Zobellella denitrificans gen. nov., sp. nov. and *Zobellella taiwanensis* sp. nov., denitrifying bacteria capable of fermentative metabolism

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Two denitrifying strains of heterotrophic, facultatively anaerobic bacteria, designated ZD1^T and ZT1^T, were isolated from sediment samples collected from mangrove ecosystems in Taiwan. The isolates were Gram-negative. Cells grown in broth cultures were straight rods that were motile by means of a single polar flagellum. The isolates grew optimally in 1–3 % NaCl, but NaCl was not an absolute requirement for growth; only strain ZT1^T grew in 13–14 % NaCl. Both isolates grew between 10 and 45 °C, with optimum growth at 30–35 °C. They were capable of anaerobic growth by denitrifying metabolism using nitrate or nitrous oxide as terminal electron acceptors or, alternatively, by fermenting glucose, sucrose or mannitol as substrates. $C_{18:1}\omega7c$ was the most abundant fatty acid (32·6–35·7 %). The other major fatty acids included $C_{16:1}\omega7c$ (27·5–29·4 %) and $C_{16:0}$ (20·1–22·0 %). The two isolates had 16S rRNA gene sequence similarity of 96·8 % and shared 94·1–96·8 % sequence similarity with the most closely related species, *Oceanimonas doudoroffii*, *Oceanimonas baumannii*, *Oceanimonas smirnovii* and *Oceanisphaera litoralis*. They could be distinguished from these species in that they were capable of fermentative metabolism, had relatively high DNA G+C contents (62·0–64·0 mol%) and contained $C_{18:1}\omega7c$ instead of $C_{16:1}\omega7c$ as the most abundant fatty acid. Characterization data accumulated in this study revealed that the two denitrifying isolates could be classified as representatives of two novel species in a new genus, *Zobellella* gen. nov., with *Zobellella denitrificans* sp. nov. (type strain ZD1^T=BCRC 17493^T=JCM 13380^T) as the type species and *Zobellella taiwanensis* sp. nov. (type strain ZT1^T=BCRC 17494^T=JCM 13381^T) as a second species.

Alteromonas-like bacteria belonging to the *Gammaproteobacteria* comprise a large group of marine, heterotrophic, polar-flagellated, Gram-negative rods that are mainly non-fermentative aerobes. Differentiation of these bacteria is impeded at the species level and even at the genus level by their similar phenotypic characteristics. Phylogeny based on 16S rRNA gene sequences, however, has been utilized to classify these bacteria into a variety of families and genera, including *Alteromonadaceae* (*Alteromonas* and *Glaciecola*), *Pseudoalteromonadaceae* (*Pseudoalteromonas* and *Algicola*), *Colwelliaceae* (*Colwellia* and *Thalassomonas*), *Ferrimonadaceae* (*Ferrimonas*), *Idiomarinaceae* (*Idiomarina*), *Moritellaceae* (*Moritella*), *Shewanellaceae* (*Shewanella*), *Psychromonadaceae* (*Psychromonas*) and *Oceanimonas* and *Oceanisphaera* [taxonomic affiliation of *Oceanimonas* and *Oceanisphaera* at the family level remains undetermined (Ivanova *et al.*, 2004)]. Among these genera, only *Ferrimonas*, *Colwellia*, *Moritella* and *Shewanella* are known to include fermentative facultative anaerobes (Ivanova *et al.*, 2004).

Denitrification is the dissimilatory reduction of nitrate or nitrite to the gaseous end product(s) nitrous oxide (N_2O) or dinitrogen gas. Many *Alteromonas*-like species classified as *Shewanella*, *Pseudoalteromonas* and *Ferrimonas* have the ability to reduce nitrate to nitrite. However, only some of them, such as *Shewanella denitrificans* (Brettar *et al.*, 2002), *Shewanella sediminis* (Zhao *et al.*, 2005) and *Shewanella decolorationis* (Xu *et al.*, 2005), are capable of denitrification. These bacteria can achieve anaerobic growth using not only nitrate, but also nitrite or nitrous oxide as the electron acceptor.

Two denitrifying bacterial strains were isolated from sediment samples collected from various estuarine mangrove ecosystems during a survey of diversity of denitrifying bacteria in estuarine and coastal environments in Taiwan. Data from this study indicated that the two isolates could achieve anaerobic growth by undertaking either denitrification or fermentation. Evidence presented in this study also

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains ZD1^T and ZT1^T are DQ195675 and DQ195676, respectively.

