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Planktonic Marine Luminous Bacteria: Species Distribution in the Water Column

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Luminous bacteria were isolated from oceanic water samples taken throughout the upper 1,000 m and ranged in density from 0.4 to 30 colony-forming units per 100 ml. Generally, two peaks in abundance were detected: one in the upper 100 m of the water column, which consisted primarily of *Beneckea* spp.; and a second between 250 and 1,000 m, which consisted almost entirely of *Photobacterium phosphoreum*. The population of *P. phosphoreum* remained relatively stable in abundance at one station that was visited three times over a period of 6 months. However, the abundance of luminous *Beneckea* spp. isolated from the upper waters fluctuated considerably; they were, as high as 30 colony-forming units per 100 ml in the spring and were not detected in the winter. Water samples from depths of 4,000 to 7,000 m contained less than 0.1 luminous colony-forming unit per 100 ml. The apparent vertical stratification of two taxa of oceanic luminous bacteria may reflect not only differences in physiology, but also depth-related, species-specific symbiotic associations.

Luminous marine bacteria are versatile heterotrophs that associate with a variety of living and nonliving sources of organic matter. They have been isolated from seawater throughout the world; from tropical, temperate, and polar regions; and from surface waters to depths of several thousand meters (5, 19). Within this extensive range they may exist as mutualistic symbionts in the light-emitting organs of fishes (10), as enteric bacteria of a variety of marine organisms (9, 15), as parasites of crustaceans (2, 7), as saprophytes on decomposing macroscopic animal matter (7, 9), or as members of the general planktonic microbial population of seawater (8, 16, 20).

Strains of the four taxa of marine luminous bacteria described herein can be found in any of several of the niches listed above, suggesting a potential ecological versatility among their progeny. Each of the associations may release luminous bacteria into the surrounding water, and also may have received its initial inoculum from cells dispersed in seawater. Therefore, knowledge of the distribution and temporal stability of luminous bacterial species in a planktonic population may be useful in understanding their success in a given niche, as well as their potential for exploiting others.

Previous studies of planktonic bacteria in neritic surface waters revealed seasonal changes in the species composition of luminous isolates that were correlated with surface water temperature (3, 4, 16). The purpose of this investigation was to characterize the luminous bacterial population found in water samples taken from depths of 0 to 7,000 m at stations in the open ocean. It was then possible to (i) compare surface luminous bacteria populations of open ocean and coastal waters, (ii) gain insights into the distribution of taxa of luminous bacteria as a function of depth, and thus (iii) determine the relationship between a spatial temperature gradient and the taxonomic composition of isolated luminous bacteria.

(A preliminary account of this work was presented at the 79th Annual Meeting of the American Society for Microbiology, Honolulu, Hawaii, 1979.)

MATERIALS AND METHODS

Collection of seawater samples. Sterile Bag Samplers (General Oceanics, Inc., Miami, Fla.) were used to collect water from various depths, and water temperatures were monitored with expendable bathythermographs (Sippican Corp., Marion, Mass.). Two stations over the Puerto Rico Trench were visited during the period 23-26 February 1978: station 4 at 20° 39′ N, 65° 07′ W, and station 9 at 19° 42′ N, 67° 22′ W (R/V *Oceanus*, cruise 40). A sampling site in the North Atlantic (in the vicinity of 38° 19′ N, 69° 41′ W) was visited three times: 21 October 1977 (R/V *Oceanus*, cruise 35), 29 February 1978 (R/V *Oceanus*, cruise 40), and 29-30 April 1978 (R/V *Oceanus*, cruise 45).

Bacterial isolations. Within 1 h after collection, 1 to 2 liters of each seawater sample was aseptically

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passed through 0.22-μm-pore-size filters (47-mm diameter; Millipore Corp., Bedford, Mass.) in portions of 20 to 200 ml per filter, depending on bacterial population densities in the samples. The filters were placed on seawater complete (SWC) agar contained in 50-mm-diameter petri dishes. SWC agar consisted of 750 ml of seawater, 250 ml of distilled water, 5 g of peptone (Difco Laboratories, Detroit, Mich.), 3 g of yeast extract (Difco), 3 ml of glycerol, and 12 g of agar (Difco) (11). The plates were incubated in the dark at 19 to 22°C for 24 to 36 h, after which the total colony-forming units (CFU) ranged from 20 to 100 per plate. Luminous colonies were enumerated in a dark room and then picked with sterile toothpicks and stabbed into SWC agar plus 0.1% $CaCO_3$ contained in screw-capped vials. The vials were stored for up to 1 month at 19°C, after which inocula were removed and streaked on SWC agar plates for the reisolation of luminous colonies.

