Actinomycetes, a Possible Hazard Encountered in Diving Operations

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Counts of viable mesophilic and thermophilic actinomycetes were made at a number of estuarine and marine stations. The total viable counts ranged from 1.5×10^5 to 0/g sediment and from 2.5×10^2 to 0/100 ml water. *Thermoactinomyces* species were used as indicators of terrestrial input into aquatic systems and changes in their viable count paralleled those of other actinomycetes. Viable counts diminished with distance from estuarine and coastal areas. Material from a location on the Demerara Abyssal Plain remote from estuarine influence did not yield actinomycetes. Members of eight genera, some of which contain pathogens, were isolated in this study. Divers operating in waters likely to be contaminated with actinomycetes, most of which are apparently allochthonous, may encounter pathogenic forms. Infections acquired in such situations could therefore be of an uncommon type caused by actinomycete bacteria.

INTRODUCTION

Actinomycetes are Gram-positive bacteria which grow typically in the form of branched elongated cells or branched filaments. Most genera form an extensive mycelium which in some cases fragments into coccoid or rod shaped elements. Certain genera occur as individual branched cells and seldom produce a mycelium.[1] A common feature of actinomycetes is the ability to produce spores. These range from the sensitive mobile spores produced by members of the genus *Actinoplanes* through a variety of structures to the resistant endospores of *Thermoactinomyces*, which can survive for many decades.[2,3]

A number of actinomycete species are human pathogens. They may be obligate pathogens such as *Mycobacterium tuberculosis* and *Mycobacterium leprae* or may exploit opportunistic infection as in the case of microaerophilic *Arachnia* and *Actinomyces* species, normally present in the oral cavity of adults. Actinomycete infections acquired from natural waters are, however, most likely to arise from exogenous species, particularly members of the genera *Nocardia, Actinomadura* and *Streptomyces*.[4]

Nocardia enter the body through wounds or by inhalation to give rise to systemic or pulmonary nocardiosis. Superficial nocardiosis may produce cutaneous or subcutaneous abscessing infections of the eye or mucous membranes and may, on some occasions, produce actinomycetoma. Infection with *Actinomadura* or *Streptomyces somaliensis* is prevailingly percutaneous, through skin lesions, giving rise to actinomycetoma. Cutaneous lesions can also be caused by certain nontuberculous mycobacteria. Infection may occur when abraded skin contacts water in a swimming pool[5] or an aquarium harboring the organism.[6]

Actinomycetes have a worldwide distribution particularly in the upper layers of soil[7,8] which are probably their primary reservoir. They are also found in decaying organic material[9] and in sewage.[10] These bacteria can also be isolated from freshwater,[11] estuarine,[12] and marine[13] situations. Our early work is directed to establishing the source and distribution of actinomycete bacteria, particularly pathogenic forms, in saline natural waters. The eventual aim is to predict situations in which such pathogens may be concentrated and assess the danger of infection from such contaminated waters.

MATERIALS AND METHODS

Collection of Samples

Samples studied were collected between July and

December 1980. The stations, in order of increasing salinity, were the Anacostia River at the U.S. Naval Yard, Washington, D.C. (salinity 0 to 2 °/00); the Chesapeake Bay, Eastern Bay (depth 12.8M, salinity 17.8 °/00), and Bloody Point (depth 31.1 M, salinity 22.8 °/00); New York Harbor, Yankee Pier (salinity 25 °/00); and the New York Bight samples, "A", longitude 73°52'W, latitude 40°24'N (depth 19.8M, salinity 30 °/00) and "B", longitude 73°49'W, latitude 40°22'N (depth 28M, salinity 30 °/00), obtained 11 weeks after sample A. Both samples A and B were from the region of a dump site for dredge spoils in New York Harbor. Sample B included core material from a 3-4 inch layer of sand deposited by the Army Corps of Engineers during operations to cap the dump site. Sediment from below the sand layer appeared to consist of the original dredge spoil and was also sampled.

Sediment samples were obtained from two regions of the Demerara Abyssal Plain. Station "A", longitude 45°48'W, altitude 10°24'N (depth 4805M, salinity 35 °/00), is considered distant enough from land to be unaffected by effluent from the Amazon River. Station "B", longitude 49°6'W, altitude 8°6'N (depth 4430M, salinity 35 °/00), is within the ocean floor area thought to be influenced by Amazon River effluent.

