92 (Britschgi & Giovannoni, 1991) (Table 2) indicate that strain T9^T has no clear affiliation with any other bacteria of this phylogenetic group.

Fig. 2. Phylogenetic dendrogram based on the results of a 16S rDNA sequence comparison. Comparative sequence data were obtained from the EMBL or RDP databases (Maidak *et al.*, 1996). Bar, 10 nucleotide substitutions per 100 nucleotides.

DISCUSSION

Several methods to clarify the relation of *F. jadensis* strain T9^T to other bacteria were used. In addition to phylogenetic analysis, we determined morphological and physiological characteristics.

The morphology of *F. jadensis* is unusual because of the great variety in the size of the rods. This phenomenon was observed in young (42 h) and old (4 d) cultures and in liquid as well as on solid media. One might think that strain T9^T belongs to the group of budding bacteria, where in some cases the daughter cells are smaller in diameter than the mother cells (Hirsch, 1974). However, as only a binary cell fission was observed, *F. jadensis* cannot be grouped along with the budding bacteria. The cause of the varying cell size of *F. jadensis* is unknown. Several other isolates obtained from mud-flat fermentations and also belonging to the gamma subclass of the *Proteobacteria* showed the same variability. In contrast to its nearest relatives *Marinomonas*, *Oceanospirillum* and *Marinobacter*, strain T9^T is not motile and has no flagella (Table 1). This may be explained by the habitat from which *Fundibacter* was isolated. In a sediment suspension, cells are often attached to particles. On account of such an immobilization, cell motility is not necessary. The *Marinomonas*, *Oceanospirillum* and *Marinobacter* species were originally isolated from seawater (Gauthier & Breittmayer, 1992; Pot *et al.*, 1992; Gauthier *et al.*, 1992), whereas strain T9^T was isolated from a sediment suspension.

The fact that *Marinobacter hydrocarbonoclasticus* and strain T9^T are able to degrade long-chain hydrocarbons like hexadecane may be explained by the presence of hydrocarbons in the environments from which the strains were derived. *Marinobacter hydrocarbonoclasticus* was isolated from the Mediterranean Sea, near a petroleum refinery outlet, and strain T9^T from a continuous suspension culture containing intertidal sediment and hexadecane as the sole carbon source. As the mud flat region is rich in organic substances, one would expect strain T9^T to be an organism that metabolizes a wide range of carbon sources, including amino acids produced by the decomposition of organic material. Strain T9^T was able to metabolize peptone, which contains proteins, amino acids and vitamins, but it was not able to grow when amino acids were presented as the sole carbon source. On the basis of the 17 carbon sources tested (Table 1), we have to conclude that the nutritional profile of strain T9^T is even more restricted than that of the genera *Marinobacter*, *Oceanospirillum* and *Marinomonas*.

Marinobacter hydrocarbonoclasticus, the nearest relative of strain T9^T, is a true denitrifier (Gauthier *et al.*, 1992) whereas *Marinomonas* and *Oceanospirillum* fail to reduce nitrate at all. We therefore tested the denitrifying properties of strain T9^T and discovered that it is able to use nitrate as an electron acceptor under anaerobic conditions. However, strain T9^T is distinguishable from *Marinobacter hydrocarbonoclasticus* because of its capacity to reduce nitrate to nitrite without further reduction to nitrogen. The denitrifying capability of strain T9^T indicates a good adaptation of this organism to its environmental conditions. The intertidal mud flat zone of the North

Sea is characterized by cyclic variations in temperature and by a sharp drop from the aerobic to the anaerobic zone in the sediment within the space of a few centimetres. The temperature changes in summer occur cyclically with the tide between approx. 17 °C in the water and 60 °C when the flats dry and become exposed to the sun. This may explain why the temperature spectrum of strain T9^T reveals a mesophilic and not a psychrophilic organism. The latter could have been expected if the bacteria had been isolated from the open sea.

