Enumeration and Characterization of Nitrogen-Fixing Bacteria in an Eelgrass (*Zostera marina*) Bed

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Abstract. Marine nitrogen-fixing bacteria distributed in the eelgrass bed and seawater of Aburatsubo Inlet, Kanagawa, Japan were investigated using anaerobic and microaerobic enrichment culture methods. The present enrichment culture methods are simple and efficient for enumeration and isolation of nitrogen-fixing bacteria from marine environments. Most-probable-number (MPN) values obtained for nitrogen-fixing bacteria ranged from 1.1×10^2 to 4.6×10^2 /ml for seawater, 4.0×10^4 to 4.3×10^5 /g wet wt for eelgrass-bed sediment, and 2.1×10^5 to 1.2×10^7 /g wet wt for eelgrass-root samples. More than 100 strains of halophilic, nitrogen-fixing bacteria belonging to the family Vibrionaceae were isolated from the MPN tubes. These isolates were roughly classified into seven groups on the basis of their physiological and biochemical characteristics. The majority of the isolates were assigned to the genus *Vibrio* and one group to the genus *Photobacterium*. However, there was also a group that could not be identified to the generic level. All isolates expressed nitrogen fixation activities under anaerobic conditions, and no organic growth factors were required for their activities.

Introduction

For many years, biological nitrogen fixation occurring in marine environments was believed to be mainly due to cyanobacteria [37]. However, the presence of bacterial nitrogen fixation has been demonstrated in many diverse marine habitats since the 1970s. Most environments where bacterial nitrogen fixation has been found are benthic, such as seagrass [5, 6, 27, 36], salt marsh [26, 28], mangrove [15, 43, 44], and coral reef [41] communities, and a variety of estuarine and marine sediments [14, 16, 23]. There are also reports that bacterial nitrogenase activity is associated with diatoms and other plankters in the pelagic zone [19, 21]. It also has been demonstrated that heterotrophic nitrogen fixation might occur in association with oxygen-poor microzones in nitrogen-depleted

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aerobic marine waters [24, 25]. These studies indicate that nitrogen-fixing bacteria may introduce significant amounts of ammonium nitrogen into various marine environments.

Several studies have been done concerning the enumeration of nitrogen-fixing bacteria in seawater [10, 21, 42], estuarine and oceanic sediments [20, 21], and salt marsh soils [8, 28]. Nitrogen-fixing bacteria associated with the roots of marsh grass *Spartina alterniflora* and the gastrointestinal tracts of sea urchins have also been enumerated [11, 28]. Early attempts were made using plate counts [21, 42]. However, these methods proved to be ineffective because many strains isolated from agar media without added combined nitrogen proved to be nitrogen-scavenging bacteria that were unable to fix nitrogen when assayed by the acetylene reduction method [17]. The most-probable-number (MPN) counts of nitrogen-fixing bacteria have also been done by analyzing nitrogenase (acetylene reduction) activity in the cultures that were inoculated with 0.2 μm filters used to concentrate the bacteria in the initial seawater samples [10]. However, only low numbers of nitrogen-fixing bacteria were counted in these studies. For example, counts of 10^{-1} – 10^2 cells/liter for seawater from Chesapeake Bay and the coast of Puerto Rico were reported by Guerinot and Colwell [10]. Nitrogen-fixing bacteria requiring NaCl for growth have been isolated from a variety of marine sources [10, 22, 39, 40]. However, nitrogenase activity of these bacteria was low, and few of them showed significant levels of nitrogenase activity (>10 nmol $\text{C}_2\text{H}_4/\text{mg}$ dry wt cells/hour) within a few hours of incubation.

During a study of nitrogen fixation in an eelgrass bed in Aburatsubo Inlet, Kanagawa, Japan, we isolated a species of marine nitrogen-fixing *Vibrio* from eelgrass (*Zostera marina*) roots using an anaerobic enrichment culture method [32]. This bacterium showed rapid growth in a nitrogen-free liquid medium and it expressed nitrogenase activity after only a few hours of incubation under anaerobic conditions. In the present study, anaerobic and microaerobic enrichment culture methods, basically similar to that of our previous report [32], were applied in the MPN technique for further enumeration and isolation of marine nitrogen-fixing bacteria distributed in the eelgrass bed and seawater of Aburatsubo Inlet. More than 100 strains of marine nitrogen-fixing bacteria were isolated from eelgrass roots, eelgrass-bed sediment, and seawater. Their taxonomic characteristics and nitrogen fixation properties were also examined.

