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Vibrio aerogenes sp. nov., a facultatively anaerobic marine bacterium that ferments glucose with gas production

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A mesophilic, facultatively anaerobic, marine bacterium, designated strain FG1^T, was isolated from a seagrass bed sediment sample collected from Nanwan Bay, Kenting National Park, Taiwan. Cells grown in broth cultures were motile, Gram-negative rods; motility was normally achieved by two sheathed flagella at one pole of the cell. Strain FG1^T required Na^+ for growth, and exhibited optimal growth at 30–35 °C, pH 6–7 and about 4% NaCl. It grew anaerobically by fermenting glucose and other carbohydrates with production of various organic acids, including acetate, lactate, formate, malate, oxaloacetate, propionate, pyruvate and succinate, and the gases CO_2 and H_2. The strain did not require either vitamins or other organic growth factors for growth. Its DNA G+C content was 45·9 mol%. It contained C12:0 as the most abundant cellular fatty acid. Characterization data, together with the results of a 16S rDNA-based phylogenetic analysis, indicate that strain FG1^T represents a new species of the genus *Vibrio*. Thus, the name *Vibrio aerogenes* sp. nov. is proposed for this new bacterium. The type strain is FG1^T (= ATCC 700797^T = CCRC 17041^T).

Keywords: *Vibrio aerogenes* sp. nov., marine bacterium, facultative anaerobe

INTRODUCTION

Bacteria inhabiting the marine environment include halophiles and non-halophiles. Only the halophiles are considered to be autochthonous organisms, i.e. true marine bacteria. The non-halophilic ones tolerate the salinity of seawater but are not considered to be of marine origin since they do not require Na^+ for growth and are always found in a non-saline environment. Most halophilic marine bacteria are either mesophilic or psychrophilic Gram-negative rods that require 70–700 mM Na^+ for optimal growth and yield in laboratory media (Baumann & Baumann, 1971). A large proportion of these bacteria are facultative anaerobes that can ferment glucose and other carbohydrates for anaerobic growth. Marine bacteria of this type are currently placed in the genera *Vibrio* (Baumann *et al*., 1984), *Photobacterium* (Baumann & Baumann, 1984), *Listonella* (MacDonell & Colwell, 1985) and *Colwellia* (Deming *et al*., 1988) of the family *Vibrionaceae* (Baumann & Schubert, 1984). These bacteria generally constitute 10–50% of heterotrophic bacteria from coastal and oceanic seawater samples that grow on ordinary plate media used in marine bacteriology (Simidu & Tsukamoto, 1985). They are also found to be closely associated with many kinds of marine organisms from plankton to fish (Cerdà-Cuéllar *et al*., 1997; Liston, 1956; MacDonald *et al*., 1986; Nair *et al*., 1988; Onarheim *et al*., 1994; Simidu *et al*., 1969, 1971; Sochard *et al*., 1979; Yoshimizu *et al*., 1976). Some species are found as symbionts in specialized luminous organs of marine fish and invertebrates (Lee & Ruby, 1994; Leisman *et al*., 1980; McFall-Ngai & Ruby, 1991; Reichelt *et al*., 1977; Ruby & Asato, 1993; Ruby & Morin, 1978), whereas quite a few other species are well-known pathogens for humans or marine animals (Blake *et al*., 1980; Egidus *et al*., 1986; Hada *et al*., 1984; Holt *et al*., 1994; Love *et al*., 1981; Schiewe *et al*., 1981). These halophilic, facultatively anaerobic, Gram-negative rods are presently known to include both nitrogen fixers and

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Abbreviations: MOPSO, 3-(*N*-morpholino)-2-hydroxysulfonic acid; PHB, poly-β-hydroxybutyrate.

The GenBank accession number for the 16S rDNA sequence of strain FG1^T is AF124005.

denitrifiers (Guerinot & Colwell, 1985; Guerinot *et al.*, 1982; Shieh & Lin, 1992, 1994; Shieh & Liu, 1996; Shieh & Yang, 1997; Shieh *et al.*, 1989, 1990; West *et al.*, 1985), although *Bergey's Manual of Systematic Bacteriology* defined in 1984 that they include neither of the two (Baumann & Baumann, 1984; Baumann & Schubert, 1984; Baumann *et al.*, 1984).

Production of gas during glucose fermentation is a characteristic commonly found among the non-halophilic, facultatively anaerobic, Gram-negative rods belonging to either *Enterobacteriaceae* (Brenner, 1984) or *Aeromonadaceae* (Colwell *et al.*, 1986). It is, therefore, intriguing why the halophilic, facultatively anaerobic, Gram-negative rods that also possess this property are not prevalent. *Vibrio gazogenes*, *Vibrio furnissii* (formerly *Vibrio fluvialis* biovar 2), *Vibrio mytili*, *Photobacterium histaminum*, *Photobacterium iliopiscarium*, *Photobacterium phosphoreum* and *Listonella damsela* (reclassified as *Photobacterium damselae* subsp. *damselae*) are the only cases among the more than forty validly described species of these bacteria that produce gas during the fermentation of glucose (Baumann & Baumann, 1984; Baumann & Schubert, 1984; Baumann *et al.*, 1984; Brenner *et al.*, 1983; Holt *et al.*, 1994; Okuzumi *et al.*, 1994; Onarheim *et al.*, 1994; Pujalte *et al.*, 1993; Urakawa *et al.*, 1999).

A new strain of halophilic, facultatively anaerobic, Gram-negative rod has been recovered in this laboratory from a sediment sample collected in a seagrass bed in Nanwan Bay, Kenting National Park, Taiwan. The present study shows that the new strain can achieve anaerobic growth by fermenting glucose and other carbohydrates with the production of various organic acids and H_2 and CO_2. Evidence presented in the study also shows that the strain represents a new species of *Vibrio*, for which the name *Vibrio aerogenes* is proposed.