Differences in abundances of luminous colonies were not observed when filter samples from colder areas of the water column (<10°C) were incubated at 4°C rather than 19 to 22°C, nor were differences observed when 0.45- rather than 0.22-μm-pore-size filters were used.

Taxonomic identification of bacterial isolates. Strains of luminous bacteria were classified by a modification (14) of the procedure developed by Reichelt and Baumann (12). The two genetically distinct luminous species *Beneckea harveyi* and *B. splendida* are not distinguished by this procedure (13). Thus the following four taxa of luminous marine bacteria were differentiated: *Photobacterium phosphoreum*, *P. leiognathi*, *P. fischeri* (*P. fischeri* and *P. logei*) (1), and *Beneckea* spp. (*B. harveyi* and *B. splendida*). In addition, the kinetics of luciferase activity in crude cell extracts of each strain were determined in order to confirm placement in either the genus *Beneckea* or the genus *Photobacterium* (8, 11, 16).

A total of 755 luminous isolates were identified as to taxon: 458 were identified as *P. phosphoreum*, 262 as *Beneckea* spp., 24 as *P. leiognathi*, and 11 as *P. fischeri*.

It was previously reported that many strains of *P. phosphoreum* exhibited a growth requirement for methionine (6, 12). Thirty-three luminous isolates from the North Atlantic and 84 from the Puerto Rico Trench grew well on SWC agar but did not grow in any of the minimal media used in this study. Fifty-two of these 117 strains were retested, using minimal media supplemented with 40 μg of L-methionine per ml, and 51 appeared to be methionine-requiring strains of *P. phosphoreum*. Growth of the remaining strain on minimal media was not stimulated by methionine.

RESULTS

Vertical samplings were performed on four different occasions: during one 4-day span, two neighboring hydrographic stations over the Puerto Rico Trench were sampled several times, and on three other occasions at different times of the year a third station located approximately 2,100 km to the north was visited. During each vertical sampling, water temperature was monitored as a function of depth, and water from a number of depths was collected for bacteriological studies.

The water temperature profiles of the upper 900 m at the two Puerto Rico Trench stations were essentially identical during the sampling period of 22–26 February 1978. The upper 125 m was characterized by temperatures between 25 and 26.5°C, and below 125 m the water decreased in temperature to 6°C at 900 m (Fig. 1B). Luminous bacteria were isolated from all of the 14 water samples taken from depths between the surface and 1,000 m. These isolates were at concentrations of 0.4 to 5.8 luminous CFU per 100 ml and constituted up to 12% of the total CFU observed. Luminous CFU were most abundant in two regions: between the surface and 100 m, and between 250 and 1,000 m. Water samples

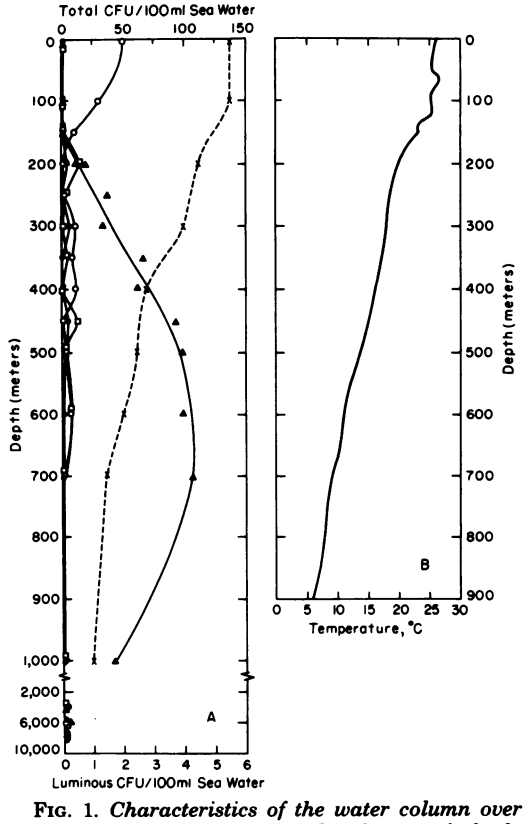

FIG. 1. *Characteristics of the water column over the Puerto Rico Trench. (A) Abundances of planktonic CFU as a function of depth: total CFU (×); CFU of P. phosphoreum (▲), P. leiognathi (□), P. fischeri (●), and Beneckea spp. (○). Data from three hydrocasts made over a period of 4 days at two hydrographic stations (see Materials and Methods). (B) Temperature profile of the water column at the time of hydrocasts.*

from 4,000, 5,000, 6,000 and 7,000 m contained less than 0.1 luminous CFU per 100 ml.

