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Distribution of Actinomycetes in Near-Shore Tropical Marine Sediments

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Actinomycetes were isolated from near-shore marine sediments collected at 15 island locations throughout the Bahamas. A total of 289 actinomycete colonies were observed, and all but 6 could be assigned to the suprageneric groups actinoplanetes and streptomycetes. A bimodal distribution in the actinomycete population in relation to depth was recorded, with the maximum numbers occurring in the shallow and deep sampling sites. This distribution can be accounted for by a rapid decrease in streptomycetes and an increase in actinoplanetes with increasing depth and does not conform to the theory that actinomycetes isolated from marine sources are of terrestrial origin. Sixty-three of the isolated actinomycetes were tested for the effects of seawater on growth. Streptomycete growth in nonsaline media was reduced by 39% compared with that in seawater. The actinoplanetes had a near obligate requirement of seawater for growth, and this is presented as evidence that actinomycetes can be physiologically active in the marine environment. Problems encountered with the enumeration of actinomycetes in marine sediments are also discussed.

It is well documented that actinomycetes can be isolated from marine sediments (see review by Goodfellow and Haynes [4] and, more recently, references 1, 14, 15, and 23), including samples from the deep sea (19). In addition, the descriptions of *Rhodococcus marinonascens* (8), along with other marine species (6), show that certain actinomycetes are indigenous to the marine environment. However, because actinomycetes are more abundant in terrestrial soils relative to marine sediments (5), show varying degrees of salt tolerance (9, 12, 13, 18), and produce spores that are undoubtedly washed in large numbers from shore into the sea, it remains unclear what component of the actinomycetes isolated from marine sources represents an autochthonous marine microflora (4, 6, 13).

In addition to uncertainties about the origin of actinomycetes in marine habitats, it is not known to what extent these bacteria represent a physiologically active component of the marine microbial community. It has been shown that actinomycetes can grow in a seawater-based medium (22) and at increased hydrostatic pressures (7) and that increased numbers of *Micromonospora* occur with increasing depth in deep-sea sediments (21, 23). Yet, due to the overall observation that actinomycetes decrease in number as distances from shore increase (19–21), and a lack of experimental evidence describing the distribution and metabolic activity of these bacteria in marine habitats, it has been concluded that, unless exceptional conditions occur, actinomycetes isolated from marine sources arise from spores or resting propagules (4, 5).

We report here the occurrence of actinomycetes in near-shore tropical marine sediments collected throughout the Bahamas. The observed actinomycetes were grouped taxonomically, and their distributions and the effects of seawater on biomass production were determined for the dominant taxonomic groups. Based on these results, conclusions are made regarding the ability of actinomycetes to grow in the marine environment.

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MATERIALS AND METHODS

Collection of samples. Marine sediments were collected from 15 island locations throughout the Bahamas as part of a research expedition aboard the R/V *Columbus Iselin* (University of Miami) in June 1989. The locations sampled included the islands of Chub Cay (sites 1 to 5), Grand Bahama (site 6), Abaco (site 7 to 9), San Salvador (sites 10 and 15), Aklins (sites 11 to 13), and Hogsty Reef (site 14). These locations included diverse environments ranging from an oceanic atoll (Hogsty Reef), ca. 65 km from the nearest island, with only a sand spit and scrub vegetation above the high-water line, to areas highly influenced by terrestrial runoff, e.g., mangrove habitats.

At each of the 15 locations, divers collected five sediment samples (total, 75 samples), one from each of the depths 0 to 1, 1 to 3, 3 to 6, 6 to 15, and 15 to 33 m, by starting near shore and heading seaward. The habitats encountered at each sampling depth varied and were categorized as sand, mangrove, seagrass, hard bottom, or reef. Sediments were collected in sterile 50-ml tubes and kept refrigerated until shipboard processing later that day.

Sample processing. Sediment samples were vigorously shaken by hand to ensure uniformity and then allowed to settle for ca. 10 min. The overlying water was decanted and used for the determination of pH and salinity. Sediment organic content was determined by drying triplicate subsamples of each sediment (1 to 9 g, dry weight) to a constant weight at 90°C, ashing at 450°C for 2 h, and reweighing. The organic contents were determined as the average difference between dry and ash weights for the triplicate subsamples.