METHODS

Culture media. Polypeptone-yeast (PY) broth contained the following ingredients (l^{-1} deionized water): 3 g Polypepton (Nihon Seiyaku); 1 g Bacto yeast extract (Difco); 25 g NaCl; and 5 g $MgCl_2.6H_2O$. The medium was adjusted to pH 7·8. Bacto agar (Difco) was added to this medium at 3·5 and 15 g l^{-1}, for the preparation of stab and plate media, respectively. Polypepton-yeast-nitrate (PYN) broth was prepared by adding KNO_3 at 0·1 g l^{-1} to PY broth. Tryptone broth contained 10 g tryptone (Sigma), 25 g NaCl and 5 g $MgCl_2.6H_2O$ dissolved in 1000 ml deionized water and adjusted to pH 7·8. Polypepton-yeast-carbohydrate (PYC) stab media were prepared from two parts. The first contained 3 g polypeptone, 1 g Bacto yeast extract, 25 g NaCl, 5 g $MgCl_2.6H_2O$, 0·24 g Tris (Sigma), 0·03 g bromothymol blue and 10 g Bacto agar dissolved in 900 ml deionized water and adjusted to pH 7·8. The second contained 5 g glucose (or any carbohydrate) dissolved in 100 ml deionized water. The two parts were autoclaved separately and mixed at about 50 °C. Glucose-mineral (GM) medium was also made up of two parts. Part 1 contained 0·54 g NH_4Cl, 25 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 $FeCl_3.6H_2O$ and 3 g (ca. 25 mmol) Tris dissolved in 900 ml deionized water and adjusted to pH 8·0, while part 2 contained 5 g glucose dissolved in 100 ml deionized water. The two parts were autoclaved separately and mixed at room temperature. However, GM-II medium differed from GM medium in that part 1 contained 5·4 g l^{-1} (ca. 25 mM) 3-(*N*-morpholino)-2-hydroxysulfonic acid (MOPSO; Sigma) instead of Tris and was adjusted to pH 7·0.

A modified PY plate medium containing $CaCl_2$ (0·1 g l^{-1}) and Tween 80 (0·1 %) was used for the lipase test. Four other modified PY plate media containing casein (4 g l^{-1}), DNA (2 g l^{-1}), gelatin (5 g l^{-1}) and starch (5 g l^{-1}), respectively, were also used to test the hydrolysis of these substrates. In addition, three stab media modified from Thornley's semi-solid arginine medium (Smibert & Krieg, 1994) were used for the tests of arginine dihydrolase, and lysine and ornithine decarboxylases. They contained the following ingredients (l^{-1} deionized water): 2 g Bacto peptone (Difco); 25 g NaCl; 5 g $MgCl_2.6H_2O$; 0·01 g phenol red; 3 g Bacto agar (Difco); and 5 g L-arginine, 5 g L-lysine or 5 g L-ornithine. All three media were adjusted to pH 7·0. All the pH adjustments of the above-mentioned media were made with HCl (1·0 M), KOH (1·0 M) or both.

Bacterial isolation. Nanwan Bay of Kenting National Park is located on the south coast of Taiwan, where fringing reefs are widely distributed. *Thalassia hemprichii* and *Halodule uninervis* are two seagrass species found to co-habit in a seagrass bed of this bay. Non-rhizosphere sediment samples were collected from the seagrass bed in the morning at low tide. Some wet mass (2 g) of each sediment sample was vigorously shaken in sterile NaCl-Tris buffer (30 g NaCl and 0·24 g Tris in 1 l deionized water, pH 8·0) containing Tween 80 (2 p.p.m.), and the shaken solutions were decimally diluted with the same buffer. A volume (1 ml) of each dilution (10^5–10^7 times) was transferred to a rimless tube (16 mm × 10 cm) containing GM medium (5 ml) in which an inverted Durham insert had been placed. All culture tubes were set in anaerobic jars (Difco) and incubated at 25 °C in the dark for 7 d. The air in each anaerobic jar was replaced by Ar before incubation. Those cultures that developed visible turbidity and produced gas (accumulated in Durham inserts) were streaked (one loopful) on PY plate medium and the plates were incubated at 25 °C in the dark for 2–3 d under air. Individual colonies appearing on the plates were picked off and purified by successive streaking on PY plates. One bacterial strain, capable of growth with gas production in GM medium under Ar, was isolated for the present study using the above-mentioned procedures. This bacterium was designated as strain FG1^T and its PY stab cultures were kept at 20 °C under aerobic conditions for maintenance.

Bacterial growth. Inoculum cultures of strain FG1^T were routinely grown in PY broth. Late exponential to early stationary phase cultures (one loopful) were inoculated into tubes containing 5 ml of the test media to determine the effects of various factors on growth. The cultures were statically incubated under either aerobic or anaerobic conditions. Anaerobic conditions were developed by flushing the culture tubes for more than 5 min with Ar gas of 99·99 % purity. The tubes were sealed with rubber stoppers that were pierced with two hypodermic needles; one needle was connected to the pipeline of Ar gas for flushing while the other was used for exhausting. Each tube was shaken vigorously on a mixer during the flushing process, after which the needles were pulled out of the stoppers. The OD_{600} of each culture was measured using a Spectronic 20

spectrophotometer (Milton Roy Company). The measurement was taken every 12–24 h until the maximal OD_{600} of each culture was passed. Specific growth rates were calculated from changes in the OD_{600} of the cultures during the exponential phase of growth. All growth experiments were performed at 30 °C in the dark unless otherwise stated. All the results presented here represent means of at least three replicates.

Bacterial characterization. Cells grown for 2–3 d on PY plate medium were used for agarase, luminescence, catalase, oxidase and swarming tests according to the procedures of Shieh *et al.* (1989). Gram reaction was determined by staining (Smibert & Krieg, 1994) and by the KOH lysis method (Buck, 1982). Cells grown for 1–3 d in GM-II medium were used for the test of poly-*β*-hydroxybutyrate (PHB) accumulation by a staining method (Smibert & Krieg, 1994). Caseinase, DNase, gelatinase and lipase tests essentially followed the methods of Smibert & Krieg (1994). Indole production was determined by Kovacs' method (Smibert & Krieg, 1994) after incubating the tryptone broth cultures for 2–3 d. Cultures grown in PYN broth were examined daily for nitrate reduction (Collins *et al.*, 1995) and denitrification (Shieh *et al.*, 1997) for up to 5 d. Sensitivity to the vibriostatic agent O/129 phosphate was tested by a disc-sensitivity method on PY plates. Any inhibition zones around the discs were read as sensitive (Baumann *et al.*, 1984). Cells grown for 2 d in PY broth and GM-II medium were observed for their shape and motility by phase-contrast microscopy, and fixed with glutaraldehyde (3%, v/v) and negatively stained with potassium phosphotungstate (1%, w/v; pH 7·0) for transmission electron microscopy.