The fatty acid composition of strain T9^T is characterized by high amounts of palmitic, palmitoleic and *cis*-vaccenic acid. C_{16:0}, C_{16:1} and C_{18:1} fatty acids were also described by Oliver & Colwell (1973) as major fatty acids in the extractable lipids of several Gram-negative marine bacteria. Furthermore, the presence of hydroxy fatty acids is also interesting. The lack of hydroxy fatty acids in *Spirillum linum* (Oliver & Colwell, 1973), formerly known as *Oceanospirillum linum* (Hylemon *et al.*, 1973), and in a deep-sea isolate belonging to the genus *Alteromonas* (Wirsen *et al.*, 1987) which is phenotypically closely related to *Marinomonas*, demonstrates that according to a chemotaxonomic marker like fatty acid profiles, strain T9^T is distinctly different from the genera *Oceanospirillum* and *Alteromonas*. According to Lechevalier (1977), the 3-OH C_{12:0} fatty acid is a typical characteristic of *Pseudomonas* species. Yet the fatty acid profiles of several *Pseudomonas* species described by Moss *et al.* (1972) and by Oliver & Colwell (1973) have a different composition from that of strain T9^T. Thus, we conclude that the fatty acid profile of *F. jadensis* is unique, when compared to other phylogenetically related marine bacteria.

Another substantial feature of strain T9^T is its G+C content, which is 63.6 mol%, and hence considerably higher than that of the phylogenetically related bacteria (52.7 mol% for *Marinobacter*, 44–48 mol% for *Marinomonas* and 42–51 mol% for *Oceanospirillum*). This discounts the inclusion of strain T9^T in the genera *Marinobacter*, *Oceanospirillum* and *Marinomonas*. The G+C content of strain T9^T is more closely related to that of the genera *Pseudomonas* (55–64 mol%) and *Alcaligenes* (52–68 mol%) (Gauthier & Breittmayer, 1992) which also belong to the gamma subclass of the *Proteobacteria*. Moreover, *Pseudomonas nautica* belongs to the *Colwellia*-assemblage of the gamma-*Proteobacteria* (Maidak *et al.*, 1996) as does strain T9^T.

Binding of different rRNA-directed oligonucleotide probes indicated a clear affiliation of strain T9^T to the gamma subclass of the *Proteobacteria*. This was confirmed by the complete analysis of the 16S rDNA. The closest relatives for which 16S rDNA sequences are presently known are *Marinobacter hydrocarbonoclasticus*, *O. beijerinckii*, *O. kriegii* and *Marinomonas vaga*, but there is no clear affiliation to one single species. Since there is a significant distance in the 16S rDNA sequence, and because of the great dissimilarity

in the morphological and physiological traits of strain T9^T compared with other marine bacteria, we propose assigning it to a new species in a new genus, as *Fundibacter jadensis* gen. nov., sp. nov.

Description of *Fundibacter jadensis* gen. nov., sp. nov.

Fundibacter (Fun'di.bac.ter. L. masc. n. *fundus* the base, in this case the sea bed; M.L. n. *bacter*, masc. equivalent of Gr. neut. n. *bakterion* rod or staff; M.L. masc. n. *Fundibacter* rod of the base). *Fundibacter jadensis* (ja.den'sis. M.L. adj. referring to the region Jade which forms part of the bay 'Jadebusen', which belongs to the German North Sea coast).

The cells are rods with sizes varying between 0.8 and 1.8 µm long and 0.3–0.7 µm wide. Colonies are circular, 0.25–1.25 mm in diameter and ivory-pigmented. The cells are Gram-negative, non-motile and possess pili. The temperature spectrum ranges from 10 to 40 °C, with optimal growth occurring at 30 °C. Shows weak halotolerance and grows at NaCl concentrations ranging from 0.5–15% (w/v), optimally at 3% (w/v). It is able to grow both aerobically and anaerobically, and reduces nitrate. It grows with the carbon sources pyruvate and acetate if SM is used and also with tetradecane, hexadecane and pristane, if the medium is supplemented with vitamins and mineral salts. The cells are resistant to ampicillin, cefazolin, cephalotin, erythromycin, linomycin, nalidixic acid, novobiocin, ofloxacin, oxacillin, penicillin and tetracycline. The G + C content of the DNA is 63.6 mol%. According to the 16S rDNA sequence and the rRNA binding to rRNA-directed oligonucleotide probes, *F. jadensis* belongs to the gamma subclass of the *Proteobacteria*. The 16S rDNA sequence of strain T9^T has been deposited at EMBL as AJ001150. The type strain is strain T9^T (= DSM 12178^T).

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