Electron micrographs of cells of strains ZD1^T and ZT1^T are available as supplementary material in IJSEM Online.

showed that the two isolates could be classified as the type strains of two species in a new genus belonging to the *Alteromonas*-like *Gammaproteobacteria*.

Polypeptone-yeast extract (PY) broth contained the following constituents (g per l deionized water): polypeptone (Nihon Seiyaku), 3; Bacto yeast extract (Difco), 1; NaCl, 20; $MgCl_2.6H_2O$, 2; $CaCl_2$, 0·005; $Na_2MoO_4.7H_2O$, 0·005; $CuCl_2.2H_2O$, 0·005; g $FeCl_3.6H_2O$, 0·005; and 3-(*N*-morpholino)-2-hydroxypropanesulfonic acid (MOPSO; Sigma-Aldrich), 4·5. The medium was adjusted to pH 7·0. Bacto agar (Difco) was added to this medium at 3 and 15 g l^{-1} for the preparation of stab and plate media, respectively. Polypeptone-yeast extract-nitrate (PYN) broth was prepared by adding KNO_3 at 2 g l^{-1} to PY broth. Polypeptone-yeast extract-glucose (PYG) broth was prepared in two parts. The first part contained 3 g polypeptone, 1 g Bacto yeast extract, 20 g NaCl, 2 g $MgCl_2.6H_2O$, 0·005 g $CaCl_2$, 0·005 g $Na_2MoO_4.7H_2O$, 0·005 g $CuCl_2.2H_2O$, 0·005 g $FeCl_3.6H_2O$ and 4·5 g MOPSO dissolved in 900 ml deionized water and adjusted to pH 7·0. The second part contained 5 g glucose dissolved in 100 ml deionized water. The two parts were autoclaved separately and mixed at room temperature. Carbohydrate-mineral (CM) liquid media were made up of two parts. Part I contained 0·54 g NH_4Cl, 20 g NaCl, 2 g $MgCl_2.6H_2O$, 3 g K_2SO_4, 0·2 g K_2HPO_4, 0·01 g $CaCl_2$, 0·005 g $FeCl_3.6H_2O$, 0·005 g $Na_2MoO_4.7H_2O$, 0·005 $CuCl_2.2H_2O$ and 4·5 g MOPSO dissolved in 900 ml deionized water and adjusted to pH 7·0, and part II contained 5 g glucose or other test carbohydrate (D-arabinose, L-arabinose, cellobiose, galactose, lactose, maltose, mannose, melezitose, melibiose, ribose, starch, sucrose, trehalose, xylose, adonitol, dulcitol, mannitol, *myo*-inositol, sorbitol) dissolved in 100 ml deionized water. The two parts were autoclaved separately and mixed at room temperature.

Sediment samples collected from various estuarine mangrove ecosystems were processed within a few hours. Some wet mass (approx. 5 g) of each sample was vigorously shaken in 95 ml sterile NaCl-MOPSO buffer (20 g NaCl and 0·45 g MOPSO in 1 l deionized water, pH 7·0). The shaken solutions were decimally diluted with the same buffer and a volume (1 ml) of each dilution (10^3–10^5 times) was transferred to a rimless tube (16 mm × 10 cm) containing PYN broth medium (5 ml) in which an inverted Durham insert had been placed. All culture tubes were incubated aerobically at 25 °C in the dark for 3–7 days. Cultures that developed visible turbidity and produced gas (accumulated in Durham inserts) were streaked (one loopful) on PY plate medium. Individual colonies appearing on each plate were picked and purified by successive streaking on PY plates. More than 100 strains that exhibited growth and produced gas in PYN broth were isolated using this enrichment cultivation method. These isolates were maintained in PY stab medium and stored at 25 °C. Two of these isolates, strains ZD1^T and ZT1^T, collected from the estuarine mangrove ecosystems of Chungkang, Miaoli County, and Kuantu, Taipei, respectively, were used for the present study.