Cells grown on PY plate medium were taken with a straight needle for inoculation into each of the PYC stab media for tests of acid production from fermentation of various carbohydrates. The surface of the medium in each tube was overlaid with sterile liquid paraffin after inoculation. The cultures were examined daily for colour changes for 7 d. Gas production was indicated by formation of gas bubbles or cracks in the medium, or by the separation of medium from the side or bottom of the tube. The cultures for tests of arginine dihydrolase and lysine and ornithine decarboxylases were also incubated under anaerobic conditions by overlaying the surface of the medium in each tube with sterile liquid paraffin after inoculation. A positive reaction was indicated by a colour change of the culture from yellow–orange to red within 7 d due to an increase in the pH value. The ability to grow at different temperatures was determined in GM-II medium and recorded after incubating the cultures for 20 d at 4 and 15 °C, and 2–7 d at 20–42 °C. The ability to grow in various NaCl concentrations was determined in GM-II medium containing 0–10% of NaCl. Utilization of various compounds as sole carbon and energy sources for growth was determined in modified GM-II media containing 0·2% (w/v) of any one of the test substrates used in place of glucose. Nitrogen source utilization was also determined in GM-II medium and its modifications containing KNO_3 at 20 mM or any one of the test amino acids at 2 g l^{-1} rather than NH_4Cl. All the test cultures were incubated at 25 °C in the dark for 7 d under aerobic conditions unless stated otherwise.

Antibiotic susceptibility. Susceptibility to various antibiotics was determined with standard 6 mm discs (Difco). The discs were placed on PY plate medium that had been spread with broth culture (0·1 ml) of strain FG1^T. Inhibition zones of growth around the discs were noted after incubating the plates at 25 °C for 24–30 h. They were interpreted as either susceptible or resistant with reference to standard data (NCCLS, 1990).

DNA base composition. This determination essentially followed the procedure described by Shieh & Liu (1996).

Cellular fatty acids. Fatty acids in cells from early stationary phase cultures grown in GM-II medium were extracted, saponified and methylated according to Suutari *et al.* (1990). GLC analysis of the fatty acid methyl esters was performed on a GC-14A (Shimadzu) equipped with an FID and a fused silica capillary column (Shieh & Jean, 1998).

Fermentation products. Strain FG1^T was anaerobically cultivated in GM-II medium under Ar. Cultures grown for 24–60 h were centrifuged to remove the cells. The supernatant samples, after filtering through a Nucleopore membrane (pore size, 0·22 μm), were analysed for organic acids produced during fermentative growth of the cultures by an HPLC equipped with an Interaction Ion-300 column (Shieh & Jean, 1998). Production of CO_2 and H_2 gases in the headspace of the GM-II culture system was, meanwhile, detected by GC using a GC-14A equipped with a thermal-conductivity detector and with either connected columns of Porapak Q and N (both 3 mm × 2 m; for CO_2 analysis) or a column of molecular Sieve 5A (3 mm × 2 m; for H_2 analysis) at 70 °C. Production of acetoin or butanediol in GM-II cultures was determined by the Voges–Proskauer reaction (Smibert & Krieg, 1994).

16S rRNA gene sequencing and phylogenetic dendrogram construction. A method similar to that of Hiraishi (1992) was used for PCR amplification and sequencing of the 16S rRNA gene of strain FG1^T. The 16S rDNA sequence was aligned manually with those of reference bacterial strains available in the GenBank database. The aligned positions from 33 to 1398 (by *Escherichia coli* sequence J01859 numbering) that were unambiguous and available for the sequences were used for the phylogenetic analysis. Distance matrices were calculated with the PHYLIP program DNADIST using the Jukes & Cantor (1969) model by assuming a transition/transversion ratio of 2·0; a neighbour-joining phylogenetic dendrogram was then constructed according to the estimated evolutionary distances (Felsenstein, 1989).

Nucleotide sequence accession numbers. The accession numbers for the sequences used to construct the phylogenetic dendrogram are as follows: *Vibrio aestuarianus* ATCC 35048^T, X74689; *Vibrio alginolyticus* ATCC 17749^T, X74690; *Vibrio anguillarum* ATCC 19264^T, X16895; *Vibrio campbellii* ATCC 25920^T, X74692; *Vibrio carchariae* ATCC 35084^T, X74693; *Vibrio cholerae* ATCC 14035^T, X74695; *Vibrio cincinnatiensis* ATCC 35912^T, X74698; *Vibrio diabolicus* HE800^T, X99762; *Vibrio diazotrophicus* ATCC 33466^T, X74701; *Vibrio fischeri* ATCC 7744^T, X74702; *Vibrio fluvialis* NCTC 11327^T, X76335; *Vibrio furnissii* ATCC 35016^T, X74704; *Vibrio gazogenes* ATCC 29988^T, X74705; *Vibrio halioticoli* IAM 14596^T, X74690; *Vibrio harveyi* ATCC 14126^T, X74706; *Vibrio hollisae* ATCC 33564^T, X74707; *Vibrio logei* ATCC 15832, X74708; *Vibrio mediterranei* CIP 103203^T, X74710; *Vibrio metschnikovii* NCTC 11170, X74712; *Vibrio mimicus* ATCC 33653^T, X74713; *Vibrio mytili* CECT 632^T, X99761; *Vibrio natriegens* ATCC 14048^T, X74714; *Vibrio navarrensis* CIP 103381^T, X74715; *Vibrio nereis* ATCC 25917^T, X74716; *Vibrio nigripulchritudo* ATCC 27043^T, X74717; *Vibrio ordalii* ATCC 33509^T,

X74718; *Vibrio orientalis* ATCC 33934^T, X74719; *Vibrio parahaemolyticus* ATCC 17802^T, X74720; *Vibrio proteolyticus* ATCC 15338^T, X74723; *Vibrio salmonicida* NCMB 2262^T, X70643; *Vibrio scophthalmi* CECT 4638^T, U46579; *Vibrio splendidus* ATCC 33125^T, X74724; *Vibrio tapetis* CECT 4600^T, Y08430; *Vibrio tubiashii* ATCC 19109^T, X74725; *Vibrio vulnificus* ATCC 27562^T, X76333; *Photobacterium histaminum* JCM 8968^T, D25308; *Photobacterium iliopiscarium* ATCC 51760^T, AB000278; *Photobacterium leiognathi* ATCC 25521^T, X74686.

RESULTS

Bacterial growth

Strain FG1^T grew over a pH range of 5·0–8·5, with optimal growth at about pH 7·0. The strain grew significantly over a temperature range of 20–35 °C, and most rapidly at 30–35 °C. Growth was weak at 15 and 38 °C, weak or negligible at 40 °C and was not observed at all at 5 and 42 °C. Both aerobic and anaerobic growth in GM-II medium were accompanied by a large decrease in medium pH (ca. 2 pH units) during the exponential phase of growth (Fig. 1). Fig. 2 shows the effect of NaCl on growth. Strain FG1^T grew aerobically in GM-II medium containing 1–6 % NaCl (ca. 0·17–1·03 M); growth was most rapid at 4 % NaCl (ca. 0·68 M) and absent at 0 %. Substitution of KCl (2–5 %) for NaCl did not support growth (not shown).