Materials and Methods

Sample Collection and Treatment

Samples of sediment and eelgrass roots were collected from an eelgrass bed in Aburatsubo Inlet, Kanagawa, Japan, in July and August of 1986, and seawater samples were collected from two stations located in this inlet in June and August of 1987. All samples were processed within a few hours. After root samples were washed free of sediment in sterile seawater, 1 g wet wt samples of the roots were washed once again by vigorous shaking in 50 ml of sterile seawater containing 1 ppm Tween 80 (root rinse water). The roots were again washed three times in sterile seawater, and then homogenized with 9 ml of sterile seawater (root homogenate solution). Samples of seawater, sediment, root rinse water, and root homogenate solution were then used for bacterial counts.

Media

To determine suitable carbon sources for obtaining the most active nitrogen-fixing bacteria, we examined 10 sugars and organic acids for their effects on the nitrogenase activities associated with eelgrass roots and eelgrass-bed sediment. Glucose and sucrose were most effective in enhancing the activities (data not shown). They were therefore used as the carbon sources in making nitrogen-free (N-free) and nitrogen-deficient (N-deficient) media for enrichment of nitrogen-fixing bacteria. The N-free liquid media contained the following components: (1) basal medium (NaCl 28 g, MgSO₄·7H₂O 5 g, CaCl₂·2H₂O 10 mg, Na₂MoO₄·2H₂O 10 mg, Tris 50 mmol, distilled water 800 ml) adjusted to pH 8.0; (2) K₂HPO₄ 0.5 g dissolved in 100 ml of distilled water; and (3) glucose or sucrose 5 g, FeCl₃·6H₂O 15 mg dissolved in 100 ml of distilled water. These were mixed after autoclaving separately. The N-deficient liquid media were prepared by supplementing N-free liquid media with 1 mg of yeast extract (Difco) per liter. The polypepton-yeast-glucose agar (PYG-A) medium contained the following two components: (1) Polypepton (Daigo, Tokyo, Japan) 2.0 g, yeast extract (Difco, Detroit, Michigan, USA) 0.5 g, Tris 50 mmol, and agar (Difco) 15 g dissolved in 900 ml of seawater and adjusted to pH 7.6; (2) 5 g glucose dissolved in 100 ml of distilled water. The two components were mixed after autoclaving separately. The polypepton-yeast agar (PY-A) medium contained 2.0 g Polypepton (Daigo), 0.5 g yeast extract (Difco), and 15 g agar (Difco) in 1 liter of 90% seawater, adjusted to pH 7.6.

Bacterial Enumeration

One milliliter seawater, 1 g wet wt sediment, 1 ml root rinse water, and 1 ml root homogenate solution were serially diluted with 9 ml of sterile seawater. Plate counts of heterotrophic bacteria were done by spreading 0.1 ml aliquots of each dilution onto duplicate PY-A or PYG-A plates. Incubation was carried out at 25°C for 7 days under either aerobic (air) or anaerobic (GasPak System, BBL, Cockeysville, Maryland, USA) conditions. The PY-A and PYG-A media were used for aerobic and anaerobic cultures, respectively. The most-probable-number (MPN) of heterotrophic bacteria in seawater and nitrogen-fixing bacteria in all samples was estimated from series with three tubes per dilution using the tables of Cochran [7]. For heterotrophic bacteria, 1 ml aliquots of each diluent were added to 5 ml of PY broth (PY-B) medium and incubated at 25°C for 7 days under aerobic conditions. For nitrogen-fixing bacteria, a 1 ml aliquot of each diluent was transferred to a 20 ml test tube containing 5 ml of each of the following liquid media: (1) N-free glucose (NFG), (2) N-deficient glucose (NDG), (3) N-free sucrose (NFS), (4) N-deficient sucrose (NDS). After the tubes were sealed with rubber stoppers, the air in each tube was replaced with N₂ (100%) or N₂/O₂ (97%:3%). All cultures were incubated in the dark at 25°C for 3–7 days. The criterion used to record an MPN tube as positive was the development of visible turbidity because non-nitrogen-fixing bacteria (usually oligotrophic bacteria) might develop invisible (but never visible) turbidity in the present N-free or N-deficient media.

