APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Aug. 1985, p. 194–201
0099-2240/85/080194-08$02.00/0

Vol. 50, No. 2

Marine Ammonia- and Nitrite-Oxidizing Bacteria: Serological Diversity Determined by Immunofluorescence in Culture and in the Environment

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Received 29 November 1984/Accepted 29 April 1985

Immunofluorescence assays for marine ammonium- and nitrite-oxidizing bacteria were used to assess the diversity of nitrifying bacteria isolated from marine environments. The antisera show relatively broad specificity, in that each reacts with several strains of the same physiological type as the strain to which the antiserum was prepared. The antisera do not, however, react with any strains of differing physiological type. Seventy percent of the 30 unidentified ammonium-oxidizing isolates tested reacted with one or both of the antisera produced to marine ammonium-oxidizing strains, and 8 of the 9 unidentified nitrite-oxidizing strains tested reacted with 1 or more of the 3 nitrite oxidizer antisera used. Ammonium- and nitrite-oxidizing bacteria were enumerated in samples taken in a depth profile (to 750 m) in the Southern California Bight by immunofluorescence assays for two ammonium oxidizers and two nitrite oxidizers. Average abundances of the two types of nitrifiers were 3.5×10^5 and 2.8×10^5 cells liter^{-1}, respectively. Nitrifiers constitute 0.1 to 0.8% of the total bacterial population in these samples.

The oxidation of ammonia to nitrate via nitrite is an essential step in the microbial nitrogen cycle in marine, freshwater, and soil environments. Organisms capable of growth at the expense of these oxidation reactions comprise a restricted group of autotrophic bacteria of two distinct "physiological types," i.e., ammonium oxidizers and nitrite oxidizers. Two of the five recognized ammonium-oxidizing genera and all four of the recognized nitrite-oxidizing genera include marine strains (23). Nitrifiers constitute a very small proportion of the total natural bacterial population in seawater, and specific identification and enumeration methods are necessary to study them in nature. Immunofluorescence is presently the only method capable of detection and enumeration of individual strains of bacteria in natural environments. This method has been applied to the study of nitrifiers in soils (3, 9, 12, 17), wastewaters (24), and seawater (20).

Diversity within a natural population of nitrifying bacteria has significant ecological implications, because serologically different strains may have important genetic differences and varying abilities to grow and nitrify under different environmental conditions (2, 4). Serological and physiological diversity within one physiological group also has methodological implications, since all members of one physiological type could not be detected with a single antiserum or isolated with a single type of medium. In this paper, we examine the diversity of ammonium- and nitrite-oxidizing bacteria from a variety of marine and estuarine habitats with antisera prepared against known marine genera. We also present data on the abundance and distribution of ammonium- and nitrite-oxidizing strains at depths to 750 m in a deep basin in the California borderland.

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MATERIALS AND METHODS

Strains. The production of antisera against two marine ammonium-oxidizing genera was described previously (20). Cultures of the homologous strains *Nitrosococcus oceanus* and *Nitrosomonas* sp. (marine) were maintained on W and W/2 media, respectively (22). Nitrite-oxidizing strains for antiserum production were graciously provided by S. W. Watson and grown on N medium (8). Data pertaining to the origin and growth characteristics of each strain used in the cross-reaction studies are summarized in Tables 1 to 3. All strains were maintained in this laboratory on the media designated in the tables.

Antisera production. Cells of nitrite-oxidizing strains were grown in pure culture for use as antigens in 20-liter glass carboys in N medium aerated gently with sterile air. Antigen production procedures for *Nitrobacter* sp. (marine), *Nitrococcus mobilis*, and *Nitrospina gracilis* were similar to those for *Nitrosococcus oceanus* and *Nitrosomonas* sp. (marine) described previously (20). Eighty liters of batch culture were needed to obtain enough bacterial protein to immunize two rabbits. Each nitrite-oxidizing strain was grown to barely detectable turbidity by periodic additions of $NaNO_2$. Cells were harvested by continuous flow centrifugation, frozen, and then thawed several times before immunization. At the time of harvest, samples of culture were innoculated into liquid CP medium (7) to check for heterotrophic contaminants. If heterotrophic growth occurred in the CP tubes, the contaminants were purified by streaking on CP plates and then maintained in culture in liquid CP or CP slants.

The immunization procedure of Ward (20) was used, and antisera were produced by Hazleton-Dutchland Laboratories (Denver, Pa.). For routine staining use as described below, no further purification of the serum beyond removal of erythrocytes was necessary.