Strains ZD1^T and ZT1^T were cultivated aerobically in PY broth at 30 °C in the dark for 2 days. The cultures were centrifuged to harvest the cells. Total genomic DNA was extracted and purified from cells using a Puregene DNA isolation kit (Gentra Systems) in accordance with the manufacturer's instructions. DNA hydration solutions of about 500 μg ml^{-1} were used for PCR amplification. PCR amplification of the bacterial 16S rRNA gene was conducted using a universal primer pair at positions 8–27 and 1488–1510 [*Escherichia coli* numbering system (Shieh *et al.*, 2003a)]. Accessory PCR amplification was performed using either of the primer pairs at positions 8–27 and 685–704 (Lane, 1991) or positions 907–926 and 1488–1511 (Lane, 1991). The PCR mixture contained 2·5 μl DNA hydration solution, 2 μl mixture of one of the primer pairs (5 μM of each primer), 1 μl mixture of the four dNTPs (Gene Teks Bioscience) (2·5 mM each), 5 μl 10× *Taq* buffer (Gene Teks Bioscience), 5 μl 10× BSA (Promega) and 0·5 μl (2 U) *Taq* DNA polymerase (Gene Teks Bioscience). Each sample was made up to 50 μl with sterile distilled water. PCR amplification was performed in a GeneAmp PCR System 2700 (Applied Biosystems) with the following temperature profile: initial denaturation at 94 °C for 10 min, 35 cycles of denaturation (1 min at 92 °C), annealing (1·5 min at 52 °C) and extension (1·5 min at 72 °C) and a final extension at 72 °C for 4 min. Aliquots (2 μl) of the PCR products were checked for size and purity by electrophoresis at 100 V for 30 min on 1 % agarose gels in TAE buffer (MDBio). Gels were stained with ethidium bromide (1 μg ml^{-1}) for 5 min in TAE buffer and DNA bands appearing on the gels were examined under an image analysing system consisting of a UV transilluminator (Spectroline), a dark box (Kodak EDAS 290) and a zoom digital camera (Kodak DC290).

Sequencing of the 16S rRNA genes, alignment and comparison of the resulting sequences and reference sequences available in the GenBank database, calculation of distance matrices for the aligned sequences and reconstruction of a phylogenetic tree by the neighbour-joining method were carried out as described by Shieh *et al.* (2004). Bootstrap confidence values (Felsenstein, 1985) were obtained with 100 resamplings with an option of stepwise addition. Phylogenetic trees were also reconstructed using maximum-parsimony (Fitch, 1971) and maximum-likelihood (Felsenstein, 1981) methods.

Cells grown in PY broth at 30 °C for 2 days were harvested by centrifugation. Total lipids in the cells were extracted by the method of Bligh & Dyer (1959). Individual lipids were separated by two-dimensional TLC in solvent systems described by Vaskovsky & Terekhova (1979). They were detected on the TLC using 10 % H_2SO_4 in methanol with subsequent heating to 180 °C and using 1·3 % molybdenum blue spray reagent (Sigma) for phospholipids, 2 % ninhydrin (Fluka) in acetone for amino-containing lipids and Dragendorf's reagent (Fluka) for choline lipids.

Phospholipids were quantified by the method of Vaskovsky *et al.* (1975). Fatty acids in whole cells grown on PY plate medium at 30 °C for 2 days were extracted, saponified and esterified, followed by GC analysis of the fatty acid methyl esters according to the instructions of the MIDI system (Sasser, 1997). This work was performed at the Bioresources Center for Research and Collection (BCRC), Food Industry Research and Development Institute, Taiwan. The DNA G+C content was determined by HPLC analysis (Shieh & Liu, 1996), which was also performed at the BCRC. DNA–DNA relatedness between strains ZD1^T and ZT1^T was determined as described by Shieh *et al.* (2003a).