Serial dilutions of the 75 sediment samples were made by aseptically removing 1.0 ml of wet sediment and adding it to 4.0 ml of sterilized filtered seawater (dilution, 2×10^{-1}), mixing, and further diluting 1:10 with sterilized filtered seawater (dilutions, 2×10^{-2} through 2×10^{-4}). The dilution procedure was repeated in triplicate for each sample, resulting in a total of 225 serial dilutions. Sediment dry weight per milliliter was determined for each sample as the average weight of two additional (2×10^{-1}) dilutions prepared with deionized water and oven dried at 90°C for 48 h.

The diluted sediments were inoculated onto two types of

nutrient agar. Medium 1 (M1; 0.25% starch, 0.1% peptone, 0.05% yeast extract, 0.01% glycerophosphate [disodium pentahydrate], 1.6% agar, 75% filtered seawater, 25% deionized water) was selected as a general medium for the enumeration of heterotrophic bacteria. Medium 2 (M2; 1.0% starch, 0.1% casein, 0.01% trace element mix [Marineland Aquarium Products], 1.6% agar, 75% filtered seawater, 25% deionized water) was chosen for the isolation of actinomycetes (6). All media contained 75 μg of filter-sterilized cycloheximide per ml, added aseptically after the media had been autoclaved and cooled, to reduce fungal contamination.

Plates of M1 and M2 were inoculated with 50 μl of dilutions 2×10^{-2} through 2×10^{-4}, and the samples were spread with a sterilized bent glass rod and plate spinner. The 2×10^{-2} and 2×10^{-3} dilutions were then heated to reduce the number of unicellular bacteria in favor of actinomycetes (2) by submersion in a water bath at 50°C for 60 min, and 50 μl of these heat-treated samples was inoculated onto M1 (M1+H).

Bacterial and actinomycete enumeration. Colonies of unicellular bacteria were counted on M1 plates after 14 and 21 days of room temperature (20 to 24°C) incubation. The average colony count at each dilution was calculated with plates with 20 to 250 colonies. CFU per milliliter of wet sediment were calculated for each sample, using the highest dilution that yielded an average of >20 colonies. CFU per gram (dry weight) were calculated based on the dry weight per milliliter of wet sediment.

Actinomycetes were recognized by their characteristic tough, leathery colonies, branched vegetative mycelia, and, when present, aerial mycelia and spore formation. Due to these criteria, only actinomycetes with well-developed and branched hyphae were included in this study. Actinomycete colonies were counted on M1, M2, and M1+H plates after 21, 30, and 45 days of room temperature incubation, and information about colony morphology, including the presence or absence of aerial mycelia, was recorded.

Due to problems associated with the use of serial dilution and plating techniques for the enumeration of actinomycetes (21; this report), only actinomycete colonies on the 2×10^{-2} dilution plates were counted. Because of the low numbers of actinomycetes observed, counts from the triplicate plates were summed. Total actinomycete colonies were calculated for each depth as the individual and collective sums (15 collecting sites) for the M1, M2, and M1+H plates. Actinomycetes were not quantified per unit sediment, and colony counts are of value in a relative sense for the comparison of depth distributions, treatments, etc.

Actinomycete isolation and taxonomic evaluation. Actinomycetes representing all colony morphologies observed from each sample were isolated by repeated transfer on medium M3 (1.0% starch, 0.4% yeast extract, 0.2% peptone, 1.6% agar, 75% filtered seawater, 25% deionized water) until pure strains were obtained as judged by colony morphology. All isolated strains were grown at room temperature in 10 ml of M3 without agar, observed microscopically for the presence of mycelial fragmentation, and frozen with 10% glycerol as a cryoprotectant.

All isolated actinomycetes with aerial mycelia, and representatives of all morphologies with only vegetative mycelia, were analyzed chromatographically for isomeric diaminopimelic acid configurations and for whole-cell sugar composition as described by Schaal (16) with the following modifications: isolates were grown in 4.0% yeast extract–4.0% glucose–75% filtered seawater–25% deionized water, and the cells were hydrolyzed by autoclaving in 1 ml of 6 N HCl at 121°C for 15 min. Chromatographies were performed by using glass-backed cellulose-coated thin-layer chromatography plates (Merck 5716-7). Based on isolate morphology and diaminopimelic acid and whole-cell sugar analyses, all but a few strains could be grouped suprageneric ally as described by Goodfellow (3) and Lechavalier (10). Taxonomic information from the representative isolates was extrapolated to the total actinomycete population observed on the 2×10^{-2} plates.

