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Luminous Enteric Bacteria of Marine Fishes: a Study of Their Distribution, Densities, and Dispersion†

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Three taxa of luminous bacteria (*Photobacterium fischeri, P. phosphoreum,* and *Beneckea* spp.) were found in the enteric microbial populations of 22 species of surface- and midwater-dwelling fishes. These bacteria often occurred in concentrations ranging between 10^5 and 10^7 colony-forming units per ml of enteric contents. By using a genetically marked strain, it was determined that luminous cells entering the fish during ingestion of seawater or contaminated particles traversed the alimentary tract and survived the digestive processes. After excretion, luminous bacteria proliferated extensively on the fecal material and became distributed into the surrounding seawater. Thus, this enteric habitat may serve as an enrichment of viable cells entering the planktonic luminous population.

The luminous bacteria are a ubiquitous and ecologically versatile group that can be isolated from a wide variety of marine heterotrophic associations. Besides their well-described mutualistic symbioses in the light organs of certain fishes (5), these bacteria also occur on the surfaces (saprophytic), in the vital fluids (parasitic), and within the gastrointestinal contents (enteric) of marine organisms (3, 4). By using a recent standardization and simplification of their taxonomic characterization (10), it has been possible to reliably identify the species of luminous bacteria isolated from a given habitat. This approach has led to an increased understanding of their ecology, including both the discovery of species specificity among light organ symbioses (2, 12, 14, 15) and reports of predictable seasonal variations in the species composition of the planktonic luminous bacterial population in certain neritic waters (16; M. Shilo, personal communication).

Past reports have noted the presence of luminous bacteria in the enteric contents of several marine vertebrates and invertebrates (10, 17), a habitat which reflects both their physiological and taxonomic designation as marine enteric bacteria (1). Because the enteric contents of ocean-dwelling organisms constitute one of the largest pools of organic carbon available to marine bacteria and are important sites of decomposition of refractory organic compounds, the microbiology of these habitats is of considerable interest in determining the role of bacteria in marine food chains. In beginning the study of luminous bacteria as enteric microorganisms it is necessary (i) to ascertain with what frequency and to what extent they occur as numerically important members of the enteric population, (ii) to identify the luminous species present, (iii) to determine their source, distribution, and stability within the alimentary tract, and (iv) to predict the importance of this association to the proliferation and distribution of luminous bacteria in the marine environment. We investigated these ecological characteristics of the enteric association as a prelude to understanding the physiological and biochemical activity of luminous enteric bacteria.

MATERIALS AND METHODS

Collection of fishes. Nearshore fishes were collected alive from the La Jolla, Calif. kelp beds; at Socorro Island, Mexico; and near the Catalina Marine Science Center on Catalina Island, Calif. In the latter two locations, specimens were collected at night by hand, whereas at the La Jolla site, fish were taken by using a small gig. Only fish with intact and undamaged gut tracts were utilized, and dissections were performed within 30 min of the death of the fish. Specimens of midwater fishes were collected by trawls at 200- to 600-m depths as previously described (14).

Isolation of bacteria. After the surfaces of the fish were swabbed with 70% ethanol, heterotrophic bacteria were isolated from the gut tracts as follows. Each fish was dissected aseptically, and the entire gastrointestinal tract was removed. About 20 to 50 μl of the contents was squeezed out of the appropriate section of the enteric tract into a tube containing 10 ml of sterile seawater. The sample was vigorously agitated for 30 s, and the particulate material was allowed to

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settle. Subsequent dilutions of the supernatant fluid into sterile seawater were plated on seawater nutrient (SWC) agar medium prepared with 75% seawater, 0.5% peptone (Difco), 0.3% yeast extract (Difco), 0.3% glycerol, and 1.5% agar (Difco). Luminous bacteria were isolated from seawater by the soft agar method, using SWC medium (16). After a 24- to 36-h incubation at 19 to 24°C, colonies were counted. Luminous strains were isolated and subjected to the taxonomic identification scheme of Reichelt and Baumann (10), as previously described (14). After the initiation of this work, Reichelt et al. (11) determined that the luminous taxon *Beneckea harveyi* was actually composed of two genetically distinct species, *B. harveyi* and *B. splendida*. These species were not clearly distinguishable by the phenotypic traits we used; thus, in our study we could differentiate only four of the five groups of luminous marine bacteria: *Photobacterium fischeri*, *P. leiognathi*, *P. phosphoreum*, and *Beneckea* spp. (*B. harveyi* and *B. splendida*).