Growth and other phenotypic characteristics of strains ZD1^T and ZT1^T were examined by the methods of Shieh *et al.* (2000) with modifications and additional tests as described below. The ability to grow at different temperatures was determined in PY broth and recorded daily for up to 7 days at 15, 20, 25, 30, 35, 40, 45 and 50 °C and for 20 days at 4 and 10 °C, unless significant growth had been observed. Growth in different NaCl concentrations was determined in PY broth containing 0–15 % (w/v) NaCl. Anaerobic growth in PY, PYN and PYG broth media in the presence or absence of N_2O was examined as described by Shieh *et al.* (2004). Growth on various carbohydrates as sole carbon and energy sources was determined in CM media. Denitrifying activity was determined by C_2H_2 blockage and N_2O reduction procedures (Shieh & Liu, 1996). H_2S production from thiosulfate was tested as described by Shieh *et al.* (2004). Urease was determined with modified Christensen urea agar (Smibert & Krieg, 1994) containing 25 g NaCl l^{-1}. Tests for arginine dihydrolase, lysine decarboxylase and ornithine decarboxylase were performed in broth media containing Bacto decarboxylase base Moeller (10·5 g l^{-1}; Difco), NaCl (20 g l^{-1}), $MgCl_2.6H_2O$ (2 g l^{-1}) and the appropriate L-amino acid (10 g l^{-1}). Other constitutive enzyme activities were detected using the API ZYM system (bioMérieux Vitek). Cell suspensions used for these tests were prepared in a mineral medium (0·54 g NH_4Cl, 20 g NaCl, 2 g $MgCl_2.6H_2O$, 3 g K_2SO_4, 0·2 g K_2HPO_4, 0·01 g $CaCl_2$, 0·005 g $FeCl_3.6H_2O$, 0·005 g $Na_2MoO_4.7H_2O$, 0·005 g $CuCl_2.2H_2O$ and 4·5 g MOPSO, dissolved in 1000 ml deionized water and adjusted to pH 7·0). Antibiotic susceptibility tests were performed by disc diffusion methods as described previously (Shieh *et al.*, 2003a, b). All test cultures were incubated aerobically at 30 °C in the dark for 7 days, unless stated otherwise.

Effects of pH, temperature and NaCl on growth were determined in PY broth under aerobic conditions. Strains ZD1^T and ZT1^T grew at pH 6–10, with optimum growth at pH 7–8. No growth was observed at pH 5·0. Growth was observed at 10–45 °C, with optimum growth at 30–35 °C. Both strains grew in 0–12 % NaCl, but only strain ZT1^T grew in 13–14 % NaCl. Although NaCl was not indispensable for growth, optimal growth was observed at 1–3 % NaCl.

Strains ZD1^T and ZT1^T exhibited good growth in PYN (maximum OD_{600} 1·02–1·05) and PYG (maximal OD_{600} 0·40–0·44) broth media under anaerobic conditions, whereas anaerobic growth in PY broth was relatively weak (maximal OD_{600} 0·14–0·17) unless N_2O was present in the culture systems (maximum OD_{600} 0·34–0·73) (Fig. 1). This indicated a lack of sufficient NO_3^- and fermentable substrates for the strains in PY broth cultures. The results also suggested that the strains were capable of anaerobic growth by carrying out denitrifying metabolism with NO_3^- or N_2O as the terminal electron acceptor. Anaerobic growth in PYG broth was accompanied by a remarkable decrease in the pH of the medium, from pH 7·0 to 5·4 within 72 h (data not shown), regardless of the large buffer content (approx. 20 mM MOPSO) in the medium. The strains could have achieved anaerobic growth in PYG by fermenting glucose, with considerable production of organic acids. In addition to glucose, both strains also showed anaerobic growth by fermenting sucrose or mannitol as substrates (data not shown).

Almost complete 16S rRNA gene sequences of strains ZD1^T and ZT1^T were determined. They were aligned and compared with all bacterial sequences available in the GenBank database. The two sequences shared 96·8 % similarity (48 differences out of 1478 nt positions). The closest relatives of strains ZD1^T and ZT1^T were species of *Oceanimonas* and *Oceanisphaera*, including *Oceanimonas doudoroffii*,