Bacterial characterization

Strain FG1^T was Gram-negative according to the staining and KOH testing methods. It produced flat, circular, off-white colonies on PY plate medium after 1–2 d incubation. Cells in late exponential to early stationary phase of growth in PY broth culture were straight or slightly curved, motile rods that were 0·6–0·8 μm in diameter by 2–3 μm in length. Electron microscopy revealed that they normally possessed two sheathed flagella at one pole of the cell (Fig. 3). Strain FG1^T was halophilic and unable to grow in the absence of NaCl. It fermented glucose and other carbohydrates with production of gas. Catalase was positive but oxidase was negative. The strain did not accumulate any appreciable amount of PHB. It was not susceptible to the vibriostatic agent O/129 at 10–150 μg. Additional biochemical and physiological characteristics and the ability to utilize various compounds as sole carbon or nitrogen sources are given in the description of *Vibrio aerogenes* sp. nov. given below.

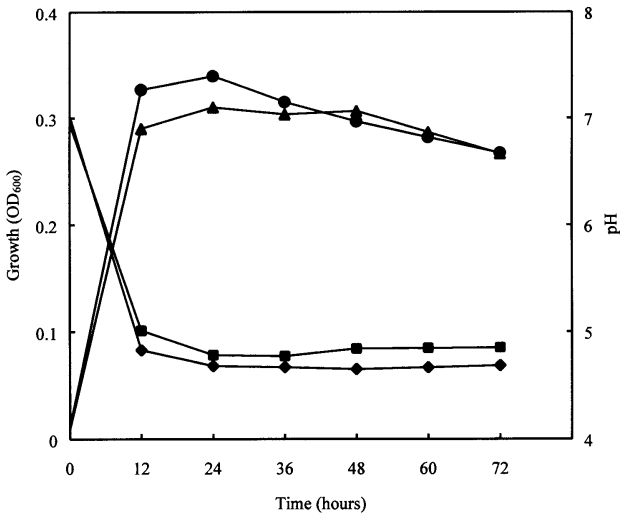

Fig. 1. Changes in OD_{600} and pH during aerobic and anaerobic growth of strain FG1^T in GM-II medium. ●, OD_{600} in aerobic culture; ▲, OD_{600} in anaerobic culture; ◆, pH in aerobic culture; ■, pH in anaerobic culture.

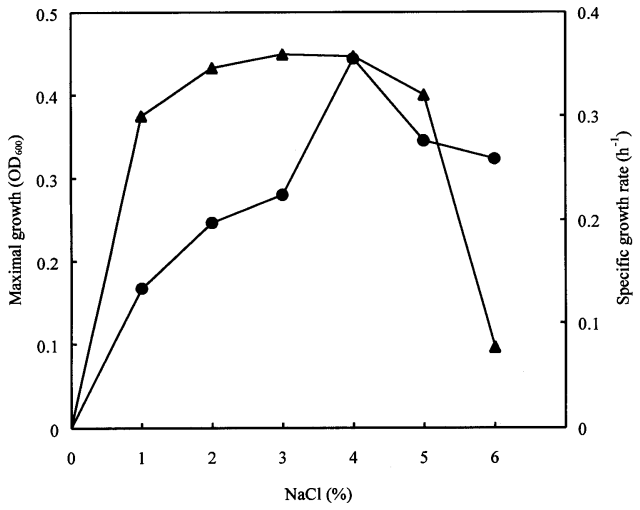

Fig. 2. Influence of NaCl concentration on aerobic growth of strain FG1^T in GM-II medium. ●, Maximal growth; ▲, specific growth rate.

Fermentation products

Anaerobic fermentative growth of strain FG1^T in GM-II medium resulted in formation of a variety of organic acids and the gases CO_2 and H_2. Acetate and lactate generally constituted more than 70 mol % of the organic acid products. Other organic acids that have been detected included formate, malate, oxaloacetate, propionate, pyruvate and succinate. No evidence indicated the production of acetoin or butanediol in these fermentative cultures.

Antibiotic susceptibility

Strain FG1^T was susceptible to ampicillin, chloramphenicol, colistin, gentamicin, nalidixic acid, polymyxin B and streptomycin but resistant to the other test antibiotics (Table 1).

Cellular fatty acids and DNA base composition

Strain FG1^T contained C12:0 as the most abundant cellular fatty acid (50·9–75·3 mol %). The other cellular fatty acids present at levels greater than 3 mol %

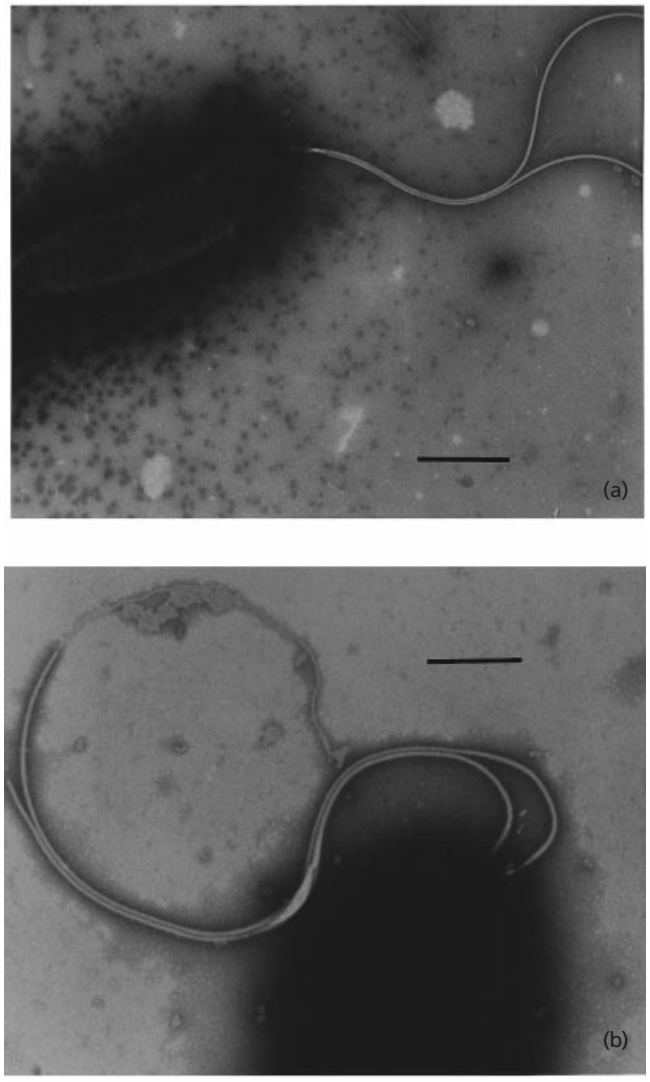

Fig. 3. (a) Electron micrograph of strain FG1^T showing two thick flagella at one pole of the rod-shaped cell. Scale bar, 1 μm. (b) Two sheathed polar flagella on one cell of strain FG1^T. One flagellum has lost the distal part of its sheath exposing the inner core. Scale bar, 0·5 μm.

included C11:0 (4·6–6·2 mol%), C14:0 (6·5–14·6 mol%), C16:0 (5·1–9·4 mol%), *iso*-C16:0 (3·5–8·2 mol%) and C18:1$^{\Delta 9}$ (7·0–10·6 mol%).