Passage of luminous bacteria through the gut tract. The fate of luminous bacteria ingested by specimens of the plankton-feeding fish *Chromis punctipinnis* was examined by using a mutant strain of *P. fischeri* derived from strain MJ-1 (15). This mutant strain is metabolically tagged since it is characteristically nonluminous until exposed to vapors of an aliphatic aldehyde (e.g., decanal), after which it becomes brightly bioluminescent within several seconds (13). Seven freshly collected *C. punctipinnis* (standard length ranging from 98 to 147 mm; standard length is the length of a fish measured from the tip of its snout to the end of its caudal peduncle) were anesthetized by immersion in seawater containing quinaldine (~100 μl/liter). By using a syringe with a 5-cm length of narrow-gauge plastic tubing (Intramedic PE-100; OD, 1.5 mm), 200 to 500 μl of sterile seawater containing 17×10^6 cells of the mutant bacteria were introduced into the stomachs of the fish. The fish were then placed in filter-sterilized seawater to recover (within 5 min). At 3-h intervals fish were sacrificed and their digestive tracts were aseptically dissected and divided into three portions, the stomach and the upper and lower halves of the intestine. The total contents of each portion was squeezed into 2 ml of sterile seawater, and appropriate dilutions were plated on SWC agar. After incubation for 36 h, the presence of the mutant strain was easily and unambiguously ascertained by exposing the plates to decanal vapors and observing the immediate increase in the number of light-emitting colonies.

Excretion of luminous bacteria. To determine whether viable luminous bacteria are excreted by fish, 12 specimens of freshly collected *C. punctipinnis* (standard length, 120 to 140 mm) were rinsed four times in sterile seawater and placed into three ethanol-sterilized aquaria (four fish each) containing 6 liters of membrane-filtered (Millipore Corp.; 0.45 μm) seawater. At intervals of 4 h, samples or dilutions of the aquarium water were plated on SWC agar medium. The plates were incubated at 19°C, and luminous and nonluminous colonies were counted after 36 h.

RESULTS

For this study 12 species of surface water and 10 species of midwater fishes were obtained from five locations in the eastern Pacific Ocean and one area of the eastern Atlantic Ocean (Table 1). Individual fishes were dissected, and the microbial populations of their enteric contents were sampled by plating serial dilutions on a SWC agar. Observations of these primary isolation plates in the dark often revealed a remarkably high proportion of light-emitting colonies, thus indicating that luminous bacteria were a common and numerically significant component of many of these enteric microbial populations (Table 1).

From the standpoint of ecology and physiological activity, the actual concentration of luminous cells in the enteric contents (Table 2) may be a more meaningful consideration than their proportion of the total number of colonies isolated. The factor by which the sample was diluted was known, so it was a simple calculation to determine the minimum concentration of luminous bacteria present in each sample. These values are probably an underestimation of the true luminous cell density since the method isolates only those bacteria that are dislodged from the macroscopic pieces of particulate enteric material by vigorous shaking. Nevertheless, concentrations of 10^5 to 10^7 luminous colony-forming units per ml of enteric contents were generally obtained from some species (Table 2).

Three taxonomic groups of luminous bacteria were present in the enteric contents of 23 specimens of three fish species sampled (Table 3). It is interesting to note that the species of planktonic luminous bacteria present in the surrounding waters were always found in the enteric contents, and at a given location the same bacterial species often predominated in both the seawater and enteric populations. These observations led to subsequent laboratory experiments designed to determine whether there is an exchange of viable luminous bacteria between the planktonic and enteric habitats.

The introduction of a tagged luminous bacterial strain into the stomachs of several *C. punctipinnus* indicated that the location of these bacteria progressed through the digestive tract rather rapidly; the tagged strain was isolated from the lower tract within 6 h (Table 4) and appeared in the feces soon thereafter. The bulk of the inoculated cells appeared to remain clustered as they travelled the length of the tract. There was no evidence that a large number of the cells colonized the stomach or upper intestinal contents, and in one case no cells of this strain were detected in the enteric contents after 12 h. In general, there appeared to be a relatively constant bacterial population size during the transit of the tract. It was not possible to determine whether this apparent stability was due to