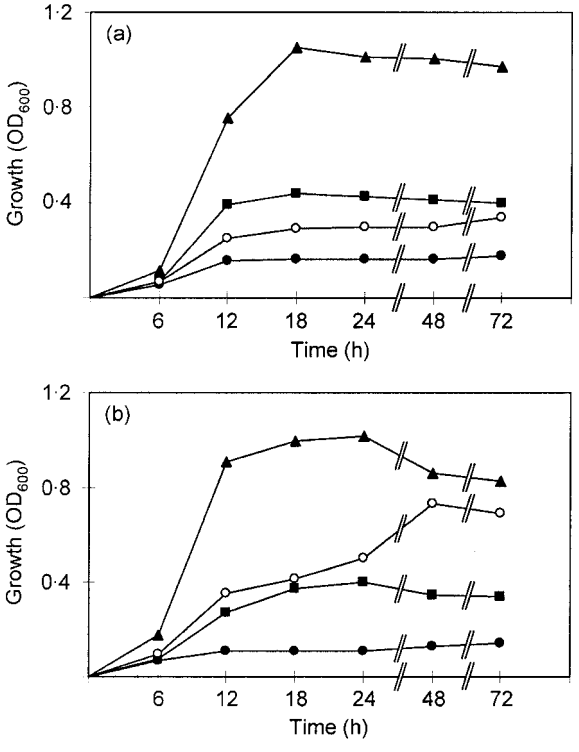

Fig. 1. Changes in OD_{600} during anaerobic growth of strains ZD1^T (a) and ZT1^T (b) in PY (●, ○), PYN (▲) and PYG (■) broth media under argon in the presence (○) or absence (●, ▲, ■) of N_2O (102·4 μmol per tube).

Oceanimonas baumannii, *Oceanimonas smirnovii* and *Oceanisphaera litoralis*. The corresponding overall 16S rRNA gene sequence similarities between the strains and these species were 94·1–96·8 %. No other known bacteria shared more than 93 % sequence similarity with the two isolates. A neighbour-joining tree (Fig. 2) shows the phylogenetic positions between strains ZD1^T and ZT1^T and species of *Oceanimonas* and *Oceanisphaera* and other related taxa belonging to the *Alteromonas*-like *Gammaproteobacteria*. Similar results were obtained from maximum-likelihood and maximum-parsimony algorithms (not shown). The 16S rRNA gene-based phylogeny revealed that the two isolates could be classified either as different species in a novel genus or as novel species of *Oceanimonas* (Fig. 2).

Strains ZD1^T and ZT1^T had DNA G+C contents of 64·0 and 62·0 mol%, respectively, which are greater than those of species of *Oceanimonas* (55·6–59·0 mol%; Ivanova *et al.*, 2005) and *Oceanisphaera* (56·4 mol%; Romanenko *et al.*, 2003). Both *Oceanimonas doudoroffii* and *Oceanimonas baumannii* had a G+C content of 54·0 mol% according to Brown *et al.* (2001), whereas the G+C contents of the two determined by Ivanova *et al.* (2005) were 59·0 and 57·0 mol%, respectively; this discrepancy between the G+C contents remains to be clarified. Strains ZD1^T and ZT1^T, like species of *Oceanimonas* and *Oceanisphaera*, contained $C_{16:0}$, $C_{16:1}\omega7c$ and $C_{18:1}\omega7c$ as the major cellular fatty acids (Table 1). However, the most abundant fatty acid of the isolates was $C_{18:1}\omega7c$ (32·6–35·7 %), whereas it was $C_{16:1}\omega7c$ (40·1–45 %) in species of *Oceanimonas* and *Oceanisphaera*. Moreover, only the novel isolates contained $C_{14:0}$ 3-OH (6·4–6·6 %). Both isolates contained phosphatidylethanolamine (47·2–51·0 %), phosphatidylglycerol (40·8–41·7 %) and diphosphatidylglycerol (8·2–11·1 %) as the major polar lipids. These polar lipids also accounted for the total phospholipids in *Oceanimonas* (Brown *et al.*, 2001; Ivanova *et al.*, 2005) and *Oceanisphaera* (Romanenko *et al.*, 2003). However, relatively low levels of diphosphatidylglycerol (trace to 3·1 %) were detected in *Oceanimonas doudoroffii* and *Oceanimonas baumannii* (Brown *et al.*, 2001).

Table 1. Cellular fatty acid contents (%) of strains ZD1^T and ZT1^T and species of *Oceanimonas* and *Oceanisphaera*

Strains: 1, ZD1^T (data from this study); 2, ZT1^T (this study); 3, *Oceanimonas doudoroffii* ATCC 27123^T (Brown *et al.*, 2001); 4, *Oceanimonas baumannii* ATCC 700832^T (Brown *et al.*, 2001); 5, *Oceanimonas smirnovii* 31-13^T (Ivanova *et al.*, 2005); 6, *Oceanisphaera litoralis* KMM 3654^T (Romanenko *et al.*, 2003). −, Not detected.