TABLE 1. Reactivity of ammonium and nitrite oxidizer antisera with ammonium-oxidizing strains and isolates

Organism	Source or location (water depth)	Type	Date of isolation	Morphology[a]	Growth medium[b]	Antiserum reactivity[c]				
						Nitrosococcus oceanus	*Nitrosomonas* sp. (marine)	*Nitrococcus mobilis*	*Nitrobacter* sp. (marine)	*Nitrospina gracilis*
Nitrosococcus oceanus	200 miles (ca. 371 km) east of Long Island (600 m)[d]	Marine	1957	C	W	++++	+++(−)	−	−	−
Nitrosomonas sp. (marine)	Continental shelf off Peru (1 m)[d]	Marine	1966	C	W/2	−	++++	−	−	−
Nitrosomonas europaea	[e]	Soil	NA[f]	R	SW	−	++	−	−	−
Nitrosomonas sp. strain WH-2	Minnesota[e]	Soil	1976	R	SW	−	++	−	−	−
Nitrosomonas sp. strain *E-K*	Morocco[e]	Soil	1973	R	SW	−	+/−	−	−	−
NH4C	lat. 47°06.8′N, long. 124°40.6′W (30 m)[g]	Marine	1979	R	W	−	+++	−	+/−	−
NH4W	lat.47°06.8′N, long. 124°40.6′W (30 m)[g]	Marine	1979	R	W	++	++	−	−	−
NO3W	lat. 47°06.8′N, long. 124°40.6′W (30 m)[g]	Marine	1979	R	W	+/−	+++	−	+++	−
URW	lat. 47°06.8′N, long. 124°40.6′W (30 m)[g]	Marine	1979	R	W	−	++	−	−	−
TT140-89A-1	lat. 47°06.9′N, long. 124°32.7′W (15 m)[g]	Marine	1979	R	W	−	++	−	−	−
TT140-89A-2	lat. 47°06.9N, long 124°32.7′W (15 m)[g]	Marine	1979	R	W	−	−	−	−	−
TT140-098-2	lat. 47°06.9′N, long. 124°18.0′W (10 m)[g]	Marine	1979	R	W	+/−	+/−	−	−	−
C-17	Galapagos Islands[d]	Marine (sediments)	1966	R	W	+++	++	−	−	−
C-19	Continental shelf off Peru[d]	Marine	1966	C	W	++++	+++(−)	−	+/−	−
C-25	200 miles (ca. 371 km) from Amazon River mouth (0 m)[d]	Marine	1964	CR	W	−	++	−	+/−	−
C-45	Gulf of Maine (1 m)[d]	Marine	1967	R	W	++	++(−)	−	−	−
C-113	Red Sea (0 m)[d]	Marine	1966	R	W	−	+++	−	−	−
3	SIO pier[h]	Marine	1964	C	CA	+++	+++(−)	−	+/−	−
5	SIO pier[h]	Marine	1964	C	CA	++++	+++(−)	−	−	−
22	lat. 50°23′N, long. 155°16′W (0.5 m)[h]	Marine	1964	R	CA	−	−	−	−	−
23	lat. 49°07′N, long. 155°36′W (0.5 m)[h]	Marine	1964	R	CA	−	−	−	−	−
24	lat. 47°38′N, long. 155°00′W (0.5 m)[h]	Marine	1964	C	CA	++++	+++(−)	−	−	−
26	lat. 44°50′N, long. 155°00′W (0.5 m)[h]	Marine	1964	C	CA	++++	+++(−)	−	−	−
27	lat. 43°28′N, long. 155°02′W (0.5 m)[h]	Marine	1964	C	CA	++++	++++(−)	−	+/−	−
32	lat. 36°49′N, long. 155°00′W (0.5 m)[h]	Marine	1964	CR	CA	+/−	−	−	−	−
36	lat. 31°29′N, long. 155°15′W (0.5 m)[h]	Marine	1964	C	CA	++++	++(−)	−	+	−
122	lat. 9°04′S, long. 83°37′W (1 m)[h]	Marine	1966	R	W	−	−	−	−	−
132	lat. 13°09′S, long. 76°47′W (30 m)[h]	Marine	1966	C	CA	−	−	−	−	−
$CaCO_3$	SIO pier[h]	Marine	1964	R	CA	−	++	−	+/−	−
T-1A	lat. 38°45′N, long. 76°8′W (0 m)[i]	Estuarine	1983	R	W/20	+/−	+++	−	−	−
T-2A	lat. 38°45′N, long. 76°8′W (0 m)[i]	Estuarine	1983	R	W/20	++	++	−	−	−
T-3A	lat. 38°45′N, long. 76°8′W (0 m)[i]	Estuarine	1983	R	W/20	+/−	+++	−−	−	−