TABLE 1. *Occurrence of luminous bacteria in the enteric contents of fishes*

Fish	Family	No. of fish harboring luminous bacteria/no. of fish observed[a]	% of total CFU that were luminous[b]
Surface species			
C. punctipinnis[c]	Pomacentridae	19/19	18 (1–20)
Chromis hirundo[d]	Pomacentridae	1/1	16
Oxyjulis californica[c,e]	Labridae	12/12	31 (8–41)
Hyperprosopon argenteum[c]	Embiotocidae	8/8	24 (3–41)
Embiotoca jacksoni[c]	Embiotocidae	2/2	~90
Cymatogaster aggregata[c]	Embiotocidae	2/2	~90
Brachyistius frenatus[c]	Embiotocidae	1/1	29
Apogon parri[d]	Apogonidae	4/4	25 (1–42)
Girella nigricans[c]	Kyphosidae	2/2	~90
Medialuna californiensis[c]	Kyphosidae	1/1	1
Atherinops affinis[c]	Atherinidae	2/2	22 (16–26)
Myripristis clarionensis[d]	Holocentridae	2/2	13 (6–15)
Midwater species			
Diaphus theta[f]	Myctophidae	1/2	1
D. rafinesquei[g]	Myctophidae	1/1	64
Lampanyctus alatus[g]	Myctophidae	1/1	2
L. crocodillus[g]	Myctophidae	1/1	70
Stenobrachius leucopsarus[h]	Myctophidae	4/10	10 (5–11)
Triphoturus mexicanus[f]	Myctophidae	1/1	6
Parvilux ingens[f]	Myctophidae	1/1	50
Chauliodus maconi[h]	Chauliodontidae	0/2	0
Argyropelecus hemigymnus[f,g]	Sternoptychidae	5/5	54 (38–100)
A. aculeatus[g]	Sternoptychidae	2/2	100

[a] Only fish specimens for which plating of dilutions of enteric contents yielded more than 20 colony-forming units were used in these calculations.
[b] CFU, Colony-forming units. Ranges of percentage values are given in parentheses.
[c] Collected from the vicinity of Catalina Island, Calif.
[d] Collected from the vicinity of Socorro Island, Mexico.
[e] Collected from the vicinity of La Jolla, Calif.
[f] Collected from the vicinity of the Southern California Bight.
[g] Collected from the vicinity east of the Canary Islands, off West Africa.
[h] Collected from the vicinity of the Santa Barbara Basin, Calif.

TABLE 2. *Concentration of luminous bacteria in the enteric contents of fishes*

Species of fish	No. of fish specimens	Total no. of colonies observed	% of total which were luminous	No. of luminous CFU per ml of enteric contents[a]
Argyropelecus hemigymnus	5	690	54	5×10^4–5×10^{5}[b]
Oxyjulis californica	12	15,600	31	5×10^5–5×10^7
C. punctipinnis	19	37,500	18	2×10^5–8×10^6

[a] CFU, Colony-forming units.
[b] Range.

a high survival rate, a growth rate similar to the death rate, or a combination of these or other factors. Similar results were obtained when the bacteria were presented on the surface of food particles (frozen brine shrimp). The tagged strain was observed to move down the tract along with the brine shrimp remains, appearing eventually in the feces and ultimately in the aquarium water.

Experiments with *C. punctipinnis* containing natural populations of enteric bacteria indicated that not only were large numbers of endemic luminous bacteria easily isolated from the fresh fecal material, but, by using photometric instruments, the feces were observed to be luminescent. This fecal luminescence required oxygen and increased in intensity over a matter of hours when the feces were incubated at room temperature. Extracts of these feces contained the enzyme bacterial luciferase in levels sufficient to

TABLE 3. *Relative abundance of taxonomic groups of luminous bacteria present in the enteric contents of fishes and in the surrounding seawater at the time of capture*

Location and date	Source of luminous bacteria	No. of fish sampled	No. of bacteria identified	% of total luminous population		
				Beneckea spp.	*P. fischeri*	*P. phosphoreum*
Catalina Island (March 1977)	*C. punctipinnis*	3	24		17	83
	Water sample (14.7°C)		18		89	11
Catalina Island (May 1978)	*C. punctipinnis*	2	21	5	19	76
	Water sample (18.7°C)		28	11	89	
Catalina Island (September 1976)	*C. punctipinnis*	12	21	95	5	
	Water sample (25°C)		82	70	30	
LaJolla kelp bed (April 1977)	*O. californica*	2	90	2	98	
	Water sample (17°C)		69	2	98	
Southern California Bight (February 1977)	*A. hemigymnus*	4	61			100
	Water sample (<6°C)		5			100

TABLE 4. *Abundance within portions of the gastrointestinal tract as a function of time of an orally introduced strain of luminous bacteria*[a]

Time after inoculation (h)	CFU ($\times 10^6$) in:[b]		
	Stomach	Upper intestine	Lower intestine
0.25	15	<0.001	<0.001
3	<0.001	10	<0.1
6	<0.001	<0.001	8
	<0.001	6.5	3.2
9	<0.0001	<0.0001	2.4
12	<0.0001	<0.0001	<0.0001
	<0.0001	<0.0001	26

[a] A total of 17×10^6 cells were introduced into the stomach of each of seven fish at zero time.