Isolation of Nitrogen-Fixing Bacteria

MPN tubes that developed visible turbidity were again serially diluted with solution containing 25 g NaCl and 5 g MgSO₄·7H₂O in 1 liter of distilled water. A 0.1 ml aliquot of each diluent of the cultures was transferred to fresh N-free or N-deficient medium and the anaerobic or microaerobic enrichment culture procedures described above were repeated. For the final isolation of nitrogen-fixing bacteria, the cultures of the highest dilution with positive growth from the second enrichment were spread on PY-A plates and the plates were incubated at 25°C for 2–5 days under aerobic conditions. All colonies from the plating of the same enrichment culture were usually of the same type, indicating that they might have been derived from the same cell. However, two types of colonies also occurred on some of the plates. Individual colonies were picked and purified by repeated streakings on PY-A plates. The nitrogenase activity of the isolates was measured by the acetylene-reduction assay method. A variety of marine nitrogen-fixing bacteria were isolated using the present enrichment culture methods.

Measurement of Nitrogenase Activity

The nitrogenase activity of the isolates was measured by the acetylene-reduction assay method using a Shimadzu G-4CM gas chromatograph [32]. Cells from PY-A plates were inoculated into 20 ml tubes containing 5 ml NFG medium. The air in the tube was replaced with N₂ (100%) or N₂/O₂ (97%:3%; v/v) after it was sealed with a rubber stopper. Incubation was carried out at 25°C in the dark. The tubes that developed turbidities (optical density at 600 nm) of 0.10–0.20 were injected with 2 ml of acetylene with a gas-tight syringe. Incubation was continued for a day, and then 0.2 ml of gas in each tube was removed with a gas-tight syringe for the analysis of ethylene production.

Characterization of Nitrogen-Fixing Isolates

Characterization of the nitrogen-fixing isolates was carried out according to the methods described in our previous report [34] with some modifications. The presence of agarase was examined by incubating the isolates on PY agar plates for 5 days, and strains that caused liquefaction or depressions were recorded as agarase-positive. Some strains that showed ambiguous reactions in this test were clarified by the iodine test [1]. The ability to produce acid and gas from different carbohydrates was determined in stab media as described previously [34]. However, broth media free of agar were also used instead of stab media for some agar-degrading isolates which produced acid from agar [33]. Poly- β -hydroxybutyrate (PHB) accumulation was tested in a medium containing the following two components: (1) 0.5 g of yeast extract (Difco), and 50 mmol Tris, dissolved in 900 ml of seawater and adjusted to pH 8.0; (2) 2 g of glucose dissolved in 100 ml of distilled water. The two components were mixed after autoclaving separately. Growth at different concentrations of NaCl was determined in yeast extract (0.4%, w/v; Difco) broth containing 0, 3, 6, and 10% NaCl.

Results and Discussion

Bacterial Enumeration

Tables 1 and 2 summarize the MPN counts of nitrogen-fixing bacteria as well as the plate counts of heterotrophic bacteria in the eelgrass bed and seawater samples. Nitrogen-fixing bacteria were found in all samples using the present anaerobic and microaerobic enrichment culture methods. The MPN values of nitrogen-fixing bacteria ranged from 1.1×10^2 to 4.6×10^2 for seawater (cells/ml) and 4.0×10^4 to 4.3×10^5 for eelgrass-bed sediment (cells/g wet wt). For eelgrass-root samples, these values (cells/g wet wt) ranged from 2.2×10^5 to 1.2×10^7 for root rinse water and 2.1×10^5 to 4.6×10^5 for root homogenate solution.

In the present study, halophilic bacteria positive in nitrogenase (acetylene reduction) activity were isolated from all the enrichment culture tubes that developed visible turbidity. Some cultures (ca. 10%) used in the isolation of nitrogen-fixing bacteria were not pure and contained nonnitrogen-fixing bacteria. However, nitrogen-fixing bacteria always appeared as the predominant groups in these mixed cultures: colony-forming units (CFU) of nitrogen-fixing bacteria were always much higher than those of nonnitrogen-fixing bacteria. The results indicate that the present counting methods are indeed applicable for the evaluation of nitrogen-fixing bacteria in marine environments. Guerinot and Colwell [10] reported aerobic plate counts of heterotrophic bacteria in