Table 1—*Continues*

TABLE 1.—*Continued*

Organism	Source or location (water depth)	Type	Date of isolation	Morphology[a]	Growth medium[b]	Antiserum reactivity[c]				
						Nitrosococcus oceanus	*Nitrosomonas* sp. (marine)	*Nitrococcus mobilis*	*Nitrobacter* sp. (marine)	*Nitrospina gracilis*
B-1A	lat. 38°45'N, long. 76°12'W (0 m)[i]	Estuarine	1983	R	W/20	−	+++	−	−	−
B-2A	lat. 38°45'N, long. 76°12'W (0 m)[i]	Estuarine	1983	R	W/20	−	+	−	−	−
B-3A	lat. 38°45'N, long. 76°12'W (0 m)[i]	Estuarine	1983	R	W/20	−	+	−	−	−

[a] Abbreviations: C, coccus; R, rod; CR, coccoid rod; SR, spiny rod; and V, vibrio.
[b] Abbreviations: W, ammonia oxidizer medium (22); SW, ammonia oxidizer medium (18); CA, ammonium oxidizer medium (8); and W/2 and W/20, W medium in 50 and 5% seawater, respectively.
[c] Symbols: −, No fluorescence; +/−, trace fluorescence; and + to ++++, increasingly bright fluorescence. Reaction in parentheses indicates result of adsorbing anti-*Nitrosomonas* serum with *Nitrosococcus* cells.
[d] S. W. Watson culture collection.
[e] E. L. Schmidt culture collection.
[f] NA, Not available.
[g] B. B. Ward culture collection.
[h] A. F. Carlucci culture collection.
[i] S. G. Horrigan culture collection.

Staining procedures. Samples of the specific antisera were stored frozen. Working stocks were diluted five times with phosphate-buffered saline (PBS; 22) and frozen between uses. Just before use, the sera were diluted with PBS to the highest dilution giving a +4 staining reaction (see below) and filtered through 0.2-μm Nuclepore filters.

(i) Cross-reaction tests. For most nitrifying strains, cultures never attained a density high enough to make the use of smears practical. Therefore, a method for rapid staining of 36 culture samples simultaneously in a Beckman microcentrifuge was developed. Microcentrifuge tubes (1.5 to 1.8 ml) were filled with Formalin-fixed samples of the appropriate test culture (2% final concentration of Formalin [vol/vol]), and the cells were washed twice with filtered PBS. After aspirating off the wash, 20 μl of serum (10 to 50 times diluted in PBS) was pipetted onto the invisible pellet; the tubes were vortexed and incubated at room temperature for 30 min. After incubation, excess antiserum was removed with two PBS washes, and the incubation was repeated with the fluorescent antibody (fluorescein isothiocyanate-conjugated sheep anti-rabbit immunoglobulin G; Miles Laboratories, Inc.). After two final PBS washes, the pellet was resuspended in PBS and carbonate buffer (1:1; 22) and stored in the refrigerator until microscopic inspection. Before resuspension, a drop of the pellet was wet mounted on a slide and viewed with an epifluorescence microscope. However, the following filtering method was also used. Slides of the stained samples were prepared by filtering the resuspended cells onto Irgalan black-dyed 0.2-μm Nuclepore filters. Half of the sample was filtered and mounted with

TABLE 2. Reactivity of ammonium and nitrite oxidizer antisera with nitrite-oxidizing strains and isolates

Organism	Source or location (water depth)	Type	Date of isolation	Morphology[a]	Growth medium[b]	Antiserum reactivity[c]				
						Nitrosococcus oceanus	*Nitrosomonas* sp. (marine)	*Nitrococcus mobilis*	*Nitrobacter* sp. (marine)	*Nitrospina gracilis*
Nitrococcus mobilis	Galapagos Islands (0 m)[d]	Marine	1966	C	N	−	−	++++	++	−
Nitrobacter sp. (marine)	Gulf of Maine[d]	Marine	1967	R	N	−	−	+++	++++	−
Nitrospina gracilis	200 miles (ca. 371 km) from Amazon River mouth (1 m)[d]	Marine	1964	SR	N	−	−	−	−	++++
Nitrospira immobilis	Gulf of Maine (200 m)[d]	Marine	1967	R	N	−	−	−	++	−
B50	SIO pier[e] sediment-water interface	Marine	1963	R	N	−	+/−	−	+++	−
26N	lat. 40°50'N, long. 155°W (0.5 m)[e]	Marine	1964	R	N	−	+	−	−	++
32N	lat. 36°49'N, long. 155°W (0.5 m)[e]	Marine	1964	R	N	−	−	−	++	−
T-1B	lat. 38°45'N, long. 76°8'W (0 m)[f]	Estuarine	1983	R	N/20	−	−	++	−	−
T-2B	lat. 38°45'N, long. 76°8'W (0 m)[f]	Estuarine	1983	CR	N/20	−	−	+++	+	++++
T-3B	lat. 38°45'N, long. 76°8'W (0 m)[f]	Estuarine	1983	CR	N/20	−	−	−	++++	++++
B-1B	lat. 38°45'N, long. 76°12'W (0 m)[f]	Estuarine	1983	R	N/20	−	−	−	−	−
B-2B	lat. 38°45'N, long. 76°12'W (0 m)[f]	Estuarine	1983	CR	N/20	−	−	++++	+++	++++
B-3B	lat. 38°45'N, long. 76°12'W (0 m)[f]	Estuarine	1983	CR	N/20	−	−	+++	++	+++