[b] Total aldehyde-stimulated luminous colony-forming units (CFU) in each third of the gut tract.

account for the fecal luminescence when assayed by the methods of Nealson et al. (9).

The possibility that feces could be a source of viable planktonic luminous bacteria in the sea was investigated by using freshly caught *C. punctipinnis* which were placed in aquaria containing filter-sterilized seawater. Fecal material began to appear almost immediately, and periodic sampling revealed a steady increase in the concentration of luminous bacteria in the aquarium water that continued for at least 20 h (Fig. 1). This increase was independent of the presence of the fish. If the animals were removed 12 h after the start of the experiment (while leaving the feces in the water), the rate of increase in number of luminous bacteria continued unabated. Conversely, if fish that had been starved for 24 h were placed in an aquarium, no fecal material accumulated, and little increase in the concentration of luminous bacteria was observed (Fig. 1).

An attempt was made to determine whether the increase in luminous cells in the water was due to reproductive proliferation of fecal bacteria or to dispersion of cells after the disintegration of the fecal mass. Visual observations of the fecal material indicated that it remained rather intact during the course of the experiments. Feces were collected, and equal portions (by volume) were placed in three beakers of sterile seawater. Into one was placed a sterile magnetic stirrer, which was used to disintegrate the feces. This beaker and one of the other two containing intact feces (no stirring) were left at 20°C. The third beaker of feces was left at 4°C and was not stirred.

The increase in concentration of bacteria in the water from this experiment was monitored by plating samples onto SWC agar (Fig. 2). Although the beaker containing the disintegrated feces started with a higher count of bacteria, the rate of increase in numbers was not significantly different from that of the unstirred beaker. Although it is likely that the rate of bacterial proliferation was inhibited by the low temperature of the beaker kept at 4°C, the natural disintegration rate of the feces at 4°C was observed to be similar to that of the unstirred feces at 20°C. In the beaker kept at 4°C, no increase in the concentration of bacterial colony numbers was observed and luminescent bacteria remained below 10 colony-forming units per ml. (Incubation at 4°C for 24 h did not decrease the viability of the species of luminous bacteria found in the enteric tract.) These observations suggested that the increase in bacterial numbers observed in Fig. 1 was due to microbial growth and not simply to disintegration of the feces.

DISCUSSION

It is widely postulated that the enteric tracts of marine fishes do not contain an autochthonous microflora, but instead serve as enrichment vessels for bacteria ingested on or in their food

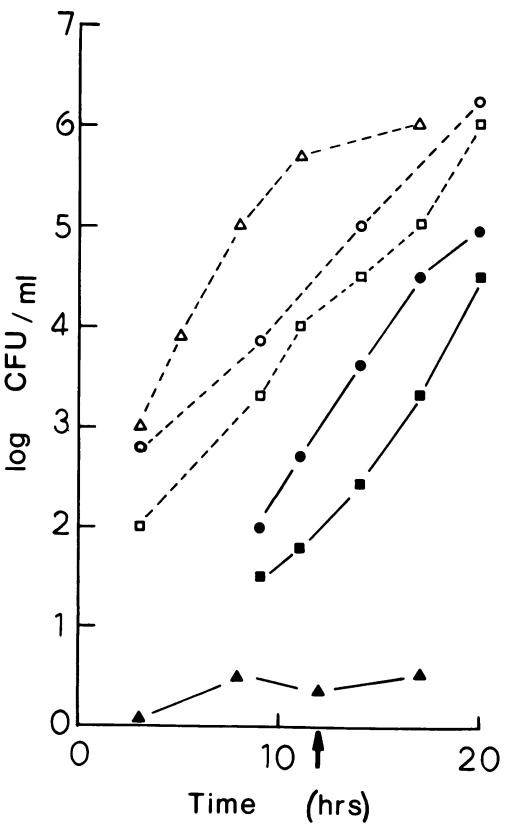

FIG. 1. *Rate of increase in the bacterial concentrations in sterile seawater after the introduction of C. punctipinnis. Counts of luminous (closed symbols) and total (open symbols) colony-forming units (CFU) were performed. Tank 1 (●, ○) contained fish throughout the 20-h experiment. Tank 2 (■, □) had fish removed at arrow. Tank 3 (▲, △) contained fish for the 20-h period, but no fecal material was produced. Similar results were obtained when the experiment was repeated 3 months later.*

(7). After a period of extended fasting, the isolation of bacteria from the enteric contents is difficult, leading to the suggestion that the tract becomes effectively sterile soon after emptying (8). Thus, the feeding history of the organism before its investigation appears to be a primary influence over the qualitative and quantitative nature of its microflora.