Effects of seawater on actinomycete growth. Representatives of the dominant suprageneric groups were grown in medium M4 (1% starch, 0.4% yeast extract, 0.2% peptone, 100% filtered seawater) and M4 substituted with deionized water to determine the effects of seawater on growth as determined by biomass production. Starter cultures were generated by inoculating 1.0 ml of frozen culture (M3 plus 10% glycerol) into 10 ml of M3. These cultures were shaken at 150 rpm and room temperature until adequate growth was obtained (3 to 10 days), at which time 5.5 ml was dispensed into 100 ml of both M4 and M4 substituted with deionized water. Because starter cultures were grown in a seawater-based medium (M3), the final concentration of seawater in 100 ml of M4 substituted with deionized water was approximately 4%; therefore, these cultures were provided with trace quantities of salts and minerals.

All 100-ml cultures were shaken at 230 rpm and room temperature for 8 days, after which the entire volumes were filtered onto 47-mm type A/E glass fiber filters (Gelman Sciences), freeze-dried, and weighed. The average dry weights of triplicate 100-ml volumes of uninoculated M4 and M4 substituted with deionized water were calculated and subtracted as medium controls.

RESULTS

A total of 289 actinomycete colonies were observed from the 75 sediment samples collected. From these, 35 colonies with aerial mycelia and 64 colonies with only substrate mycelia were isolated as morphological representatives. All 35 isolates with aerial mycelia and 18 of 64 isolates with only substrate mycelia, including representatives of all morphologies observed, were examined chemotaxonomically. The combined morphological and chemotaxonomic evaluations of the representative isolates were extrapolated to the entire observed population. From this, it was determined that 91 of the 289 observed colonies had both aerial and substrate mycelia, did not fragment when grown in liquid culture, possessed predominantly the LL isomer of diaminopimelic acid, and had no diagnostic whole-cell sugar pattern. Based on these results, 91 of the total observed actinomycetes were assigned to the suprageneric group streptomycetes or the genus *Kitasatosporia* (10). For the purpose of this study, all 91 of these actinomycetes will be considered to belong to the streptomycete group, within which there are five genera, including the genus *Streptomyces* (3).

Of the remaining 198 observed actinomycetes, 192 lacked aerial mycelia, possessed predominantly the *meso* isomer of diaminopimelic acid, and had xylose and arabinose as diagnostic whole-cell sugars. These bacteria commonly displayed orange to red-brown mycelia and, when present, spores that blackened the surface of the colonies. Based on these results, 192 of the observed actinomycetes belong to the suprageneric group actinoplanetes (3). There are six genera within this suprageneric group and, based on morphological characteristics, most of the observed strains appear to belong to the genus *Micromonospora*. In total,

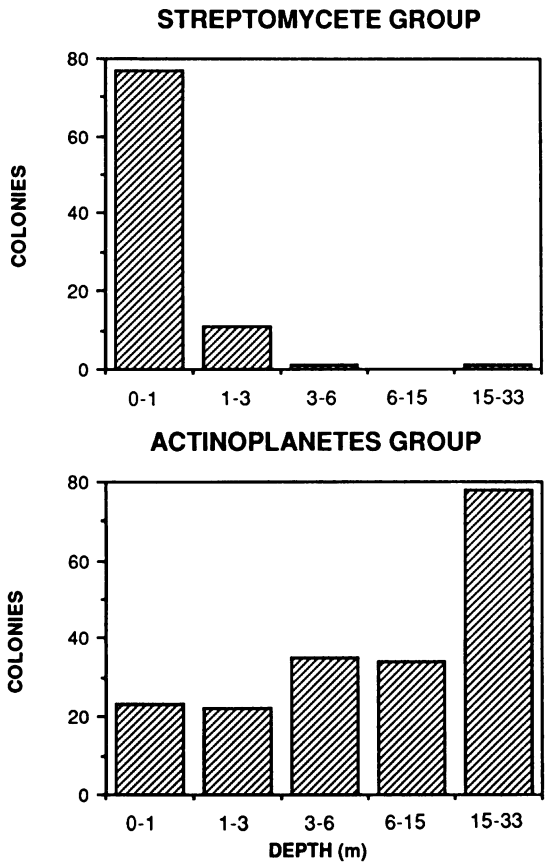

FIG. 1. Distribution versus depth for streptomycetes and actinoplanetes calculated as the total number of colonies observed from 15 locations (2×10^{-2} dilution).