[a] See footnote *a* of Table 1 for abbreviations.
[b] Abbreviations: N, nitrite oxidizer medium (8); and N/20, N medium in 5% seawater.
[c] See footnote *c* of Table 1 for an explanation of symbols.
[d] S. W. Watson culture collection.
[e] A. F. Carlucci culture collection.
[f] S. C. Horrigan culture collection.

TABLE 3. Reactivity of ammonium and nitrite oxidizer antisera with heterotrophic bacterial strains and isolates

Organism	Source or location (water depth)	Type	Date of isolation	Morphology[a]	Growth medium[b]	Antiserum reactivity[c]				
						Nitrosococcus oceanus	*Nitrosomonas* sp. (marine)	*Nitrococcus mobilis*	*Nitrobacter* sp. (marine)	*Nitrospina gracilis*
Escherichia coli	[d]	Enteric	NA[e]	R	CP	−	−	−	−	−
Vibrio harveyi	[f]	Marine	NA	V	CP	−	−	−	−	−
Beneckea gazogenes	[i]	Marine	1978	V	CP	−	−	−	−	−
Serratia marinarubra	SIO pier[h]	Marine	NA	R	CP	−	−	−	−	−
Xmas	[g]	Marine	NA	V	CP	−	−	−	−	−
URW HET	[g]	Marine	1979	R	CP	−	−	+/−	−	−
BVI-SJG	[i]	Marine	1978	V	CP	−	−	+/−	−	−
297-1	[g]	Marine	1983	R	CP	−	−	−	−	−
297-3	[g]	Marine	1983	R	CP	−	−	+/−	−	−
297-2Y	[g]	Marine	1983	R	CP	−	−	−	−	−
297-20	[g]	Marine	1983	R	CP	−	−	−	−	−
C3-1	[g]	Marine	1983	R	CP	−	−	−	−	−
C3-2	[g]	Marine	1983	R	CP	+/−	−	−	−	+
LN150	lat. 32°53'N, long. 117°15'W (7 m)[h]	Marine	1971	R	CP	−	−	−	−	−
LN161	lat. 30°59'N, long. 155°24'W (100 m)[h]	Marine	1972	R	CP	−	−	−	−	−
HET11	Central North Pacific (0 m)[h]	Marine	1964	R	CP	−	−	−	−	−
HET15	Central North Pacific (0 m)[h]	Marine	1964	R	CP	−	−	−	−	+/−

[a] See footnote *a* of Table 1 for abbreviations.
[b] CP, Heterotroph medium (7).
[c] See footnote *c* of Table 1 for an explanation of symbols.
[d] Provided by D. Cramer, University of Washington.
[e] NA, Not available.
[f] K. H. Nealson culture collection.
[g] B. B. Ward culture collection.
[h] A. F. Carlucci culture collection.
[i] Provided by S. J. Giovanonni, University of Oregon.

glycerol and carbonate buffer (22), and half was filtered and stained with acridine orange (1% in distilled water; 10). The acridine orange preparation served as a check to ensure that a lack of immunofluorescence reaction did not mean that the cells had been lost or destroyed in the staining procedure and to note the morphology of cells which were not visible by immunofluorescence.

A Zeiss epifluorescence microscope with a 100-W tungsten halogen lamp and Zeiss filter set no. 487709 was used to view both the immunofluorescence and acridine orange preparations. A subjective intensity scale, based on the brightness of the fluorescent cells (+4 = maximum fluorescence, +/− = trace fluorescence) was used to assess the strength of the immunofluorescent reaction. A +2 observation was chosen as the cutoff for designating significant reactions (see below). All cross-reaction tests were performed at least twice to check the reproducibility of the subjective scale.