Because one of the ultimate goals of this study was to determine the presence and pattern of dispersion of luminous enteric bacteria under native feeding conditions, feral fishes were chosen. Unless otherwise noted, the specimens utilized in this study were dissected within 1 h of collection. It was observed that the presence of food material in the alimentary tract often correlated with high concentrations of enteric microflora; however, no systematic study of this

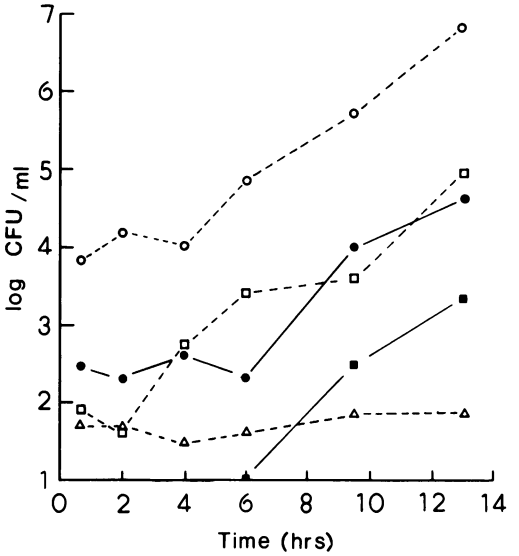

FIG. 2. *Rate of appearance of luminous (closed symbols) and total (open symbols) colony-forming units (CFU) in platings of sterile seawater containing feces of C. punctipinnis. Beaker 1 (■, □) contained intact feces incubated at 20°C; beaker 2 (●, ○) contained disintegrated feces incubated at 20°C; and beaker 3 (△) contained intact feces incubated at 4°C.*

relationship was made. *C. punctipinnis*, the primary subject of this investigation, is a diurnal, planktivorous organism (6). Individuals collected after dusk were found to contain a good deal of food material in their enteric tracts, and the density of microbial isolates from different specimens was relatively reproducible. Thus, these fish were well suited to the purposes of this study.

This report establishes four important points concerning the ecology of enteric luminous bacteria. (i) They are commonly present in the gastrointestinal tracts of many marine fishes, occurring in concentrations up to at least 5×10^7 cells per ml of fluid in the enteric tracts of several species of surface and midwater fishes. (ii) There are at least three luminous bacterial taxa that can be found in these habitats. (iii) Their presence in fish gastrointestinal tracts can arise from ingestion of luminous bacteria (from the surrounding water or on food particles). And (iv), in experiments run in sterilized seawater, the luminous bacteria expelled on fecal particles are capable of extensive proliferation (possibly due to their association with the concentrated organics in the feces) and subsequent distribution into the surrounding water. These observations suggest that the luminous bacteria are well adapted for existence in the enteric habitat and that this association may be a significant source of luminous cells in the marine environment. In addition, the same species of bacteria

are generally present both in the enteric tract and the surrounding water. This may indicate that there exists a rapid cycling of cells between the nutrient-rich enteric contents and the widely distributed planktonic populations (Table 3). Because the concentration of luminous cells in the excreted feces may be 10^5 to 10^6 times greater than the surrounding water, this cycling between enteric and planktonic habitats may have an important impact on the numerical ecology of populations of luminous bacteria. The input of cells to the planktonic luminous pool could affect both the distribution and the species composition of luminous populations in other associations (e.g., parasitic or saprophytic) in the marine environment (4). The increase in numbers of luminous bacteria appears to derive from two activities: (i) the ingestion of luminous cells and their association with organic material concentrated in the enteric tract; and (ii) the proliferation of the bacteria, perhaps in the enteric tract, but certainly after excretion while still in the presence of fecal matter.