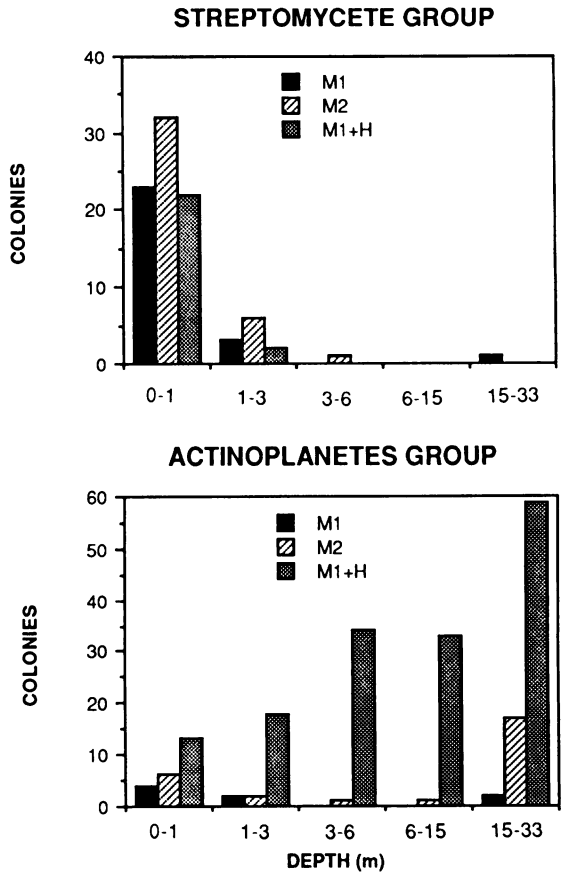

FIG. 2. Distribution versus depth for streptomycetes and actinoplanetes calculated as the total number of colonies observed from 15 locations (2×10^{-2} dilution) for M1, M2, and M1+H.

97% of the observed actinomycete population could be grouped suprageneric ally within the streptomycetes or actinoplanetes. Of the remaining six colonies, one was placed in the suprageneric group nocardioforms, two could not be grouped, and three could not be grown in sufficient quantities for chemotaxonomic evaluation.

The highest numbers of actinomycetes were observed from the 0- to 1- and the 15- to 33-m sampling sites (Fig. 1). This bimodal maxima in the actinomycete population can be accounted for by the taxon-specific distributions of the suprageneric groups. The number of streptomycetes decreased rapidly with increasing water depth, with 86% being recovered from 0 to 1 m and 12% from 1 to 3 m. In all, 98% of the streptomycetes observed were from water $\leq$3 m deep. Conversely, the number of actinoplanetes increased with increasing water depth, with 41% of the 192 observed colonies coming from the deepest sampling site (15 to 33 m). However, unlike the streptomycetes, the actinoplanetes were more evenly distributed throughout the sampling zones, with 12, 11, 18, and 18% of the total isolates coming from 0 to 1, 1 to 3, 3 to 6, and 6 to 15 m, respectively.

The large number of actinomycetes observed from the deep sediments can, for the most part, be accounted for by the high number of actinoplanetes obtained from the heat-treated samples (Fig. 2). If heat treatment as a selective isolation method had not been used, the extent of the deep-water actinomycete population would not have been recognized. Both the heat-treated and non-heat-treated samples resulted in approximately equal numbers of streptomycete colonies, with the numbers of these bacteria decreasing rapidly in all cases with increasing depth.

Actinomycetes were observed and isolated from each of the 15 sampling locations (Table 1) including site 14, an oceanic atoll far removed from terrestrial influences, from which all of the actinomycetes observed belonged to the actinoplanetes group. Of the five types of habitats sampled, none proved to be a rich source of actinomycetes, and the numbers observed from any one habitat were variable.

The salinity, pH, and percent organic content of the sediments became less variable as depth increased (Table 2); however, these parameters appeared to have little effect on the number of actinomycetes observed. High numbers of bacteria were recorded from organic rich sediments, yet there was no correlation between percent organic content and number of actinomycetes or in the ratio of actinomycetes to bacteria as a function of depth as reported by Weyland (21).

