**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 FG1T, 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 FG1T 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 CO₂ and H₂. 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 FG1T 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 FG1T (= ATCC 700797T = CCRC 17041T).

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

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**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...
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 furnissi (formerly Vibrio fluvialis biovar 2), Vibrio mytilli, Photobacterium histaminum, Photobacterium ilioptiscarum, Photobacterium phosphoreum and Listonella damsela (reclassified as Photobacterium damsela subsp. damsela) 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 of 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 H₂ and CO₂. 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 MgCl₂·6H₂O. 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. Polypeptone-yeast-nitrate (PYN) broth was prepared by adding KNO₃ at 0.1 l−¹ to PY broth. Tryptone broth contained 10 g tryptone (Sigma), 25 g NaCl and 5 g MgCl₂·6H₂O dissolved in 1000 ml deionized water and adjusted to pH 7.8. Polypeptone-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 MgCl₂·6H₂O, 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 NH₄Cl, 25 g NaCl, 2 g MgCl₂·6H₂O, 3 g K₂SO₄, 0.2 g K₃HPO₄, 0.001 g CaCl₂, 0.005 g FeCl₃·6H₂O 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 g g l−¹ (ca. 25 mM) 3-(N-morpholinol)-2-hydroxysulfonic acid (MOPS) (Sigma) instead of Tris and was adjusted to pH 7.0.

A modified PY plate medium containing CaCl₂ (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 (1−¹ deionized water): 2 g Bacto peptone (Difco); 25 g NaCl; 5 g MgCl₂·6H₂O; 0.01 g phenol red; 3 g Bacto agar (Difco); and 5 g l−¹-arгинine, 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 M), KOH (1 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 (10⁻⁵–10⁻¹ times) was transferred to a sterile 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 morphological characteristics were used to identify the strain. 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 FG1² and its PY stab cultures were kept at 20 °C under aerobic conditions for maintenance.

Bacterial growth. Inoculum cultures of strain FG1² 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 in a mixer during the flushing process, after which the needles were pulled out of the stoppers. The OD₆₀₀ 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_{max} of each culture was passed. Specific growth rates were calculated from changes in the OD_{max} 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 tryptophan 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 noted after incubating the cultures for 20 d at 4 and 15 °C. The ability to grow at different temperatures was determined in GM-II medium and its modifications containing KNO₃ (20 mM or any one of the test amino acids at 2 g l⁻¹) 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 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 CO₂ and H₂ 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 CO₂ analysis) or a column of molecular Sieve 5A (3 mm × 2 m; for H₂ analysis) at 70 °C. Production of acetoin or butanediol in GM-II cultures was determined by the Voges-Proskauer reaction (Smibert & Krieg, 1994).

**16S rDNA gene sequencing and phylogenetic dendrogram construction.** A method similar to that of Hiraishi (1992) was used for PCR amplification and sequencing of the 16S rDNA gene of strain FG1. 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 PHYLP program PHYLIP using the Jukes & Cantor (1969) model by assuming a transition/transversion ratio of 2:0; a neighbour-joining construction 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 35048³, X74689; *Vibrio anginolyticus* ATCC 17749³, X74690; *Vibrio anguillarum* ATCC 19264³, X16895; *Vibrio campbellii* ATCC 25920, X74692; *Vibrio carlirhariae* ATCC 35084³, X74693; *Vibrio choleae* ATCC 14035³, X74695; *Vibrio cincinnatiensis* ATCC 35912, X74698; *Vibrio diabolicus* ATCC 34266³, HE800³, X99762; *Vibrio diazotrophicus* ATCC 33466³, HE74701; *Vibrio fischeri* ATCC 7744³, X74702; *Vibrio fluvialis* NCTC 11327³, X76335; *Vibrio halioticoli* ATCC 3369³, X74704; *Vibrio gazogenes* ATCC 29988³, X74705; *Vibrio halotoleranti IAM 14596³, X74690; *Vibrio harveyi* ATCC 14126³, X74706; *Vibrio helvola* ATCC 33564³, X74707; *Vibrio logei* ATCC 15832³, X74708; *Vibrio mediterraneen CIP 10320³, X74710; *Vibrio metschnikovii* NCTC 11170, X74712; *Vibrio mimicus* ATCC 33653³, X74713; *Vibrio mytits CECT 632³, X99761; *Vibrio natriegens* ATCC 14048³, X74714; *Vibrio navarrensis* CIP 103381³, X74715; *Vibrio nereis* ATCC 25917³, X74716; *Vibrio nigripulchritudo* ATCC 27043³, X74717; *Vibrio ordalii* ATCC 33509³.
RESULTS

Bacterial growth

Strain FG1\textsuperscript{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\textsuperscript{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\textsuperscript{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\textsuperscript{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\textsubscript{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.