Whether the relationship between the host fish and its enteric luminous bacteria is mutually beneficial remains an open question at this time. Certainly the bacteria may gain from their presence in the nutrient-rich enteric contents, but it has also been speculated by Hastings and Nealson (4) that the luminous bacteria may contribute a service to the host by virtue of their ability to digest chitin (18), the ubiquitous polysaccharide that among other things composes the exoskeletons of marine crustaceans. It has also been hypothesized that the light emitted by the bacteria associated with a fecal pellet might serve as an attraction to fishes or other marine organisms so that these particles would be consumed, hence recycling and dispersing the bacteria to other nutrient-rich enteric tracts (4; B. Robison and J. Morin, manuscript in preparation).

Since we have determined that concentrations of greater than 10^5 luminous cells per ml are generally present in the enteric contents of several species of marine fishes, experiments can be designed to investigate the metabolic activities of these bacteria in pure culture under physiological and nutritional conditions approximating those found in the enteric tract. In this way the contribution that these microbes make to the degradation and mobilization of organic compounds can be estimated. In addition, the potential of the enteric association as the source of nutrients and bacterial inocula for fecal matter must be recognized in considering the population dynamics of luminous bacteria and other heterotrophic microbial species in the bacterioplankton.

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LITERATURE CITED

1. **Baumann, P., and L. Baumann.** 1977. Biology of the marine enterobacteria: genera *Beneckea* and *Photobacterium*. Annu. Rev. Microbiol. **31**:39–61.
2. **Fitzgerald, J. M.** 1977. Classification of luminous bacteria from the light organ of the Australian pinecone fish, *Cleidopus gloriamaris*. Arch. Microbiol. **112**:153–156.
3. **Harvey, E. N.** 1952. Bioluminescence. Academic Press Inc., New York.
4. **Hastings, J. W., and K. H. Nealson.** 1977. Bacterial bioluminescence. Annu. Rev. Microbiol. **31**:549–595.
5. **Herring, P. J., and J. G. Morin.** 1978. Bioluminescence in fishes, p. 273–329. *In* P. J. Herring (ed.), Bioluminescence in action. Academic Press Inc., New York.
6. **Hobson, E. S., and J. R. Chess.** 1976. Trophic interactions among fishes and zooplankters near shore at Santa Catalina Island, California. Fish. Bull. **74**:567–598.
7. **Horsley, R. W.** 1977. A review of the bacterial flora of teleosts and elasmobranchs, including methods for its analysis. J. Fish Biol. **10**:529–553.
8. **Margolis, L.** 1953. The effect of fasting on the bacterial flora of the intestine of fish. J. Fish. Res. Board Can. **10**:62–63.
9. **Nealson, K. H., T. Platt, and J. W. Hastings.** 1970. Cellular control of the synthesis and activity of the bacterial luminescent system. J. Bacteriol. **104**:313–322.
10. **Reichelt, J. L., and P. Baumann.** 1973. Taxonomy of the marine, luminous bacteria. Arch. Mikrobiol. **94**: 283–330.
11. **Reichelt, J. L., P. Baumann, and L. Baumann.** 1976. Study of genetic relationships among marine species of the genera *Beneckea* and *Photobacterium* by means of *in vitro* DNA/DNA hybridization. Arch. Microbiol. **110**:101–120.
12. **Reichelt, J. L., K. H. Nealson, and J. W. Hastings.** 1977. The specificity of symbiosis: pony fish and luminescent bacteria. Arch. Microbiol. **112**:157–161.
13. **Rogers, P., and W. D. McElroy.** 1955. Biochemical characteristics of aldehyde and luciferase mutants of luminous bacteria. Proc. Natl. Acad. Sci. U.S.A. **41**:67–70.
14. **Ruby, E. G., and J. G. Morin.** 1978. Specificity of symbiosis between deep-sea fishes and psychrotrophic luminous bacteria. Deep Sea Res. **25**:161–167.
15. **Ruby, E. G., and K. H. Nealson.** 1976. Symbiotic association of *Photobacterium fischeri* with the marine luminous fish *Monocentris japonica*: a model of symbiosis based on bacterial studies. Biol. Bull. **151**:574–586.
16. **Ruby, E. G., and K. H. Nealson.** 1978. Seasonal changes in the species composition of luminous bacteria in nearshore sea water. Limnol. Oceanogr. **23**:530–533.
17. **Singleton, R. J., and T. M. Skerman.** 1973. A taxonomic study by computer analysis of the marine bacteria from New Zealand waters. J. Roy. Soc. N. Z. **3**:129–140.
18. **Spencer, R.** 1961. Chitinoclastic activity in the luminous bacteria. Nature (London) **190**:938.