The species composition of the luminous bacterial isolates varied as a function of depth (Fig. 1A). In the upper 150 m, *Beneckea* spp. predominated, whereas at depths of 250 to 1,000 m, *P. phosphoreum* was the most abundant species isolated. Strains of the two other taxa of luminous bacteria, *P. fischeri* and *P. leiognathi* were isolated from the 200- through 600-m water samples, but they appeared at relatively low cell densities.

Although the total CFU decreased as a function of depth between 100 and 1,000 m, the abundance of luminous *P. phosphoreum* actually increased in this region (Fig. 1A). Thus, the factor(s) responsible for the increase in numbers of *P. phosphoreum* did not similarly affect the abundance of the nonluminous bacterial population forming colonies on SWC agar.

Another series of vertical samples was obtained at the North Atlantic sampling site, which was visited in fall 1977 and in winter and early spring 1978. During the fall visit the temperature profile (Fig. 2) was similar to those observed at the Puerto Rico Trench stations (Fig. 1B). The vertical distribution of taxa of luminous bacterial isolates was also similar: *Beneckea* spp. predominated in upper waters, and *P. phosphoreum* was the most abundant taxon found in water samples obtained from 200 m and below (Fig. 3A).

During the late winter, samples at the North Atlantic station were taken from three depths. Although CFU of *P. phosphoreum* again occurred in relatively high concentration at 500 m, no luminous *Beneckea* spp. were isolated from the surface sample (Fig. 3B). At this time the

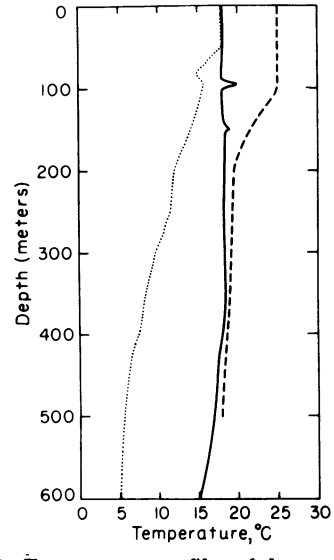

FIG. 2. *Temperature profiles of the water column at the North Atlantic sampling site in the fall* (- - -), *winter* (——), *and early spring* (· · ·).

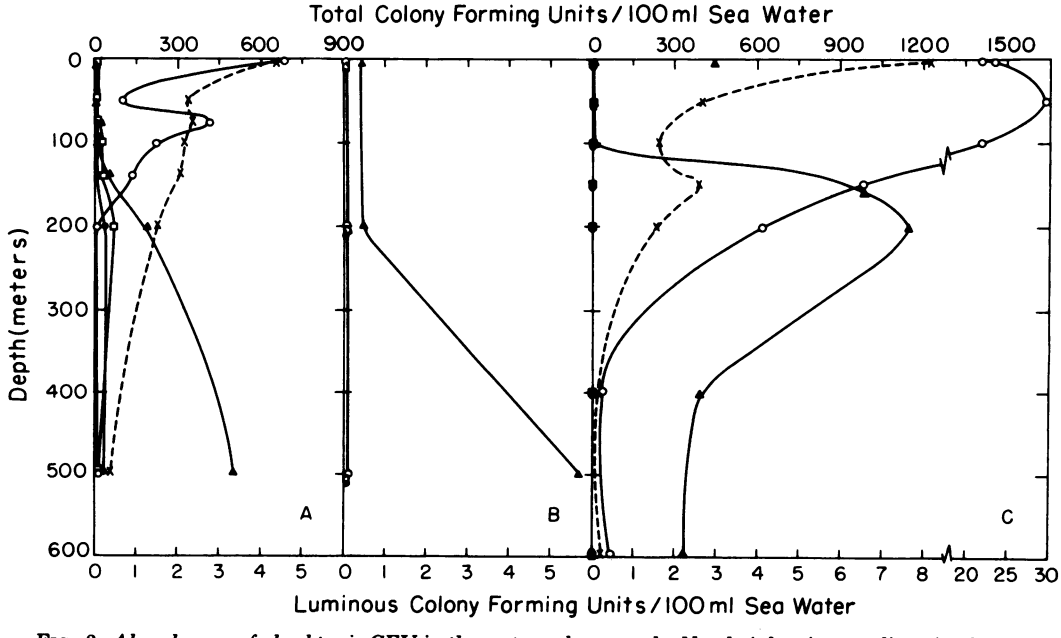

FIG. 3. *Abundances of planktonic CFU in the water column at the North Atlantic sampling site during the fall (A), winter (B), and early spring (C) visits. Total CFU* (×); *CFU of P. phosphoreum* (▲), *P. leiognathi* (□), *P. fischeri* (●), *and Beneckea* spp. (○).

temperature profile of the water column at depths below 200 m was similar to that observed in the fall; however, the temperatures in the upper 150 m were lower than during the fall sampling (Fig. 2).