Strain FG1^T had a G+C content of 45·9 mol%.

16S rDNA-based phylogenetic analysis

An almost complete 16S rDNA sequence (ca. 89%; estimated by comparison with the *E. coli* sequence J01859) of strain FG1^T was obtained. Phylogenetic analysis based on the 16S rDNA sequence comparisons showed that the strain is a member of the genus *Vibrio* in the gamma subclass of the *Proteobacteria*. The dendrogram in Fig. 4 shows the phylogenetic position of strain FG1^T within the radiation of the genus *Vibrio* and some related bacteria. The levels of 16S rDNA sequence similarity between strain FG1^T and the known *Vibrio* species were never greater than 96·2% (not shown); the highest similarity level found (96·2%) was observed with *V. mytili*.

Table 1. Susceptibility of strain FG1^T to various antibiotics

Antibiotic	Disc content	Inhibition zone diameter (mm)	Reaction*
Ampicillin	10 μg	25	S
Chloramphenicol	30 μg	30	S
Clindamicin	2 μg	6	R
Colistin	10 μg	20	S
Erythromycin	15 μg	12	R
Gentamicin	10 μg	24	S
Kanamycin	30 μg	13	R
Lincomycin	5 μg	6	R
Nalidixic acid	30 μg	20	S
Novobiocin	30 μg	12	R
Penicillin G	10 U	15	R
Polymyxin B	300 U	20	S
Streptomycin	10 μg	18·5	S
Tetracycline	30 μg	12	R

* S, susceptible; R, resistant.

DISCUSSION

The mesophilic strain FG1^T could be categorized as a slightly halophilic bacterium (Kushner & Kamekura, 1988) since it required NaCl for growth with optimum growth at 4·0% (ca. 0·51–0·68 M). The requirement for NaCl could not be substituted by KCl, indicating that the strain required Na^+ and not Cl^- for growth and it also showed that the Na^+ requirement was not for osmotic function.

Strain FG1^T grew significantly in GM-II medium under both aerobic and anaerobic conditions, which indicated that the strain is a facultative anaerobe and does not require vitamins or other organic growth factors. The anaerobic growth in GM-II medium was accompanied by a remarkable decrease in medium pH (ca. 2 pH units) during the exponential phase of growth, regardless of the large buffer content (25 mM MOPSO) in the medium. This indicated that the strain achieved anaerobic growth in the medium by fermenting glucose with the production of considerable amounts of acids. Detection of various organic acids including acetate, lactate, succinate, formate, malate, oxaloacetate, propionate and succinate from the anaerobic GM-II cultures further indicated that strain FG1^T is a mixed acid fermenter. Similar acidification occurring in the aerobic GM-II cultures during bacterial growth was possibly due to the creation of an oxygen-depleted microenvironment in such static cultures.

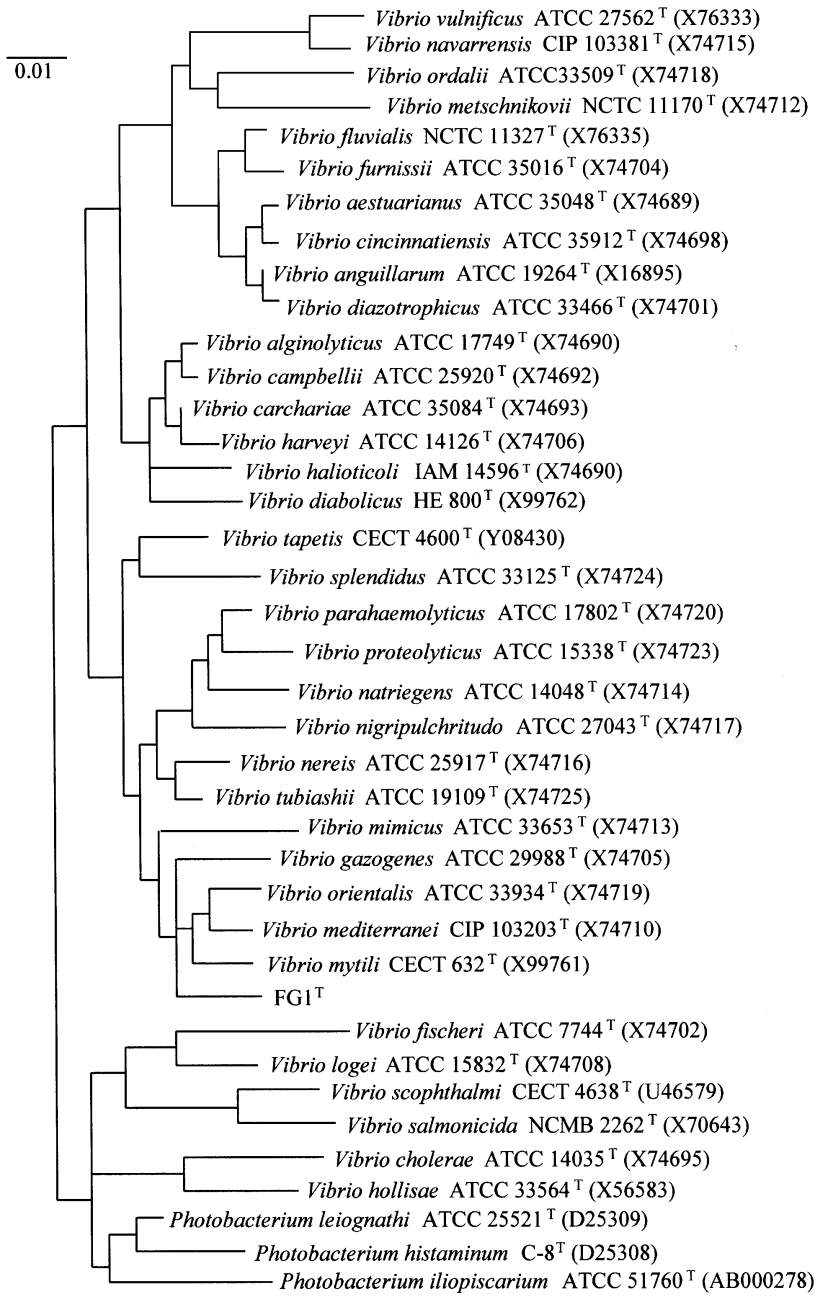

Fig. 4. 16S rDNA-based phylogenetic dendrogram showing the position of strain FG1^T within the radiation of the *Vibrio* species and some reference bacteria. Scale bar, evolutionary distance (K_{nuc}) of 0·01.