Fatty acid	1	2	3	4	5	6
$C_{12:0}$	5·3	4·8	7·0	3·0	1·5	−
$C_{12:0}$ 3-OH	0·2	−	−	−	−	−
$C_{14:0}$	0·6	0·9	−	−	0·6	−
$C_{14:0}$ 3-OH	6·6	6·4	−	−	−	−
$C_{15:0}$ anteiso	−	−	−	−	0·5	−
$C_{15:0}$	−	−	−	−	0·6	1·8
$C_{16:1}\omega7c$	27·5	29·4	42·0	45·0	40·1	41·0
$C_{16:0}$	20·1	22·0	21·2	20·0	24·1	21·6
$C_{16:0}$ iso	−	−	−	−	−	1·6
$C_{17:0}$	−	−	−	−	0·7	1·9
$C_{17:0}$ anteiso	−	−	−	−	0·9	−
$C_{17:1}\omega8c$	−	−	−	−	−	1·9
$C_{18:0}$	1·1	2·0	−	−	0·9	−
$C_{18:1}\omega7c$	35·7	32·6	29·9	32·0	29·2	27·5
$C_{20:1}\omega7c$	0·2	0·2	−	−	−	−

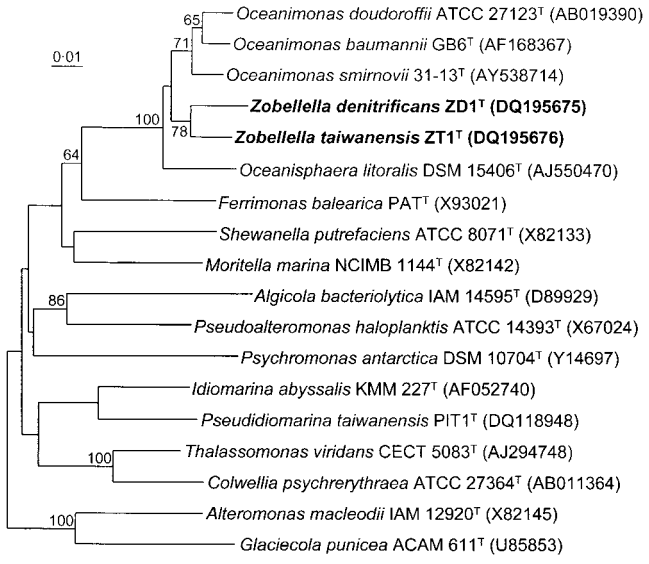

Fig. 2. Unrooted phylogenetic tree derived from neighbour-joining analysis of the 16S rRNA gene sequences of strains ZD1^T and ZT1^T and related genera of the *Alteromonas*-like *Gammaproteobacteria*. GenBank accession numbers are given in parentheses. Numbers above nodes represent bootstrap confidence values obtained with 100 resamplings; values below 60 are not shown. Bar, 1 % estimated sequence divergence.

Based on G+C contents, profiles of fatty acids and polar lipids and 16S rRNA gene-based phylogeny, it is proposed that strains ZD1^T and ZT1^T represent two novel species in a new genus rather than novel species of *Oceanimonas* or *Oceanisphaera*. The DNA relatedness value between strains ZD1^T and ZT1^T was 58·5 % when genomic DNA of the former strain was used as a probe. The strains could be classified as two different genomic species according to this data.

Strains ZD1^T and ZT1^T were Gram-negative and oxidase- and catalase-positive. Colonies produced on PY plate medium were circular, off-white and non-luminescent. Cells grown in PY broth, which appeared to be straight, motile rods, normally possessed a single polar flagellum, as revealed by TEM (see electron micrographs available as Supplementary Fig. S1 in IJSEM Online). Both strains were facultative anaerobes capable of fermenting glucose, sucrose, ribose, maltose, melezitose, starch, mannitol and

myo-inositol. Their activity to reduce NO_3^- to N_2O and further to N_2 was detected by the C_2H_2 blockage and N_2O reduction procedures. Additional phenotypic characterization data are given below in the species descriptions.

Phenotypically, strains ZD1^T and ZT1^T could be differentiated from each other by different reactions in several phenotypic tests, including growth in 14 % NaCl, fermentation of galactose, melibiose and trehalose and utilization of galactose, melibiose, trehalose and adonitol as sole carbon and energy sources. Both strains could be distinguished from species of *Oceanimonas* and *Oceanisphaera* in that they were capable of fermentative metabolism. The ability to grow in the absence of NaCl also distinguished the strains from these species, with the exception of *Oceanimonas smirnovii*. Other phenotypic characteristics useful for differentiating strains ZD1^T and ZT1^T from species of *Oceanimonas* and *Oceanisphaera* are listed in Table 2.