If cross-reaction tests indicated that adsorption of the sera was necessary, then the procedure of Belly et al. (1), involving three sequential adsorptions with the cross-reacting strain, was followed. Adsorption of antisera with a marine heterotrophic strain (URW HET; 19) helped minimize general background and nonspecific fluorescence. *Nitrobacter* and *Nitrococcus* antisera both showed slight but significant cross-reactions with heterotrophic contaminants isolated from the batch cultures used for immunization. These sera were adsorbed as above with cells of the cross-reacting strain. Where noted (see below), anti-*Nitrosomonas* serum was adsorbed with *Nitrosococcus* cells by the same procedure (1).

(ii) Environmental samples. Seawater samples were collected on cruise SCBS-21 of the R/V New Horizon in the Southern California Bight in November 1982. Samples from station 205 (lat. 33°18.7' N, long. 118°09.6' W; water depth 777 m) were collected in 30-liter Niskin bottles; subsamples (250 ml) were fixed with buffered 2% Formalin and stored in the dark. Replicate 10- or 20-ml samples were stained, each with different antiserum, so that each strain was enumerated on a separate sample. For field samples, the final dilution of the specific antiserum was completed by adding filtered, partially hydrolyzed gelatin (6) (final gelatin concentration, 1%). The membrane filter staining procedure of Ward and Perry (22) was modified to omit the preliminary incubation with gelatin. This modification improved slide quality by drastically reducing background nonspecific staining and decreased the amount of time required for staining by deleting the incubation step with gelatin and reducing the wash time between antisera incubations. A multiple-filter rig (10 places; Hoefer Scientific Instruments, Inc., San Francisco, Calif.), which allowed individual control of suction on each filter, was most convenient for this procedure.

Stained cells in the field samples were identified by morphology; for example, stained rods on slides stained with anti-*Nitrosomonas* serum were designated *Nitrosomonas* organisms, and any other cells on these filters were ignored (since the specific antisera did react with other strains of the same physiological type, *Nitrosococcus*-type cells were sometimes seen on *Nitrosomonas* slides; see below). Counts reported here are the means of three replicates for each antiserum at each depth; 150 fields were counted on each slide. Nonparametric tests (19) for comparisons of means (Mann-Whitney U test) were used to determine whether significant differences existed among counts ($\alpha = 0.05$ was chosen as the criterion for significance).

RESULTS

Specificity and diversity. The five antisera were tested against a total of 72 strains (Tables 1 to 3), including 35 ammonium-oxidizing strains, 13 nitrite-oxidizing strains, and 17 heterotrophic strains. (A few of the results in Table 1 for ammonium oxidizer antisera were reported previously [20] but are included here for completeness.) The results for all sera against ammonium-oxidizing strains are presented in Table 1, results for all sera against nitrite-oxidizing strains are presented in Table 2, and results for all sera against heterotrophs are presented in Table 3. The major findings from these tests may be summarized as follows.

(i) Antisera produced against nitrifying bacteria did not cross-react (fluorescence of $<+2$, usually $-$ or $+/-$) with randomly isolated marine heterotrophs or selected heterotrophic marine isolates from long-term culture collections (Table 3).

(ii) Antisera produced against ammonium-oxidizing strains had negligible cross-reactions with nitrite-oxidizing strains (Table 2), and antisera produced against nitrite-oxidizing strains did not react significantly with ammonium-oxidizing strains (Table 1).

(iii) Not all of the ammonium- and nitrite-oxidizing isolates reacted with antisera produced to genera of their respective physiological type, although in some cases there were no morphological differences between the isolates and the homologous strains which could be detected with the epifluorescence microscope. Of the strains that did react (fluorescence, $\geq+2$), many were stained by more than one of the antisera (Tables 1 and 2); this was particularly true for the ammonium oxidizers, of which 70% reacted with anti-*Nitrosomonas* serum and 33% reacted with anti-*Nitrosococcus* serum. Most of our test organisms were originally collected from the North Pacific Ocean; however, our antisera react with organisms from all geographic regions represented (Table 1). All of the strains reacting with anti-*Nitrosococcus* serum also reacted with anti-*Nitrosomonas* serum, so that 33% reacted with both sera. The anti-*Nitrosomonas* serum is evidently less specific than the other. Based on morphology, 11 of the ammonia-oxidizing strains were of the *Nitrosococcus* type, and 9 were of the *Nitrosomonas* type. Anti-*Nitrosomonas* serum which had been adsorbed with *Nitrosococcus* organisms did not react with the 11 coccoid, ammonium-oxidizing strains. Adsorption did not affect the homologous reaction with *Nitrosomonas* organisms nor the reactions between anti-*Nitrosomonas* serum and the ammonium-oxidizing strains of rod morphology. For nitrite oxidizer antisera, eight of the nine isolates tested reacted with at least one of the sera, and four reacted with all three of them.