Strain FG1^T is characterized as a mesophilic, slightly halophilic, facultatively anaerobic, Gram-negative rod that is motile by sheathed polar flagella and unable to accumulate PHB, indicating that the strain is probably a species of *Vibrio* in the family *Vibrionaceae* (Baumann & Schubert, 1984; Baumann *et al.*, 1984). A 16S rDNA-based phylogenetic analysis has revealed that the sequence of strain FG1^T indeed falls inside the cluster made up of the representative sequences of the known *Vibrio* species (Fig. 4). Data analysis also supports the establishment of a new species since the levels of 16S rDNA sequence similarity between strain FG1^T and all known species of *Vibrio* are never greater than 96·2%. Thus, the name *Vibrio aerogenes* is proposed for this new bacterium. *V. aerogenes* possesses some phenotypic characteristics quite uncommon among the vibrios, including production of gas from glucose, two polar flagella on each cell, resistance to O/129 and a negative oxidase reaction (Holt *et al.*, 1994). *V. gazogenes* and *V. metschnikovii* are the only two *Vibrio* species previously reported to be negative for the oxidase reaction. Only the former produces gas from glucose. However, *V. gazogenes* is not resistant to O/129 and has only single polar flagellum on each cell (Baumann *et al.*, 1984; Harwood, 1978). Moreover, it produces red to orange colonies on plate media. Colonies produced by *V. aerogenes*, on the other hand, are off-white. More detailed characteristics useful for

Table 2. Phenotypic characteristics useful for differentiating *Vibrio aerogenes* FG1^T from previously described oxidase-negative and/or gas-producing species in the family *Vibrionaceae*

Data from this study and previous studies (Baumann & Baumann, 1984; Baumann *et al.*, 1984; Brenner *et al.*, 1983; Harwood, 1978; Holt *et al.*, 1994; Okuzumi *et al.*, 1994; Pujalte *et al.*, 1993; Urakawa *et al.*, 1999). Strains: 1, *Vibrio aerogenes* FG1^T; 2, *Vibrio furnissii*; 3, *Vibrio gazogenes*; 4, *Vibrio mytili*; 5, *Vibrio metschnikovii*; 6, *Photobacterium histaminum*; 7, *Photobacterium iliopiscarium*; 8, *Photobacterium phosphoreum*; 9, *Photobacterium damselae* subsp. *damselae* (formerly *Listonella damsela*). +, Positive; −, negative; V, variable; ND, no data available.

Characteristic	1	2	3	4	5	6	7	8	9
Gas from glucose	+	+	+	+	−	+	+	+	+
Oxidase	−	+	−	+	−	+	ND	ND	+
O/129 sensitivity (150 μg)	−	+	+	+	+	+	+	ND	+
Red–orange pigment	−	−	+	−	−	−	−	−	−
PHB accumulation	−	ND	−	ND	−	+	+	+	ND
Nitrate reduction	+	+	−	+	−	+	+	+	+
Sheathed polar flagella	+	+	+	ND	+	−	−	−	+
Amylase	+	ND	+	−	+	+	ND	−	V
Gelatinase	+	+	+	−	+	−	−	−	−
Lipase	+	+	+	ND	+	ND	ND	−	−
Arginine dihydrolase	+	+	−	+	−	ND	+	ND	+
Growth at:									
4 °C	−	−	−	−	−	−	+	+	−
35 °C	+	+	+	+	+	+	−	−	+
Growth in 10% NaCl	−	+	−	+	−	−	−	ND	−
Utilization of:									
Cellobiose	+	−	+	+	−	+	−	−	−
Sucrose	+	+	+	+	+	−	−	−	−
Mannose	+	+	+	−	V	+	+	+	+
Xylose	+	−	+	+	−	−	−	−	−
Mannitol	+	+	+	+	+	−	−	−	−
Inositol	+	−	−	−	V	ND	−	−	−
Citrate	+	+	+	V	V	−	−	−	−

differentiating *V. aerogenes* from *V. gazogenes* and other oxidase-negative or gas-producing species in the family *Vibrionaceae* are listed in Table 2.

V. aerogenes is presently found only in the marine sediment. However, the requirement for NaCl and a moderate temperature indicates that this species may also occur in shallow coastal and oceanic seawaters.

Description of *Vibrio aerogenes* sp. nov.

Vibrio aerogenes (a.e.ro′ge.nes. Gr. masc. n. *aer* air; Gr. v. *gennanio* to produce; M.L. adj. *aerogenes* gas-producing).

Straight to slightly curved rod. Cells stain Gram-negative and are 0·6–0·8 μm wide by 2–3 μm long. Swarming does not occur on solid agar media while motility normally occurs by means of two sheathed, polar flagella when grown in liquid media. Colonies produced on agar media are flat, circular, off-white and non-luminescent with an entire margin. The species is a facultative anaerobe capable of both aerobic and anaerobic fermentative growth. Acid and gas are produced from fermentation of glucose and other carbohydrates such as cellobiose, galactose, inositol, mannitol, mannose, sucrose and xylose. However, D-arabinose, dulcitol, lactose, sorbitol and trehalose are not fermented. Voges–Proskauer reaction is negative. Accumulation of PHB inside the cells is not observed. Catalase, arginine dihydrolase, amylase, caseinase, DNase, gelatinase and lipase are positive while oxidase, agarase, and lysine and ornithine decarboxylases are negative. Indole is produced. Nitrate is reduced to nitrite but not further to N_2O or N_2. The species is mesophilic, growing well at 20–35 °C but not at all at 4 or 42 °C. It is able to grow in a mineral medium containing glucose and ammonium or nitrate salts. It grows at NaCl levels of 1–7% with optimal growth at about 4% while no growth occurs at 0 and 10% NaCl. It is resistant to the vibriostatic agent O/129 (10 or 150 μg per disc). The most abundant cellular fatty acid is C12:0. The following compounds are utilized as sole carbon and energy sources: cellobiose, galactose, glucose, sucrose, mannose, xylose, citrate, fumarate, pyruvate, inositol, mannitol, L-alanine, L-aspartate, L-glutamate and L-threonine. The following compounds

are not utilized as sole carbon and energy sources: D-arabinose, lactose, melibiose, trehalose, acetate, β-hydroxybutyrate, DL-malate, malonate, tartrate, adonitol, dulcitol, sorbitol, L-arginine, L-glycine, L-lysine, L-methionine, L-ornithine, L-phenylalanine, L-tryptophan and L-valine. The species utilizes ammonium, nitrate and the L-form amino acids alanine, arginine, aspartate, glutamate, lysine, methionine, ornithine, phenylalanine, threonine, tryptophan and valine as sole nitrogen sources for growth. The DNA G+C composition is about 46·0 mol%. The species is found in coastal sediment. The type strain is FG1^T, which has been deposited in the American Type Culture Collection as strain ATCC 700797^T and in the Culture Center for Research and Collection, Taiwan as strain CCRC 17041^T.