Table 1. MPN and plate counts of various bacterial groups in the eelgrass bed of Aburatsubo Inlet (cells/g wet wt sediment or root sample)

			Bacterial counts		
Bacterial group and counting method	Month (in 1986)	Medium ^a	Sediment	Root rinse water	Root homogenate solution
Plate counts of heterotrophs					
Aerobic	July	PY-A	2.2×10^7	3.4×10^7	2.6×10^7
Aerobic	Aug.	PY-A	2.6×10^7	2.4×10^7	2.8×10^7
Anaerobic	July	PYG-A	4.2×10^6	6.2×10^6	3.8×10^6
Anaerobic	Aug.	PYG-A	2.5×10^6	5.5×10^6	2.8×10^6
MPN counts of N ₂ -fixing bacteria					
Anaerobic	July	NFS	9.0×10^4	1.2×10^7	2.4×10^5
Anaerobic	July	NDS	2.3×10^5	5.5×10^5	2.4×10^5
Anaerobic	Aug.	NFG	9.0×10^4	4.6×10^5	4.3×10^5
Anaerobic	Aug.	NDG	1.5×10^5	2.2×10^5	2.3×10^5
Microaerobic	July	NFS	2.4×10^5	1.2×10^6	4.6×10^5
Microaerobic	July	NDS	4.0×10^4	2.3×10^6	2.4×10^5
Microaerobic	Aug.	NFG	9.0×10^4	2.2×10^5	2.1×10^5
Microaerobic	Aug.	NDG	4.3×10^5	7.5×10^5	4.3×10^5

^a PY-A, PY agar medium; PYG-A, PYG agar medium; NFS, liquid N-free sucrose medium; NFG, liquid N-free glucose medium; NDS, liquid N-deficient sucrose medium; NDG, liquid N-deficient glucose medium

seawater from Chesapeake Bay (from 1.9×10^4 to 5.1×10^4 cells/ml) which were similar to those in Aburatsubo Inlet seawater (from 3.3×10^4 to 1.6×10^5 cells/ml; Table 2). However, in their study, the MPN values of nitrogen-fixing bacteria for seawater samples collected from Chesapeake Bay (from 2×10^{-3} to 1.05×10^{-1} cells/ml) were far lower than those in Aburatsubo Inlet seawater (from 1.1×10^2 to 4.6×10^2 cells/ml) obtained here (Table 2). The relatively low MPN values they obtained may be expected because they used enrichment media containing 100 mg of yeast extract per liter. Although the high amount of yeast extract might support more diverse species of nitrogen-fixing bacteria, bacteria other than nitrogen-fixing bacteria might grow in these media much more rapidly to surpass the latter.

Nitrogen Fixation Activity

A total of 127 strains of nitrogen-fixing bacteria were isolated from the above sources. All these isolates reduced acetylene into ethylene under anaerobic conditions (>200 nmol C₂H₄/culture/day). Most of the isolates showed significant levels of nitrogenase activity after only a few hours of incubation when using the previous measurement method [34]. All isolates grew in the NFG medium under N₂. This indicates that they could fix N₂ as the sole nitrogen source under anaerobic conditions, and no organic growth factors were required by these isolates. Some of the isolates have been demonstrated to be tolerant to low levels of oxygen [34], but none of them showed significant growth in this medium under air. On the other hand, all isolates grew in PYG medium under both aerobic (air) and anaerobic (GasPak System, BBL) conditions. These

Table 2. MPN and plate counts of various bacterial groups in Aburatsubo Inlet seawater (cells/ml)

Bacterial group and counting method	Month (in 1987)	Medium ^a	Bacterial counting value in	
			Station I	Station II
Plate counts of heterotrophs				
Aerobic	June	PY-A	1.6×10^5	3.3×10^4
Aerobic	Aug.	PY-A	1.2×10^5	6.0×10^4
Anaerobic	June	PYG-A	1.3×10^4	1.8×10^3
Anaerobic	Aug.	PYG-A	3.8×10^4	2.5×10^3
MPN counts of heterotrophs				
Aerobic	June	PY-B	2.3×10^5	2.1×10^5
Aerobic	Aug.	PY-B	1.5×10^6	7.5×10^5
MPN counts of N ₂ -fixing bacteria				
Anaerobic	June	NFG	2.4×10^2	1.1×10^2
Anaerobic	Aug.	NFG	1.5×10^2	4.6×10^2