Standard serial dilution and plating techniques were used in this study for the purpose of isolating and quantifying actinomycetes. However, the actinomycete counts were not a quantitative function of dilution as would be expected when using these techniques. A similar observation was reported by Weyland (21) for actinomycetes isolated from deep-sea sediments. Of the 225 serial dilutions plated, the incidence of a more dilute sample within one dilution series

TABLE 1. CFU of unicellular bacteria (10^4) reported per gram (dry weight) of sediment and actinomycetes reported as the sum of all colonies observed (M1, M2, M1+H, 2×10^{-2} dilution)[a]

Site	Depth														
	0–1 m			1–3 m			3–6 m			6–15 m			15–33 m		
	Habitat	Bacteria	Actinomycetes	Habitat	Bacteria	Actinomycetes	Habitat	Bacteria	Actinomycetes	Habitat	Bacteria	Actinomycetes	Habitat	Bacteria	Actinomycetes
1	MG	455.7	1	SG	179.9	0	SG	243.9	1	RF	19.8	1	RF	184.1	2
2	MG	560.2	28	SG	23.2	2	HB	60.3	4	RF	14.9	3	RF	70.6	1
3	SD	3.1	34	SG	30.0	0	RF	34.4	4	SD	33.7	3	RF	125.4	1
4	SD	3.3	4	HB	64.3	8	HB	34.3	3	SG	19.3	5	SD	45.8	1
5	MG	403.7	0	HB	886.7	5	HB	281.3	0	SG	6.1	0	RF	203.9	0
6	MG	194.7	0	RF	181.5	1	RF	70.9	1	RF	89.3	2	RF	101.2	6
7	MG	192.5	18	HB	190.8	2	HB	127.9	9	RF	589.3	15	RF	163.2	11
8	MG	547.4	2	HB	79.1	5	HB	65.2	3	RF	141.1	0	RF	91.6	3
9	SD	74.4	7	HB	20.0	1	HB	194.3	2	HB	75.8	1	SD	44.9	8
10	SD	41.7	5	SD	54.3	1	SD	34.9	0	SD	32.2	1	RF	25.2	5
11	SD	1.1	0	SD	40.4	6	HB	95.0	0	RF	23.1	0	RF	50.1	24
12	SD	0.5	0	SD	2.2	1	SD	67.3	7	RF	80.3	0	RF	68.8	0
13	SD	3.2	0	SD	11.6	6	SG	1.8	2	RF	5.7	2	RF	32.2	1
14	SD	1.7	1	HB	12.2	0	RF	7.2	0	RF	142.6	0	RF	125.6	7
15	SD	0.6	2	HB	79.7	0	SD	57.8	0	RF	113.3	0	RF	65.6	8

[a] Habitats were categorized as mangrove (MG), sand (SD), seagrass (SG), hard bottom (HB), or reef (RF).

yielding an equal or greater number of actinomycetes than a less dilute sample in that same series was high. For the purpose of this report, we have called this observation a nonquantitative dilution. There were 14 nonquantitative dilutions on M1 of 27 serial dilutions that yielded actinomycetes; for M2, 17 of 39 actinomycete-yielding serial dilutions were nonquantitative, and for M1 following heat treatment, 49 of 129 were nonquantitative.

Nonquantitative dilutions were not observed for unicellular bacteria or streptomycetes. They were only observed for the actinoplanetes, occurring on both media and for the heat-treated samples, and usually on plates with few total bacteria. For example, a sample diluted to 2×10^{-3} and plated would yield 20 colonies of unicellular bacteria and one actinoplanete. This same sample further diluted to 2×10^{-4} would yield two unicellular bacteria and four actinoplanetes. In many cases, the apparent inhibition of the actinoplanetes on the plates inoculated with the more concentrated samples was not caused by the overgrowth of unicellular bacteria, which were few in number and formed well-isolated, individual colonies.

Because of unexplained irregularities in the serially diluted actinoplanete colony counts and the low numbers of total actinomycetes observed, actinomycetes were counted only at the 2×10^{-2} dilution and were not quantified per unit sediment. Of the three isolation methods used, M1 following heat treatment produced a total of 185 actinomycete colonies, with an average of 2.2 colonies on 85 plates. Medium 2 produced a total of 69 actinomycete colonies, with an average of 2.5 colonies on 28 plates; and M1 yielded 35 colonies, with an average of 2.5 colonies on 14 plates.