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REFERENCES

Baumann, P. & Baumann, L. (1971). Taxonomy of marine bacteria: the genus *Beneckea*. *J Bacteriol* **107**, 268–294.

Baumann, P. & Baumann, L. (1984). Genus II *Photobacterium* Beijerinck 1889, 401^{AL}. In *Bergey's Manual Systematic Bacteriology*, vol. 1, pp. 516–550. Edited by N. R. Krieg & J. G. Holt. Baltimore: Williams & Wilkins.

Baumann, P. & Schubert, R. H. W. (1984). Family II *Vibrionaceae* Veron 1965, 5245^{AL}. In *Bergey's Manual Systematic Bacteriology*, vol. 1, pp. 516–517. Edited by N. R. Krieg & J. G. Holt. Baltimore: Williams & Wilkins.

Baumann, P., Furniss, A. L. & Lee, J. V. (1984). Genus I *Vibrio* Pacini 1854, 411^{AL}. In *Bergey's Manual Systematic Bacteriology*, vol. 1, pp. 518–538. Edited by N. R. Krieg & J. G. Holt. Baltimore: Williams & Wilkins.

Blake, P. A., Weaver, R. E. & Hollis, D. G. (1980). Disease of humans (other than cholera) caused by vibrios. *Annu Rev Microbiol* **34**, 341–367.

Brenner, D. J. (1984). Family I. *Enterobacteriaceae* Rahn 1937, Nom. fam. Cons. Opin. 15, Jud. Comm. 1958, 73; Ewing, Farmer, and Brenner 1980, 674; Judicial Commission 1981, 104. In *Bergey's Manual Systematic Bacteriology*, vol. 1, pp. 518–538. Edited by N. R. Krieg & J. G. Holt. Baltimore: Williams & Wilkins.

Brenner, D. J., Hickman-Brenner, F. W., Lee, J. V. & 7 other authors (1983). *Vibrio furnissii* (formerly aerogenic biogroup of *Vibrio fluvialis*), a new species isolated from human feces and the environment. *J Clin Microbiol* **18**, 816–824.

Buck, J. D. (1982). Nonstaining (KOH) method for determination of Gram reactions of marine bacteria. *Appl Environ Microbiol* **44**, 992–993.

Cerdà-Cuéllar, M., Rosselló-Mora, R. A., Lalucat, J., Jofre, J. & Blanch, A. (1997). *Vibrio scophthalmi* sp. nov., a new species from turbot (*Scophthalmus maximus*). *Int J Syst Bacteriol* **47**, 58–61.

Collins, C. H., Lyne, P. M. & Grange, J. M. (editors) (1995). Chapter 7. Identification Methods. In *Collins and Lyne's Microbiological Methods*, 7th edn, pp. 102–120. London: Butterworth–Heinemann.

Colwell, R. R., MacDonell, M. T. & De Ley, J. (1986). Proposal to recognize the family *Aeromonadaceae* fam. nov. *Int J Syst Bacteriol* **36**, 473–477.

Deming, J. W., Somers, L. K., Straube, W. L., Swartz, D. G. & MacDonell, M. T. (1988). Isolation of an obligately barophilic bacterium and description of a new genus, *Colwellia* gen. nov. *Syst Appl Microbiol* **10**, 152–160.

Egidus, E., Wilk, R., Andersen, K., Hoff, K. A. & Hjelnes, B. (1986). *Vibrio salmonicida* sp. nov., a new fish pathogen. *Int J Syst Bacteriol* **36**, 518–520.

Felsenstein, J. (1989). PHYLIP – phylogeny inference package. *Cladistics* **5**, 164–166.

Guerinot, M. L. & Colwell, R. R. (1985). Enumeration, isolation, and characterization of N_2-fixing bacteria from seawater. *Appl Environ Microbiol* **50**, 350–355.

Guerinot, M. L., West, P. A., Lee, J. V. & Colwell, R. R. (1982). *Vibrio diazotrophicus* sp. nov., a marine nitrogen-fixing bacterium. *Int J Syst Bacteriol* **32**, 350–357.

Hada, H. S., West, P. A., Lee, J. V., Stemmler, J. & Colwell, R. R. (1984). *Vibrio tubiashii* sp. nov., a pathogen of bivalve mollusks. *Int J Syst Bacteriol* **34**, 1–4.

Harwood, C. S. (1978). *Beneckea gazogenes* sp. nov., a red, facultatively anaerobic, marine bacterium. *Curr Microbiol* **1**, 233–238.

Hiraishi, A. (1992). Direct automatic sequencing of 16S rDNA amplified by polymerase chain reaction from bacterial cultures without DNA purification. *Lett Appl Microbiol* **15**, 210–213.

Holt, J. G., Krieg, N. R., Sneath, P. H., Staley, J. T. & Williams, S. T. (editors) (1994). Group 5 facultatively anaerobic Gram-negative rods. Subgroup 2: family *Vibrionaceae*. In *Bergey's Manual of Determinative Bacteriology*, 9th edn, pp. 190–194. Baltimore: Williams & Wilkins.

Jukes, T. H. & Cantor, C. R. (1969). Evolution of protein molecules. In *Mammalian Protein Metabolism*, pp. 21–132. Edited by H. N. Munro. New York: Academic Press.

Kushner, D. J. & Kamekura, M. (1988). Physiology of halophilic eubacteria. In *Halophilic Bacteria*, vol. 1, pp. 109–138. Edited by F. Rodriguez-Valera. Boca Raton, FL: CRC Press.

Lee, K. H. & Ruby, E. G. (1994). Competition between *Vibrio fischeri* strains during initiation and maintenance of a light organ symbiosis. *J Bacteriol* **176**, 1985–1991.

Leisman, G., Cohn, D. H. & Nealson, K. H. (1980). Bacterial origin of luminescence in marine animals. *Science* **208**, 1271–1273.

Liston, J. (1956). Quantitative variations in the bacterial flora of flat fish. *J Gen Microbiol* **15**, 305–314.