(iv) Both marine and nonmarine (i.e., estuarine or soil origin) nitrifying isolates were tested. Of the ammonium-oxidizing isolates (Table 1), 11 of 24 strains of marine origin reacted with *Nitrosococcus* antiserum, and 16 of 24 reacted with *Nitrosomonas* serum. Anti-*Nitrosococcus* serum stained only one isolate of estuarine origin, whereas anti-*Nitrosomonas* serum stained six of nine nonmarine strains. Only three unidentified marine nitrite-oxidizing strains were included in the study (Table 2). Two of these reacted with anti-*Nitrobacter* serum, and the third strain reacted with anti-*Nitrospina* serum, whereas none reacted with anti-*Nitrococcus* serum (Table 2). Most of the six estuarine strains tested reacted with one or more of the nitrite oxidizer antisera, including anti-*Nitrococcus* serum.

(v) Of the ammonium-oxidizing isolates tested, 24% were coccoid and the rest were rods, usually of a plump elliptical shape. In culture, the nitrifying bacterial cells are relatively shorter and fatter than most heterotrophs grown in rich medium or observed in environmental samples. No cocci were found among the nonmarine ammonium-oxidizing isolates. All of the marine nitrite-oxidizing isolates were narrow rods; the estuarine strains were mostly coccoid rods. The unique nitrite-oxidizing strain, *Nitrospina gracilis*, had a distinct morphology shared by none of the other isolates available to us. However, *Nitrospina* antiserum did stain five of the heterologous nitrite-oxidizing isolates; these cross-reacting cells were of coccoid rod morphology.

(vi) In addition to enumerating nitrifying cells in preserved seawater samples collected on a cruise in the Southern California Bight (see below), we also tested our antisera on enrichment cultures obtained from water column, sediment trap, and sediment-water interface samples at a nearby station on a subsequent cruise. These cultures have not been purified and thus cannot be included in the isolate tests reported above. However, every enrichment culture obtained (10 in all) was found to contain cells which reacted with one or more of our antisera.

Distribution and abundance in seawater. The depth distributions of four of the five strains of nitrifying bacteria for which we possess antisera are shown in Fig. 1. Cells reacting with *Nitrospina* antiserum were very rare and difficult to identify due to the highly pleomorphic cells of this strain. Therefore, no quantitative data are presented for the distribution of *Nitrospina* organisms; however, they were detected in some of the samples. Based on three replicate counts for each antiserum at each depth, the coefficient of variation (CV) for all counts averaged 40%. Counts for ammonium oxidizers were more variable (CV averaged 48%) than counts for nitrite oxidizers (CV averaged 33%). There was no significant difference in the distribution pattern of total ammonium-oxidizing and total nitrite-oxidizing cells in the water column (Kolmogorov-Schmirnov test, $\alpha > 0.20$). Cells generally occurred singly or, more rarely, in groups of two or four. Large clusters of the kind observed in sediment trap samples (13) were not found in these water column samples.

The total abundance of ammonium-oxidizing cells (*Nitrosococcus* plus *Nitrosomonas* organisms) averaged 3.5×10^5 cells liter^{-1} and varied by only a factor of 2 over the water column. Total cell concentration appeared highest in the surface (1-m) sample, while the lowest total counts were found in the 9- and 26-m samples (Fig. 1A). Due to the high variability associated with these counts, there were no statistically significant differences with depth in the total counts.

Nitrosomonas organisms were generally more abundant than *Nitrosococcus* organisms, but the difference varied from zero to a factor of 3. *Nitrosomonas* organisms were significantly more abundant than *Nitrosococcus* organisms at only two depths, i.e., 9 and 700 m. *Nitrosomonas* organisms were relatively constant with depth; no significant differences among depths were found. However, *Nitrosococcus* abundance varied significantly over the water column; differences were found between 1 and 9 m and between 700 and 750 m.

Total nitrite-oxidizing cells (*Nitrobacter* plus *Nitrococcus* organisms) averaged 2.8×10^5 cells liter^{-1} over the water column (Fig. 1B), an amount not significantly different from the total for ammonium-oxidizing cells. The total for nitrite oxidizers showed a maximum at 9 m (significantly different from 1 m) and a minimum at 150 m. Abundance at 700 m was