TABLE 2. Range and mean (x) values for salinity, pH, and sediment organic content

Depth (m)	Salinity (‰)	x	pH	x	Organic content (%)	x
0–1	36–46	38.8	7.4–8.3	7.9	0.3–5.9	2.5
1–3	36–39	37.4	7.5–8.1	7.9	1.6–5.9	2.8
3–6	36–38	37.1	7.8–8.1	7.9	1.4–5.2	2.4
6–15	36–38	36.5	7.7–8.2	7.9	1.6–2.4	2.3
15–33	36–37	36.4	7.7–8.0	7.9	1.6–2.4	2.1

The effect of seawater on biomass production was determined for 32 of the streptomycete isolates and 31 of the actinoplanetes. Of the streptomycetes tested, biomass production in all but five of the isolates was reduced when streptomycetes were grown in deionized water (Fig. 3). The average decrease in biomass for the 32 isolates was 39%. The actinoplanetes (with the exception of isolate 394) grew poorly or not at all in deionized water (Fig. 4), producing on average 92% more biomass when grown in seawater. The dry weights recorded for the actinoplanetes grown in deionized water can, for the most part, be accounted for by the biomass of the initial inoculum.

DISCUSSION

The isolation of actinomycetes from marine sediments is well documented, yet it remains unclear what component of these bacteria represents an indigenous marine microflora. This question persists, in part, because there is little published information describing the distribution, growth, and ecological role of actinomycetes in marine habitats. In addition, because actinomycetes represent a small component of the total bacterial population in marine sediments (4, 5), their role in the marine environment is difficult to assess.

It has been proposed that most actinomycetes isolated from marine sources are of terrestrial origin and reside in the sea as spores or resting propagules (4, 5). Goodfellow and Haynes (4) support this proposition by showing that actinomycetes isolated from North Atlantic sediments, including streptomycetes, and *Micromonospora* and *Rhodococcus* species show no specific seawater requirements, growing equally well on media prepared with either distilled water or seawater. In addition, because actinomycetes are common soil bacteria, produce resistant spores, and are known to be salt tolerant (9, 12, 13, 18), it is likely that they are washed in large numbers from shore into the sea, where some portions remain viable. This concept is supported by the observations that actinomycetes are less common in marine sediments

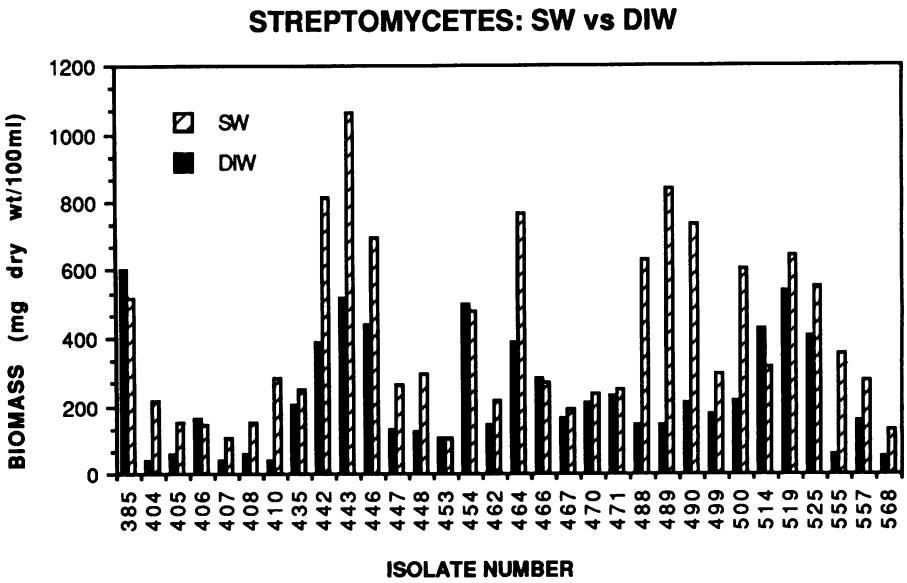

FIG. 3. Dry weight biomass for streptomycetes grown in a seawater-based medium (SW) and the same medium substituted with deionized water (DIW).

relative to terrestrial soils (5) and that they are encountered less frequently as distances from shore increase (19–21).