Water samples were collected from 1 M below the surface of the water column and 1 M above the bottom by means of a Niskin aseptic sampler (General Oceanics, Inc., Miami, Florida). Sediment was collected by means of a Ponar grab sampler except in the case of the Demerara and New York Bight "B" material which was collected by type U.S.N.E.L. box corer and diver hand corer, respectively.

Salinity was measured by using a salinity refractometer (American Optical, Buffalo, New York).

Bacterial Enumeration

To isolate actinomycetes, water samples were inoculated directly onto agar plates[14] and triplicate 10 cm^3 and 1-0 cm^3 volumes were passed through 0.45 m membrane filters (Schleicher and Schuell; Keene, H.H.). Membranes were incubated grid surface uppermost for the isolation of *Thermoactinomyces* species,[15] but an imprint technique gave greater recoveries of other isolates.[16] Sediment samples were suspended in phosphate-buffered saline (7.2 g NaCl, 1.48 g Na_2HPO_4, 0.43 g K_2HPO_4, distilled water 1 dm^3, pH 7.2) before inoculation onto agar plates.

Mesophilic actinomycetes were isolated on M3 agar[14] incubated at 30°C and at 15°C; *Thermoactinomyces* species were isolated on C.Y.C. medium[17] incubated at 50°C. Microaerophilic actinomycetes were cultured on Brain Heart infusion agar (Difco Laboratories, Detroit, Mich.) in Gaspak Jars with H_2 and CO_2 generator at 37°C (G.M. Schofield, Pers. Comm.).

Viable aerobic heterotrophic marine eubacteria were enumerated by membrane filtration using Marine Ag 2216 (Difco) and incubation at 25°C.

Thermoactinomycete isolates were assigned to speci by means of characteristics suggested by Cross a Unsworth.[18] Mesophilic isolates were grouped acco ing to criteria set out by Cross and Goodfellow[9] prior more precise identification.[19]

RESULTS AND DISCUSSION

Our preliminary findings (Tables 1 and 2) confi previous observations showing that actinomycetes c be isolated from estuarine and marine sediments a waters.[12,13,20]

The numbers recovered ranged from 1.5×10^5 to 0/g sediments and from 2.5×10^2 to 0/100ml in waters amined. The viable counts presented above indicate t actinomycete species are particularly numerous in se ments. For eubacteria isolated on marine agar, the ra of the number recovered per gram of sediment to t number per milliliter of water ranged from 1.3:1 (N Bight B, sediment: top water) up to 3.6×10^2:1 (N Bight A, sediment: bottom water). The correspondi ratio for actinomycetes range from 1×10^2:1 (N.Y. Bi A, surface sediment: bottom water) up to 5.8×10 (Anacostia, sediment: bottom water) in waters carryi these organisms. This concentration of actinomyce in sediment may be attributable to active proliferation the bacteria, as there is evidence for activity of so species in aquatic sediments.[12,21] However, the majo of actinomycetes isolated from aquatic situations considered to be allochthonous organisms washed

Table 1. Numbers of actinomycete and heterotrop marine bacteria colony forming units per gram of sediment.

Station	*Thermo-actino-myces* sp.	*Actino-mycetes* 30°C	*Actino-mycetes* 15°C	Mari bacte
Anacostia	2.5×10^4	2.8×10^4	5.2×10^3	4.0 ×
Chesapeake Bay				
Eastern Bay	7.1×10^3	2.3×10^4	3.5×10^4	NT
Bloody Point	1.8×10^4	1.0×10^5	3.2×10^4	NT
New York				
Yankee Pier	2.5×10^3	1.2×10^4	2.8×10^3	7.0 ×
N.Y. Bight A	4.0×10^1	7.0×10^1	3.1×10^1	8.0 ×
N.Y. Bight B Upper	2.0×10^1	1.7×10^2	1.2×10^2	8.0 ×
N.Y. Bight B Lower	1.1×10^3	1.1×10^4	5.3×10^3	NT
Demerara Abyssal Plain				
A	0	0	0	1.0 ×
B	1.5×10^1	0	0	1.5 ×

*NT = Not tested.

Table 2. Numbers of actinomycete and heterotrophic marine bacteria colony-forming units per 100 milliliters of water.