^a PY-A, PY agar medium; PYG-A, PYG agar medium; PY-B, PY broth medium; NFG, liquid N-free glucose medium

results indicate that all isolates were typical facultatively anaerobic, nitrogen-fixing bacteria. Microaerophilic, nitrogen-fixing bacteria requiring NaCl for growth have been isolated from the gland of *Deshayes* in shipworms [39] and the roots of marsh grass *Spartina alterniflora* [22] in the past several years. However, none of these bacteria could be isolated using the present microaerobic culture methods. Some nitrogen-fixing bacteria isolated from the rhizosphere of terrestrial plants [18, 38] have been demonstrated to fix nitrogen only in the presence of combined nitrogen or certain vitamins but these bacteria were also unable to be isolated in this study. Considering the high MPN counts of facultatively anaerobic, nitrogen-fixing bacteria without the requirement of organic growth factors, these bacteria might be important in providing inputs of ammonium nitrogen into the present study sites. In our previous report [35], we suggested that the in situ nitrogenase activity of eelgrass rhizome-root complexes might be primarily attributed to root-associated nitrogen-fixing bacteria. We also suggested that facultatively anaerobic nitrogen-fixing bacteria, including the present isolates, might be the most important nitrogen fixers associated with eelgrass roots.

Characterization of Nitrogen-Fixing Isolates

All the nitrogen-fixing isolates were gram-negative rods which were motile by means of a single polar flagellum in PY-B medium. They fermented glucose and required NaCl for growth. These characteristics clearly indicate that all isolates belong to the family Vibrionaceae. All isolates were roughly divided into seven groups (Table 3), based mainly on the results of the following tests: PHB accumulation, utilization of β -hydroxybutyrate, and production of agarase, oxidase, catalase, and gas from glucose fermentation.

Table 3. Characteristics of marine nitrogen-fixing bacteria isolated in this study

Bacterial group ^a (No. of strains)	I (5)	II (4)	III (10)	IV (12)	V (73)	VI (15)	VII (8)
Characteristic							
PHB accumulation	+ ^b	+	—	—	—	—	—
Gas from glucose	—	—	—	—	—	—	+
Agarase	—	—	+	+	—	—	—
Catalase	+	+	+	+	+	+	—
Oxidase	+	+	v	+	+	—	—
Gelatinase	+	+	+	—	v	—	+
Lecithinase	+	v	—	—	—	—	—
Lipase	+	+	+	+	v	—	—
Amylase	v	v	w	+	+	—	—
DNase	+	v	w	v	+	—	+
Chitinase	w	—	—	—	—	—	—
Arginine dihydrolase	+	v	—	—	v	—	—
Sensitivity to 0/129 ^c (150 µg)	v	v	v	v	v	+	—
Growth at							
4°C	v	v	v	—	v	v	—
20°C	+	+	+	+	+	+	+
40°C	—	v	v	v	v	v	+
Growth in							
0% NaCl	—	—	—	—	—	—	—
3% NaCl	+	+	+	+	+	+	+
6% NaCl	+	+	+	+	+	+	+
10% NaCl	—	v	—	—	v	—	+
Acid production from							
Glucose	+	+	+	+	+	+	+
Sucrose	+	+	—	—	+	+	+
Mannitol	+	v	+	+	+	+	+
Inositol	—	v	—	—	v	+	—
D-Arabinose	—	—	—	—	—	+	—
Cellobiose	+	+	+	+	+	+	+
Melibiose	+	v	+	+	v	+	+
Lactose	—	v	v	w	v	w	+
Utilization as sole carbon source							
β-Hydroxybutyrate	—	+	—	—	—	—	—
Glucose	+	+	+	+	+	+	+
Mannitol	+	v	+	+	+	+	+
Galactose	+	+	+	+	v	+	+
Mannose	+	v	v	—	v	+	v
L-Rhamnose	—	v	—	—	v	—	v
Glycerol	+	v	v	—	v	v	+
Inositol	+	v	—	—	v	+	—
Trehalose	+	+	—	+	v	—	—
Dulcitol	—	—	—	—	—	—	+
Tartrate	—	—	—	—	—	—	+
Acetate	+	+	v	—	v	—	—
Citrate	v	+	+	—	v	—	+

^a All strains were positive for glucose fermentation and nitrogenase and were negative for Gram stain, swarming, pigmentation and luminescence