Phylogenetic, chemotaxonomic and phenotypic data accumulated in this study strongly support the establishment of two different species in a novel genus. It is, therefore, proposed that strains ZD1^T and ZT1^T should be classified in the genus *Zobellella* gen. nov., as the type strains of *Zobellella denitrificans* sp. nov. and *Zobellella taiwanensis* sp. nov., respectively, with *Zobellella denitrificans* as the type species.

Table 2. Characteristics useful for differentiating strains ZD1^T and ZT1^T from species of *Oceanimonas* and *Oceanisphaera*

Strains: 1, ZD1^T (data from this study); 2, ZT1^T (this study); 3, *Oceanimonas doudoroffii* ATCC 27123^T (Brown *et al.*, 2001); 4, *Oceanimonas baumannii* ATCC 700832^T (Brown *et al.*, 2001); 5, *Oceanimonas smirnovii* 31-13^T (Ivanova *et al.*, 2005); 6, *Oceanisphaera litoralis* KMM 3654^T (Romanenko *et al.*, 2003). +, Positive; −, negative; W, weakly positive; ND, no data available. All species grow at 10–40 °C and in 3 % NaCl.

Characteristic	1	2	3	4	5	6
Cell shape	Rod	Rod	Rod	Rod	Rod	Coccus
Growth at:						
4 °C	−	−	−	−	−	+
45 °C	+	+	−	−	+	−
Growth in NaCl at:						
0 %	+	+	−	−	+	−
9 %	+	+	−	−	+	+
12 %	+	+	−	−	+	−
14 %	−	+	−	−	−	−
Denitrification	+	+	−	ND	−	−
Urease	W	+	−	−	ND	+
Fermentation of:						
Glucose	+	+	−	−	−	−
Cellobiose	+	+	−	−	−	−
Galactose	+	−	−	−	−	−
Mannose	+	+	−	−	−	−
Melibiose	+	−	−	−	−	−
Trehalose	+	−	−	−	−	−
Sorbitol	+	+	−	−	−	−
Utilization of:						
Glucose	+	+	−	−	+	−
Galactose	+	−	−	+	−	−
Maltose	+	+	−	−	+	−
Mannitol	+	+	−	−	+	−
Mannose	+	+	−	−	−	−
Sucrose	+	+	−	−	+	−
Trehalose	+	−	ND	ND	+	−
Adonitol	−	+	ND	ND	−	−
Fatty acids (% of total)						
$C_{18:1}\omega7c$	35·7	32·6	27·5	29·9	29·2	32·0
$C_{16:1}\omega7c$	27·5	29·4	41·0	42·0	40·1	45·0
$C_{14:0}$ 3-OH	6·6	6·4	−	−	−	−
DNA G+C content (mol%)*	64·0	62·0	54·0 (59·0)	54·0 (57·0)	55·6	56·4

*Data in parentheses are from Ivanova *et al.* (2005).

Zobellella denitrificans and *Zobellella taiwanensis* are the only species in the *Alteromonas*-like *Gammaproteobacteria* that possess DNA G+C contents greater than 60 mol%. They have so far been found only in the sediment of estuarine mangrove forests. However, their tolerance of rather wide ranges of temperatures and salinities and ability to grow in the absence of oxygen and organic growth factors suggest that these bacteria may also occur in other habitats, including saline and non-saline environments.

Description of *Zobellella* gen. nov.

Zobellella (Zo.bell.el′la. N.L. dim. ending *-ella*; N.L. fem. n. *Zobellella* named after C. E. ZoBell, a pioneer marine microbiologist).

Members are heterotrophic, Gram-negative rods belonging to the *Gammaproteobacteria*. Cells grown in broth cultures are motile by means of a single, polar flagellum. NaCl stimulates growth, but is not an absolute requirement. Facultative anaerobes capable of both respiratory and fermentative metabolism. Mesophilic; grow optimally at 30–35 °C, but do not grow at 4 or 50 °C. Oxidase- and catalase-positive. $C_{18:1}\omega7c$ is the most abundant fatty acid; $C_{16:1}\omega7c$ and $C_{16:0}$ are the next most abundant fatty acids. Major constituents of polar lipids are the phospholipids phosphatidylglycerol, phosphatidylethanolamine and diphosphatidylglycerol. The DNA G+C contents of the strains so far examined are 62·0–64·0 mol%. The type species is *Zobellella denitrificans*.