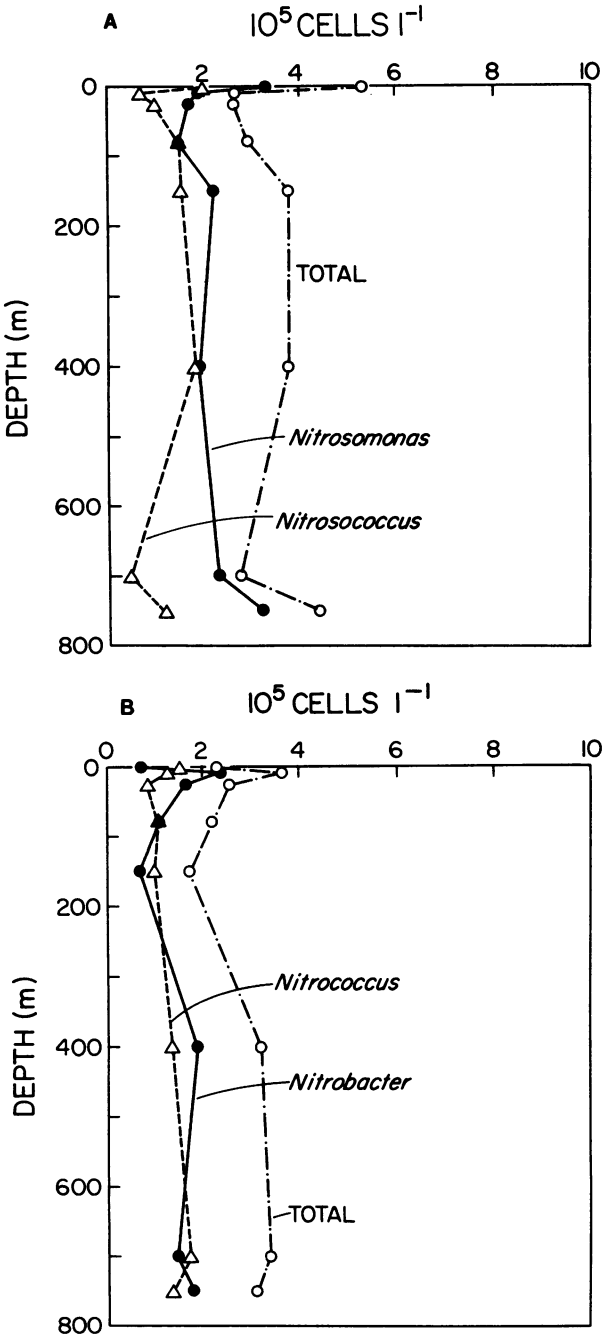

FIG. 1. Nitrifying bacteria abundance at station 205 (lat. 33°18.7′ N, long. 118°09.6′ W). (A) Ammonium oxidizers. Symbols: (○) total, (△) *Nitrosococcus* organisms, and (●) *Nitrosomonas* organisms. (B) Nitrite oxidizers. Symbols: (○) total, (△) *Nitrococcus* organisms and (●) *Nitrobacter* organisms.

significantly higher than at 150 m. Neither nitrite-oxidizing strain was consistently more abundant than the other throughout the water column. Significant differences in abundance between the strains were found at 1 m (*Nitrococcus* organisms were more abundant) and at 400 m (*Nitrobacter* organisms were more abundant). *Nitrobacter* abundance varied more among different depths (significant differences between successive depths of 1 and 9 m, 400 and 700 m, and 700 and 750 m), while *Nitrococcus* abundance was relatively constant with depth (no significant differences among depths) and did not show the subsurface maximum found in *Nitrobacter* abundance.

DISCUSSION

The degree of cross-reactivity between antisera prepared against one strain and other related strains of the same physiological type reported here is greater than that observed by Belser and Schmidt (3) in a study on the serological diversity of soil ammonium-oxidizing bacteria or by Josserand and Cleyet-Marel (12) for soil nitrite-oxidizing populations. Belser and Schmidt (3) found very little cross-reactivity across genus lines and relatively little cross-reactivity among different strains of the same genus. Josserand and Cleyet-Marel (12) identified at least four unique serotypes among nitrite oxidizers of identical morphology. The isolates we tested have not been identified to genus; however, they include a variety of discernibly different morphological types under similar culture conditions and span an unknown degree of taxonomic diversity. Several reasons are possible for the greater cross-reactivity that we observed. (i) The marine ammonium-oxidizing population is less diverse than the soil population. The water column environment may present less habitat diversity than the soil environment, resulting in a relatively less diverse microbial assemblage. Although consistent with the data presented here, this conclusion is not warranted by these data alone, since the diversity of isolates available from any environment depends on isolation medium and method as well as the biological diversity of the natural population from which the isolates derive. Thus, we cannot rule out (ii) a bias in the culture methods leading to a nondiverse culture collection. (iii) The marine isolates may share an outer antigen somehow associated with their existence in seawater. Broad specificity was found in both our ammonium and nitrite oxidizer antisera, which might implicate a common environmental factor.