Contrary to these results, it has been shown by Weyland (22) that nocardioform actinomycetes isolated from marine sediments possess features of bacteria indigenous to the sea and that, with respect to seawater tolerance, *Rhodococcus* species behave like typical marine bacteria (23). We believe that various taxonomic groups of actinomycetes, when isolated from marine sources, differ in their degree of adaptation to the marine environment, and we conclude, based on the results presented here, that certain actinomycetes found in near-shore tropical marine sediments are well-adapted and functional members of the marine microbial community.

Nearly all of the actinomycetes observed in this study could be assigned to the suprageneric groups streptomycetes and actinoplanetes. The streptomycetes decreased in number with increasing distance from shore and were not observed from site 14, the area farthest removed from terrestrial influence. Although the streptomycetes were capable of growth in the absence of seawater, better growth was observed in media containing seawater. This is in agreement with the results of Weyland (22) for streptomycetes isolated from deep-sea sediments.

Based on the streptomycete distribution and their ability to grow in seawater, we speculate that these bacteria are mainly of terrestrial origin but under appropriate conditions

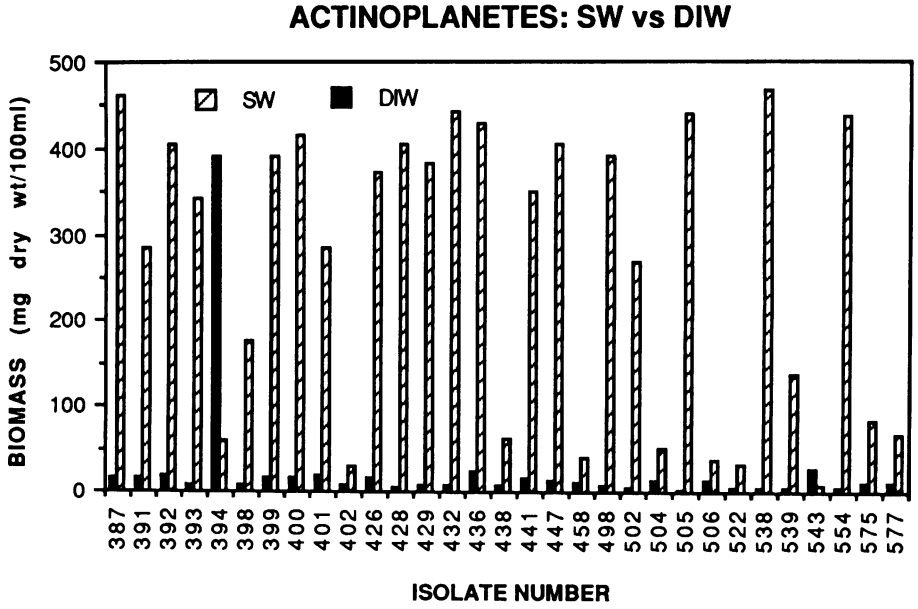

FIG. 4. Dry weight biomass for actinoplanetes grown in a seawater-based medium (SW) and the same medium substituted with deionized water (DIW).

can grow in the marine environment. This conclusion is supported by the finding that terrestrial streptomycetes can adapt to salt-supplemented media by stepwise exposure to increasing concentrations of sodium chloride (13). It is curious, however, that the streptomycete distribution was limited to a narrow range of sediments collected between 0 and 3 m. Although variable spore precipitation rates have been reported for actinomycetes (11), it is difficult to explain this distribution by the limits of dispersal alone. It is likely, considering that streptomycetes grow well in seawater, that other as yet unknown factors affect the occurrence of these actinomycetes in marine sediments.

The actinoplanetes were found in highest numbers in the deepest sediments sampled. This distribution is difficult to explain if these bacteria are of terrestrial origin. The actinoplanetes were most commonly observed from the heat-treated samples, and based on morphology, most of these appear to belong to the genus *Micromonospora*. This genus is more heat resistant than other actinomycetes (2, 17), and higher numbers of *Micromonospora* in deeper water are consistent with the results of Weyland (21) and Weyland and Helmke (23) for sediments collected between 0 to 200 and >2,000 m.

The finding that the actinoplanetes grow poorly or not at all in the absence of seawater suggests that these bacteria are adapted to the marine environment, an accomplishment that would be difficult to achieve without metabolic activity. This near obligate requirement of seawater for growth contradicts the results of Weyland (22), who reported that *Micromonospora* from deep-sea sediments grew better in the absence of seawater than in 100% seawater. Based on a comparison of these results, it appears that actinoplanetes isolated from near-shore tropical habitats are better adapted to grow in the marine environment than strains isolated from deep-sea sediments.