In the early spring, the surface waters were characterized by a seasonal bloom of algae with an accompanying increase in planktonic invertebrates and an abundance of microplanktonic life. At this time the concentration of luminous *Beneckea* spp. found in the upper 200 m of the water column was much greater than at the other two sampling times, and *P. phosphoreum* was present at higher concentrations in the 150- and 200-m samples than previously noted (Fig. 3). The temperature of the water at any given depth was as low as or lower than it was during either of the earlier visits (Fig. 2).

DISCUSSION

These studies on the vertical distribution of luminous bacterial isolates from oceanic waters have led to a striking finding: the luminous species *P. phosphoreum* was found in greater abundance in deep water, peaking in cell density between 200 and 1,000 m (Fig. 1 and 3). To our knowledge, this sort of depth-related distribution of a heterotrophic marine bacterial species is unprecedented. A second luminous taxon, *Beneckea* spp., did not exhibit this type of distribution. Rather, luminous *Beneckea* were found in greatest abundance in waters above the thermocline (Fig. 1-3). This vertical stratification of luminous bacteria may be presumed to reflect differences in their responses to a variety of physicochemical and biological factors.

P. phosphoreum has been previously noted to occur in samples of deep water (19) and to be more psychrotrophic than other luminous species (12, 14). In addition, *P. phosphoreum* has been demonstrated to occur as the specific luminous bacterial symbiont in the light organs of several species of macrourid and opisthoproctid fishes that inhabit depths of 200 to 1,200 m (10, 14). Studies of the ultrastructure of similar light-organ associations have revealed the presence of dividing bacterial cells (B. M. Tebo, D. S. Linthicum, and K. H. Nealson, BioSystems, in press), and apparently, luminous bacteria growing in light organs are continuously shed into the surrounding water (K. Nealson, personal communication). This information, together with the observed abundance of *P. phosphoreum* at 200 to 1,000 m (Fig. 1), supports the hypothesis of Singleton and Skerman (19) that light organs of certain marine fishes may serve as a specific source of inoculum of *P. phosphoreum* into the midwater planktonic population. *P. phosphoreum* from other symbiotic niches such as the enteric tracts of midwater fishes (15) may also serve to inoculate the planktonic population. It is also possible that the planktonic population of *P. phosphoreum* serves as a source of inoculum for the light organs and/or enteric tracts of midwater fishes.

As previously mentioned, luminous members of the genus *Beneckea* were found mainly in waters above the thermocline (Fig. 1-3). The highest concentration of these organisms detected was 30 per 100 ml (Fig. 3C), still considerably lower than the 100 to 1,600 per 100 ml found in surface waters off San Diego, Calif., and Woods Hole, Mass. (16; E. G. Ruby, E. P. Greenberg, and J. W. Hastings, unpublished data). Since levels of dissolved nutrients in oceanic waters are usually lower than in neritic waters (17), this information is consistent with the hypothesis of Chumakova and Getelson (5) that the proliferation of oceanic luminous bacteria is limited by the concentration of dissolved organic matter.

Ruby and Nealson (16) reported that in Mission Bay, Calif., the planktonic population of luminous members of the genus *Beneckea* exhibited fluctuations in abundance that correlated with seasonal changes in water temperature. In general, as water temperature went up or down within the range of 13 to 21°C, so did the abundance of luminous *Beneckea* isolates. Likewise, in oceanic waters of the North Atlantic, the decrease in populations of these organisms in the surface waters in the winter (Fig. 3) corresponded to a drop in water temperature (Fig. 2). However, in the spring the water temperature was as low as or lower than in the winter, but luminous *Beneckea* were found in greater abundance than in water samples collected in the fall when the highest water temperature was recorded (Fig. 2 and 3). On the other hand, luminous *Beneckea* spp. were isolated only rarely from water deeper than 200 m, even where temperatures were relatively high. This information supports previous indications that variations in temperature alone are not sufficient to explain observed fluctuations in densities of luminous *Beneckea*, but that other factors such as salinity and the concentration of dissolved nutrients can also be important (18).

In general, *P. fischeri* and *P. leiognathi* were encountered as only a minor percentage of the luminous isolates (Fig. 1 and 3). *P. fischeri* has been observed as a major part of the planktonic surface population isolated from neritic waters off San Diego, Calif. (16), and Woods Hole, Mass. (Ruby et al., unpublished data), whereas *P. leiognathi* has been found in relative abundance in surface waters of the Indo-Pacific,

where fishes that harbor this species in their light organs are known to occur (8) and off the coast of Israel (20).

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