(iv) Methodological differences in immunization procedures and antigen preparation may have resulted in antisera of different specificity. It is possible that the sera used in the present study were more complex (i.e., contained antibodies from a greater number and variety of antibody-producing clones) due to a difference in the immunogenicity of the antigen preparation or the scheduling of the immunization procedure. It was previously reported (20) that the two ammonium oxidizer antisera did not react across genus lines. We have since found that the specificity varies among rabbits. Of two rabbits immunized with the same *Nitrosomonas* antigen preparation, one produced serum that reacted with *Nitrosococcus* organisms, whereas serum from the other did not. The fact that adsorption of anti-*Nitrosomonas* serum with *Nitrosococcus* cells removed the cross-reaction between this serum and all coccus-shaped ammonium-oxidizing strains with no detectable effect on the homologous reaction probably indicates that a few shared antigens were responsible for the cross-reaction. However, it is a logical and potentially useful result that many marine, ammonium-oxidizing strains have some surface antigens in common. Fliermans et al. (9) found that antisera raised against one strain of *Nitrobacter* isolated from soils cross-reacted (+2) with another strain of the same genus. The cross-reaction could be removed by adsorption to obtain a serotype-specific serum. Josserand and Cleyet-Marel (12) pursued this approach to show that, by progressive adsorp-

tion of cross-reacting sera, several "serotypes" could be defined within a soil nitrite-oxidizing population. Unfortunately, the interpretation and utility of defining serotypes in this way is questionable. A complex serum can be made as narrowly specific as desired by progressive adsorption. But is the specificity thus obtained characteristic of the target bacterial strain or an artifact of the serological techniques? We maintain that for ecological applications, serum of rather broad specificity is most useful; one that stains all strains of a particular physiological type but none of any other physiological type would be optimal for some objectives. Since complex sera of the type used here can vary in strength and specificity, even when raised against identical antigen preparations, it is very important to fully characterize a particular serum before use with environmental samples. For some applications, purification of the immunoglobulins or the use of monospecific or monoclonal antibodies may be necessary.

The true diversity of the marine nitrifying population is impossible to know, and we do not know to what extent cultures obtained by commonly used enrichment methods are representative of the natural population. However, the sera used here did react with most strains tested and yielded ecologically reasonable estimates of in situ nitrifier abundances. We recognize that using immunofluorescence to enumerate nitrifying bacteria in seawater will always lead to an underestimate of the total cell concentration of any physiological type. However, the demonstrated and potential specificity of the assays means that cells of a particular serological similarity can be quantitatively detected and that qualitative aspects of the strain composition of the in situ population can be determined.

The limit of detection in the quantitative analysis of field samples depends on the volume filtered, the filter area, and the number of cells and microscope fields counted. The filter staining method used here resulted in a limit of detection of ca. 10^4 cells liter^{-1} when a 25-ml sample is filtered. This limit could be lowered by optimizing the variables listed above (e.g., by using a smaller filter area). The counts presented here generally exceeded the limit of detection by several times. However, the CV of counts at this level is still high; the concentration of samples before staining to increase the number of cells counted per slide and thus decrease the CV (14) might improve counting statistics.

Despite the large error in the counts, the ammonium-oxidizing bacterial abundance estimates presented here are higher than estimates for a station in the Southern California Bight reported previously (20). This difference may in part be due to improvements in the enumeration procedures. Total counts by acridine orange (A. F. Carlucci and D. B. Craven, unpublished data) of the bacterial population in San Pedro Basin (another basin in the California borderland, near the station sampled for nitrifier counts) ranged from 3×10^8 cells liter^{-1} at the surface to 4×10^7 cells liter^{-1} at 850 m. Given the relative constancy of nitrifier abundance with depth, ammonium oxidizers and nitrite oxidizers would each constitute an increasing proportion of the total population, i.e., 0.1% at the surface and 0.8% at 750 m. This changing proportion of nitrifying bacteria is probably due to the differential influence of light, oxygen concentration and substrate availability on nitrifiers and the heterotrophs which compose most of the bacterial population.

Although nitrifying bacteria exhibit photoinhibition in laboratory and field studies of nitrification rates (5, 11, 15, 16, 21), nitrifiers are frequently isolated from surface waters (8, 11) and are detected there by immunofluorescence (20; see above). In fact, most of the nitrifying strains used in this study were obtained from relatively shallow depths, and our sera detected high numbers of nitrifiers in surface samples. Other strains which do not react with our sera may exist in the ocean and have different depth distributions than the strains detected in this study.

ACKNOWLEDGMENTS

Technical assistance by D. B. Craven and C. E. Murphy is appreciated.

This work was supported by the National Science Foundation (OCE81-17697).

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