Description of *Zobellella denitrificans* sp. nov.

Zobellella denitrificans (de.ni.tri′fi.cans. N.L. v. *denitrifico* to denitrify; N.L. part. adj. *denitrificans* denitrifying).

Description is as for the genus with the following additional characteristics. Cells during late exponential to early stationary phase of growth in broth cultures are straight rods, approximately 1·6–2·6 μm long by 0·6–0·8 μm wide. Colonies produced on PY agar plates at 30 °C for 48–60 h are approximately 1·5–4·0 mm in diameter, circular, off-white and non-luminescent, with an entire edge. Swarming does not occur. Capable of complete denitrification, i.e. capable of reducing NO_3^- to N_2 via NO_2^- and N_2O. Able to ferment glucose, cellobiose, galactose, maltose, mannose, melezitose, melibiose, ribose, sucrose, trehalose, starch, mannitol, *myo*-inositol and sorbitol with production of acid, but no gas. Unable to ferment D-arabinose, L-arabinose, lactose, xylose or dulcitol. Growth occurs at 10–45 °C, with optimum growth at 30–35 °C. Growth occurs at pH 6–10, with optimum growth at pH 7–8. Growth occurs in 0–12 % NaCl, with optimum growth in 1–3 %; no growth is observed in 13–14 % NaCl. Weakly positive for urease. Negative for arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, agarase, amylase, DNase, gelatinase and lipase. H_2S is not produced from thiosulfate. Indole is not produced from tryptophan. Cellobiose, glucose, galactose, maltose, mannose, mannitol, melezitose, melibiose, *myo*-inositol, ribose, sorbitol, starch, sucrose and trehalose can be utilized as sole carbon and energy sources, but D-arabinose, L-arabinose, lactose, xylose, adonitol and dulcitol cannot. The following constitutive enzyme activities are detected in API ZYM tests: acid phosphatase, alkaline phosphatase, esterase (C4), esterase lipase (C8), α-galactosidase, α-glucosidase, leucine arylamidase, naphthol-AS-BI-phosphohydrolase, trypsin and valine arylamidase. Susceptible to ampicillin (10 μg), carbenicillin (100 μg), cephalothin (30 μg), chloramphenicol (30 μg), colistin (10 μg), gentamicin (10 μg), nalidixic acid (30 μg), neomycin (30 μg), penicillin G (10 U), polymyxin B (300 U) and tetracycline (30 μg); intermediate susceptibility to kanamycin (30 μg); resistant to clindamycin (2 μg), erythromycin (15 μg), lincomycin (2 μg), novobiocin (30 μg), oxacillin (1 μg), streptomycin (10 μg) and vancomycin (30 μg).

The type strain is ZD1^T (=BCRC 17493^T=JCM 13380^T), isolated from a sediment sample collected from the estuarine mangrove ecosystem of Chungkang, Miaoli County, Taiwan. It has a DNA G+C content of 64·0 mol%.

Description of *Zobellella taiwanensis* sp. nov.

Zobellella taiwanensis (tai.wan.en′sis. N.L. fem. adj. *taiwanensis* pertaining to Taiwan, where the type strain was isolated).

Description is as for the genus and the species description of *Zobellella denitrificans* with the following modifications. Growth occurs in 0–14 % NaCl, with optimum growth at 1–3 %. Cells are 1·0–1·9 μm long by 0·6–0·7 μm wide. Positive for urease test. Unable to ferment galactose, melibiose or trehalose. Adonitol can be utilized as a sole carbon and energy source, but not galactose, melibiose or trehalose. In API ZYM tests, α-galactosidase, esterase lipase (C8), naphthol-AS-BI-phosphohydrolase and trypsin activities are not detected. Resistant to ampicillin (10 μg), carbenicillin (100 μg) and penicillin G (10 U).

The type strain is ZT1^T (=BCRC 17494^T=JCM 13381^T), isolated from a sediment sample collected from the estuarine mangrove ecosystem of Kuantu, Taipei, Taiwan. It has a DNA G+C content of 62·0 mol%.

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