Love, M., Teebken-Fisher, D., Hose, J. E., Hickman, F. W. & Fanning, G. R. (1981). *Vibrio damsela*, a marine bacterium, causes skin ulcers on the damselfish *Chromis punctipinnis*. *Science* **214**, 1139–1140.

MacDonald, N. L., Stark, J. R. & Austin, B. (1986). Bacterial microflora in the gastro-intestinal tract of Dover sole (*Solea solea* L.), with emphasis on the possible role of bacteria in the nutrition of the host. *FEMS Microbiol Lett* **35**, 107–111.

MacDonell, M. T. & Colwell, R. R. (1985). The phylogeny of the *Vibrionaceae* and recommendation for two new genera, *Listonella* and *Shewanella*. *Syst Appl Microbiol* **6**, 171–182.

McFall-Ngai, M. J. & Ruby, E. G. (1991). Symbiont recognition and subsequent morphogenesis as early events in an animal-bacterial mutualism. *Science* **254**, 1491–1494.

Nair, S., Kita-Tsukamoto, K. & Simidu, U. (1988). Bacterial flora of healthy and abnormal chaetognaths. *Nippon Suisan Gakkaishi* **54**, 491–496.

NCCLS (1990). *Performance Standards for Antimicrobial Disk Susceptibility Tests*, 4th edn, Approved Standard M2-A4. Villanova, PA: National Committee for Clinical Laboratory Standards.

Okuzumi, M., Hiraishi, A., Kobayashi, T. & Fujii, T. (1994). *Photobacterium histaminum* sp. nov., a histamine-producing marine bacterium. *Int J Syst Bacteriol* **44**, 631–636.

Onarheim, A. M., Wiik, R., Burghardt, J. & Stackebrandt, E. (1994). Characterization and identification of two *Vibrio* species indigenous to the intestine of fish in cold sea water; description of *Vibrio iliopiscarius* sp. nov. *Syst Appl Microbiol* **17**, 370–379.

Pujalte, M. J., Ortigosa, M., Urdaci, M.-C., Garay, E. & Grimont, P. A. D. (1993). *Vibrio mytili* sp. nov., from mussels. *Int J Syst Bacteriol* **43**, 358–362.

Reichelt, J. L., Nealson, K. H. & Hastings, J. W. (1977). The specificity of symbiosis: pony fish and luminescent bacteria. *Arch Microbiol* **112**, 157–161.

Ruby, E. G. & Asato, L. M. (1993). Growth and flagellation of *Vibrio fischeri* during initiation of the sepiolid squid light organ symbiosis. *Arch Microbiol* **159**, 160–167.

Ruby, E. G. & Morin, J. G. (1978). Specificity of symbiosis between deep-sea fishes and psychrotrophic luminous bacteria. *Deep Sea Res* **25**, 161–167.

Shieh, W. Y. & Jean, W. D. (1998). *Alterococcus agarolyticus* gen. nov., sp. nov., a halophilic thermophilic bacterium capable of agar degradation. *Can J Microbiol* **44**, 567–575.

Shieh, W. Y. & Lin, Y. M. (1992). Nitrogen fixation (acetylene reduction) associated with the zoanthid *Palythoa tuberculosa* Esper. *J Exp Mar Biol Ecol* **163**, 31–41.

Shieh, W. Y. & Lin, Y. M. (1994). Association of heterotrophic nitrogen-fixing bacteria with a marine sponge of *Halichondria* sp. *Bull Mar Sci* **54**, 557–564.

Shieh, W. Y. & Liu, C. M. (1996). Denitrification by a novel halophilic fermentative bacterium. *Can J Microbiol* **42**, 507–514.

Shieh, W. Y. & Yang, J. T. (1997). Denitrification in the rhizosphere of the two seagrass *Thalassia hemprichii* (Ehrenb.) and *Halodule uninervis* (Forsk.) Aschers. *J Exp Mar Biol Ecol* **218**, 229–241.

Shieh, W. Y., Simidu, U. & Maruyama, Y. (1989). Enumeration and characterization of nitrogen-fixing bacteria in an eelgrass (*Zostera marina*) bed. *Microb Ecol* **18**, 249–259.

Shieh, W. Y., Simidu, U. & Maruyama, Y. (1990). A *Photobacterium*-like bacterium able to fix nitrogen. *Antonie Leeuwenhoek* **57**, 51–54.

Schiewe, M. H., Trust, T. J. & Crosa, J. H. (1981). *Vibrio ordalii* sp. nov. – a causative agent of vibriosis in fish. *Curr Microbiol* **6**, 346–348.

Simidu, U. & Tsukamoto, K. (1985). Habitat segregation and biochemical activities of marine members of the family *Vibrionaceae*. *Appl Environ Microbiol* **50**, 781–790.

Simidu, U., Kaneko, E. & Aiso, K. (1969). Microflora of fresh and stored flat-fish (*Kareus bicoloratus*). *Bull Jpn Soc Sci Fish* **35**, 77–82.

Simidu, U., Ashino, K. & Kaneko, E. (1971). Bacterial flora of phyto- and zoo-plankton in the inshore water of Japan. *Can J Microbiol* **17**, 1157–1160.

Smibert, R. M. & Krieg, N. R. (1994). Systematics: phenotypic characterization. In *Manual of Methods for General and Molecular Bacteriology*, pp. 607–654. Edited by P. Gerhardt. Washington, DC: American Society for Microbiology.

Sochard, M. R., Wilson, D. F., Austin, B. & Colwell, R. R. (1979). Bacteria associated with the surface and gut of marine copepods. *Appl Environ Microbiol* **37**, 750–759.

Suutari, M., Liukkonen, K. & Laakso, S. (1990). Temperature adaptation in yeasts: the role of fatty acids. *J Gen Microbiol* **136**, 1469–1474.

Urakawa, H., Kita-Tsukamoto, K. & Ohwada, K. (1999). Reassessment of the taxonomic position of *Vibrio iliopiscarius* (Onarheim *et al*. 1994) and proposal for *Photobacterium iliopiscarium* comb. nov. *Int J Syst Bacteriol* **49**, 257–260.

West, P. A., Brayton, P. R., Twilley, R. R., Bryant, T. N. & Colwell, R. R. (1985). Numerical taxonomy of nitrogen-fixing 'decarboxylase-negative' *Vibrio* species isolated from aquatic environments. *Int J Syst Bacteriol* **35**, 198–205.

Yoshimizu, M., Kimura, T. & Sakai, M. (1976). Studies on the intestinal microflora of Salmonids. III. The intestinal microflora of salmon living in the open sea. *Bull Jpn Soc Sci Fish* **42**, 875–884.