	TOP WATER				BOTTOM WATER			
Station	*Thermoactinomyces* sp.	*Actinomycetes* 30°C	*Actinomycetes* 15°C	Marine bacteria	*Thermoactinomyces* sp.	*Actinomycetes* 30°C	*Actinomycetes* 15°C	Marine bacteria
Anacostia	7.2×10^1	1.8×10^1	2.5×10^1	2.0×10^6	6.0×10^1	2.5×10^1	1.3×10^1	$<1 \times 10^4$
New York								
Yankee Pier	1.0×10^1	8	NT*	8.5×10^3	NT	NT	NT	NT
N.Y. Bight A	0	0	0	1.0×10^6	1.0×10^2	1.2×10^1	3.0×10^1	2.2×10^5
N.Y. Bight B	0	1.2×10^1	0	6.3×10^5	0.7	9	2.5×10^2	4.2×10^6

*NT = Not tested.

from terrestrial sources[11,20,22] and which may accumulate in the form of spores produced by most species.

We have used members of the genus *Thermoactinomyces (Tha.)* as indicators of input from terrestrial sources. Growth and sporulation of *Tha.* species are restricted to decaying organic material at temperatures in the region 35-60°C. The spores are common and often numerous in soil, from which they are washed into flowing surface waters and ultimately deposited in sediment.[23] They are unable to proliferate in the sediment because of low temperatures and the sparsity of nutrients and oxygen. They can, however, remain viable for many years and, therefore, accumulate.[17] Procedures which differentiate between spore and vegetative forms of thermoactinomycetes, to be reported elsewhere, provide further evidence that the organism exists as a spore in sediments. *Thermoactinomyces vulgaris, Tha. sacchari,* and *Tha. thalpophilus* were all found in excess of 1.0×10^3/g of Anacostia River sediment. Similar numbers of the former two species were also isolated from Chesapeake Bay sediments, but *Tha. thalpophilus* was absent. It therefore seems likely that the ratio in which various *Tha.* species occur may indicate something about the nature and source of the terrestrial input.

Actinomycetes are numerous in estuarine and coastal effluents. The resistance and longevity of *Tha.* spores accounts for their persistence in more remote locations, such as the Demerara Abyssal Plain station "B" at a depth of 4430M where other actinomycetes are less prevalent or absent. Members of the genera *Nocardia, Micromonospora, Microbispora,* and *Streptomyces* were discovered from marine sediments, including deep sediments off the East African coast.[13] Actinomycetes were not isolated from Demerara station "A," considered free of coastal and estuarine influence.

Isolates characteristic of eight genera and also a microaerophilic form have been encountered in this study so far (Table 3). Organisms identified as mycobacteria have been isolated from the Chesapeake Bay during earlier work carried out in our laboratory. Members of certain of the genera listed in Table 3 are human pathogens and specific identification is to be made and reported elsewhere.

Table 3. Actinomycetes isolated at each station examined.

	Thermoactinomyces	*Dactylosporangium*	*Microbispora*	*Micromonospora*	*Nocardia*	*Saccharomonospora*	*Streptomyces*	Microaerophilic *sporangiu*	isolate
Anacostia	✓*	0†	✓	✓	✓	0	✓	✓	✓
Chesapeake									
Eastern Bay	✓	0	0	✓	0	0	✓	0	0
Bloody Point	✓	0	0	✓	0	0	✓	0	0
New York									
Yankee Pier	✓	✓	✓	✓	✓	0	✓	✓	✓
N.Y. Bight A	✓	0	✓	✓	✓	✓	✓	✓	✓
N.Y. Bight B	✓	0	0	✓	✓	0	✓	✓	0
Demerara Abyssal Plain									
A	0	0	0	0	0	0	0	0	0
B	✓	0	0	0	0	0	0	0	0

* ✓ = Isolated
† 0 = Not isolated

It appears that actinomycetes, including genera containing potential pathogens, are prevalent in estuarine and coastal regions. These bacteria may be associated with terrestrial run-off, sewage effluent,[10] polluted waters,[12] and with material such as coastal dredging spoil dumped at sea. The latter is evident in the figures for N.Y. Bight B given in Table 1: 1.7×10^4 actinomycetes/g were recovered from the lower sediments, i.e., dumped dredging spoil, but only 3.2×10^2g were recovered from the upper capping material. Many of the organisms encountered produce spores which may remain viable for long periods, and accumulate.

In view of the above observations, it seems advisable for divers working in situations likely to be heavily contaminated to wear suits which minimize direct contact with the water. It appears wise to avoid disturbing sediment and to wash the diving suit in clean water before removal. It is perhaps most important to be aware that the possibility of actinomycete infection exists, because such infections are uncommon and in consequence often not diagnosed.[24,4] Wounds in particular provide a port of entry for organisms such as pathogenic *Nocardia* and *Mycobacterium* species. Correct and early diagnosis followed by appropriate therapy are vital in overcoming potentially serious actinomycete infections.

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