^b +, positive; —, negative; w, weakly positive; v, variable between strains

^c 2,4-diamino-6,7-diisopropylpteridine (phosphate)

The strains included in group I were plump, straight rods. They accumulated PHB as an intracellular product and produced lecithinase. They utilized glucose, mannose, and glycerol but not β -hydroxybutyrate as the sole carbon sources. Gas was not produced during glucose fermentation. These strains could be assigned to the genus *Photobacterium* [2]. They were not luminous; however, this cannot exclude them from the genus because bioluminescence is no longer treated as a generic-key characteristic of *Photobacterium* [2]. Inclusion of nitrogen-fixing strains into the genus *Photobacterium* requires a redefinition of the genus because the current definition of *Photobacterium* states that no species fix N_2 [2]. Strains belonging to this group have been isolated from sediment and eelgrass roots but not from seawater.

Group II contained strains that were able to accumulate PHB and utilize β -hydroxybutyrate as the sole carbon source. All strains included in this group were positive in oxidase and catalase reactions, and they did not produce gas during glucose fermentation. This group could be placed into the genus *Vibrio* [3]. All strains included in this group were isolated from eelgrass roots; none of these bacteria was isolated from samples of sediment or seawater.

Strains that could neither accumulate appreciable amounts of PHB nor utilize β -hydroxybutyrate were placed into the other five groups. Groups III, IV, V, and VI contained strains that produced acid but no gas during the fermentation of glucose. All these groups could also be assigned to the genus *Vibrio*. Groups III and IV contained strains that produced agarase but did not produce acid from sucrose. They might represent new species of the genus *Vibrio* because all recognized *Vibrio* species lack agarase activity. The strains included in group III liquefied agar. When the strains were streaked on PY agar plates and grown at 25°C, deep holes were formed by the colonies in a few days. The agar in the plates was completely liquefied after incubation for 1–2 months. Some strains belonging to this group have been demonstrated to fix nitrogen anaerobically using agar as the sole carbon source [33]. Strains included in this group were isolated from either sediment or seawater, and none of them was isolated from eelgrass roots. Group IV contained strains that softened but did not liquefy agar. Colonies on PY-A plates were circular and were surrounded by shallow depressions; their agarase reaction was confirmed using the iodine test [1]. All strains included in group IV utilized trehalose and they could neither hydrolyze gelatin nor utilize citrate. These characteristics clearly distinguished them from group III. This group and groups V–VII were isolated from all the present samples, including seawater, sediment, and eelgrass roots.

Group V included the majority of the nitrogen-fixing isolates. The strains produced oxidase, amylase, and DNase, but not agarase. Most of the strains also hydrolyzed gelatin. This group could be further divided into several subgroups because so many characteristics among the group were variable. Group VI included strains that were oxidase-negative. Acid production from D-Arabinose distinguished this group from all the other groups. All strains included in this group did not produce DNase, amylase, lipase, and gelatinase. They did not utilize acetate and citrate as the sole carbon sources. These strains might represent a new species of *Vibrio* because only two oxidase-negative species are included in this genus [3], neither of which has been reported to produce nitrogenase. *Vibrio diazotrophicus* [12] and *Vibrio natriegens* [40] are

the only two recognized species of *Vibrio* capable of fixing N₂. Of the two species, the former can be included in group V and the latter is similar to the strains of group II. The *Vibrio*-like, nitrogen-fixing bacterium described in our previous report [32] can also be included in group II.

The strains included in group VII contained curved rods that produced not only acid but also gas during the fermentation of glucose. Catalase and oxidase were negative. They utilized dulcitol and tartrate as sole carbon sources. Amylase and lipase (Tween 80 hydrolysis) were not produced. At present, this group cannot be identified to the generic level. The strains included in this group either represent a new genus of the family Vibrionaceae or they are new members of the genus *Vibrio* [34].

Members of the halophilic, facultatively anaerobic bacteria comprise one of the predominant bacterial groups in many marine habitats. Some of them are known as pathogens of humans [4] or marine animals [9, 13, 31]. Their symbiotic relationship with luminous marine fishes has also received considerable attention [29, 30]. The present results, as well as those of others [10, 40], indicate that halophilic, facultatively anaerobic bacteria may also be important in providing ammonium nitrogen into estuarine and coastal environments.

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