It should be emphasized that the actinomycetes reported in this study represent only a portion of the total population. Because all isolation methods are selective for certain groups of bacteria, and because of the criteria used for the recognition of actinomycete colonies, certain marine genera that produce fragmenting mycelia, e.g., *Rhodococcus marinonascens* (8), along with other nocardioform actinomycetes, may have been excluded. Certainly if members of the suprageneric group actinobacteria (3), e.g., *Micrococcus*, *Arthrobacter*, etc., were present in the marine environments sampled, they were not included in this study.

Another potential cause for the underestimation of actinomycetes is that spores may have been suspended during the sediment mixing process. If these spores did not settle within the 10 min allocated, they would have been discarded with the supernatant. Conversely, because samples were plated on two media prior to heat treatment and again on one medium following heat treatment, actinomycetes able to grow under all of these conditions would be better represented than those that could not. Although all of these factors may have influenced the results to some extent, it is emphasized that the numbers reported here are not an attempt to define the total actinomycete population but rather to show how the distributions of certain subgroups of this population change with increasing depth as observed by using consistent methodologies for all samples.

A number of problems regarding the enumeration of actinomycetes were encountered during the course of this study. The first such problem is caused by the relatively low numbers and slow growth of actinomycetes in relation to many common unicellular bacteria (2). Neither of the methods used for the isolation of actinomycetes in this study yielded sufficient numbers of colonies for the extrapolation of plate counts to CFU per unit sediment. Of the methods used, mild heat treatment of samples prior to plating was best for the growth of actinomycetes and was especially effective for the actinoplanetes, while having no obvious effect on the streptomycete population.

An additional problem was that the actinoplanete counts were not quantitatively correlated when serial dilution and plating techniques were used. In our experience, we commonly observe that actinomycetes appear to be inhibited from forming colonies on plates that are crowded with unicellular bacteria. However, the nonquantitative dilution as reported here often occurred on plates with few total bacteria and was not necessarily due to overgrowth of the agar surface by unicellular bacteria, but rather some other mechanism that remains to be determined.

The implications of the nonquantitative dilution can be considered in terms of the concept that actinomycetes represent only a small fraction of the bacterial population in marine sediments (5). In the most dilute samples, where the inverted dilution was often observed, the actinomycete numbers, although low, approached or in some cases exceeded the numbers of unicellular bacteria. From this, it can be speculated that the actinomycete population is greater than that predicted based on the use of standard colony counting methods and that their numbers have been underestimated because colony development is inhibited on plates used for the enumeration of unicellular bacteria. Evidence has not been presented to support this conclusion, and it remains possible that other factors are involved. For example, fragmentation of actinomycete hyphae during vortex mixing could result in multiple colonies arising from what would have been a single colony. This theory may have some validity as we only observed the nonquantitative dilution for the actinoplanetes, and it has been shown that *Micromonospora* strains are present in soils and lake mud as a mixture of spores and mycelia, while streptomycetes are largely spores (17).

It is clear that questions concerning the true numbers of actinomycetes in marine sediments and the best methods for their quantification remain unanswered. Based on the problems reported in this study and elsewhere in the literature, we caution the use of serial dilution and plating for the quantification of actinomycetes in marine sediments. However, if consistent methodologies are used, this technique can provide valuable information concerning relative actinomycete distributions.

We conclude that actinomycetes have taxon-specific distributions in near-shore tropical marine sediments. These distributions, and the extent that seawater is required for growth, cannot be explained by the generalized theory that actinomycetes isolated from marine sources are of terrestrial origin and physiologically inactive in the marine environment. The streptomycetes observed in this study were severely limited to shallow sediments and are probably of terrestrial origin. However, these bacteria grew better when seawater was present in the medium, and therefore it can be assumed that they are capable of growth under the appropriate conditions in the marine environment. Clearly, the factor limiting the distribution of streptomycetes is not their inability to grow in seawater. The actinoplanetes appeared in highest numbers in the deepest waters sampled and had a near obligate requirement of seawater for growth. These results lead us to believe that the actinoplanetes are physiologically adapted to the marine environment and metabol-

ically active members of the marine microbial community. Based on these conclusions, it appears that the role of actinomycetes in the marine environment is more complex than previously believed.

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