Fermentation products

Anaerobic fermentative growth of strain FG1\textsuperscript{T} in GM-II medium resulted in formation of a variety of organic acids and the gases CO\textsubscript{2} and H\textsubscript{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\textsuperscript{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\textsuperscript{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%
Vibrio aerogenes sp. nov.

Fig. 3. (a) Electron micrograph of strain FG1T 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 FG1T. One flagellum has lost the distal part of its sheath exposing the inner core. Scale bar, 0.5 µm.

included C11:0 (46–62 mol%), C14:0 (65–146 mol%), C16:0 (51–94 mol%), iso-C16:0 (35–82 mol%) and C18:1ω9c (70–106 mol%).

Strain FG1T 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 FG1T 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 FG1T within the radiation of the genus Vibrio and some related bacteria. The levels of 16S rDNA sequence similarity between strain FG1T 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 FG1T to various antibiotics

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Disc content</th>
<th>Inhibition zone diameter (mm)</th>
<th>Reaction*</th>
</tr>
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<tbody>
<tr>
<td>Ampicillin</td>
<td>10 µg</td>
<td>25</td>
<td>S</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>30 µg</td>
<td>30</td>
<td>S</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>2 µg</td>
<td>6</td>
<td>R</td>
</tr>
<tr>
<td>Colistin</td>
<td>10 µg</td>
<td>20</td>
<td>S</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>15 µg</td>
<td>12</td>
<td>R</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>10 µg</td>
<td>24</td>
<td>S</td>
</tr>
<tr>
<td>Kanamycin</td>
<td>30 µg</td>
<td>13</td>
<td>R</td>
</tr>
<tr>
<td>Lincomycin</td>
<td>5 µg</td>
<td>6</td>
<td>R</td>
</tr>
<tr>
<td>Nalidixic acid</td>
<td>30 µg</td>
<td>20</td>
<td>S</td>
</tr>
<tr>
<td>Novobiocin</td>
<td>30 µg</td>
<td>12</td>
<td>R</td>
</tr>
<tr>
<td>Penicillin G</td>
<td>10 U</td>
<td>15</td>
<td>R</td>
</tr>
<tr>
<td>Polymyxin B</td>
<td>300 U</td>
<td>20</td>
<td>S</td>
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<tr>
<td>Streptomycin</td>
<td>10 µg</td>
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<td>S</td>
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<tr>
<td>Tetracycline</td>
<td>30 µg</td>
<td>12</td>
<td>R</td>
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* S, susceptible; R, resistant.

DISCUSSION

The mesophilic strain FG1T could be categorized as a slightly halophilic bacterium (Kushner & Kamekura, 1988) since it required NaCl for growth with optimum growth at 40% (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 FG1T 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 FG1T 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.
Strain FG1<sup>T</sup> 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<sup>T</sup> 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<sup>T</sup> 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<sub>129</sub> 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<sub>129</sub> 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

![Fig. 4. 16S rDNA-based phylogenetic dendrogram showing the position of strain FG1<sup>T</sup> within the radiation of the *Vibrio* species and some reference bacteria. Scale bar, evolutionary distance (K<sub>sub</sub>) of 0.01.](image-url)
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 anaerobic 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 negative and are 0±8 µm wide by 2–3 µm long. It is able to grow in a 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 O₁₂₉ (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

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

<table>
<thead>
<tr>
<th>Characteristic</th>
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<td>Oxidase</td>
<td>-</td>
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<td>Red–orange pigment</td>
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<td>PHB accumulation</td>
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<td>Growth in 10% NaCl</td>
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<td>Utilization of:</td>
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<td>Cellobiose</td>
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<td>Mannose</td>
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<td>Citrate</td>
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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-l-syne, 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 FG1T, which has been deposited in the American Type Culture Collection as strain ATCC 700797\(^7\) and in the Culture Center for Research and Collection, Taiwan as strain CCRC 17041\(^7\).

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REFERENCES


