APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Aug. 1993, p. 2684–2689
0099-2240/93/082684-06$02.00/0

Vol. 59, No. 8

Identification of *Vibrio splendidus* as a Member of the Planktonic Luminous Bacteria from the Persian Gulf and Kuwait Region with *luxA* Probes

K. H. NEALSON,* B. WIMPEE, AND C. WIMPEE

Department of Biological Sciences and Center for Great Lakes Studies, University of Wisconsin—Milwaukee, 600 East Greenfield Avenue, Milwaukee, Wisconsin 53204

Received 1 February 1993/Accepted 16 April 1993

Hybridization probes specific for the *luxA* genes of four groups of luminous bacteria were used to screen luminous isolates obtained from the Persian Gulf, near Al Khiran, Kuwait. Nine of these isolates were identified as *Vibrio harveyi*, a commonly encountered planktonic isolate, while three others showed no hybridization to any of the four probes (*V. harveyi*, *Vibrio fischeri*, *Photobacterium phosphoreum*, or *Photobacterium leiognathi*) under high-stringency conditions. Polymerase chain reaction amplification was used to prepare a *luxA* probe against one of these isolates, K-1, and this probe was screened under high-stringency conditions against a collection of DNAs from luminous bacteria; it was found to hybridize specifically to the DNA of the species *Vibrio splendidus*. A probe prepared against the type strain of *V. splendidus* (ATCC 33369) was tested against the collection of luminous bacterial DNA preparations and against the Kuwait isolates and was found to hybridize only against the type strain and the three unidentified Kuwait isolates. Extensive taxonomic analysis by standard methods confirmed the identification of the 13 isolates.

The ecology of the luminous bacteria is complex (10, 11, 19); even a given species can exhibit a variety of life-styles and inhabit several niches, including planktonic, saprophytic, symbiotic, and parasitic niches. Neither the dynamics of the bacterial populations nor the importance of the different niches to the overall ecology of the luminous bacteria is understood, even though a great deal of information is available concerning the distribution and abundance of various luminous species. The taxonomic scheme of Reichelt and Baumann (25) and slight modifications of it (17) have been used widely to identify the luminous bacteria of four major groups: *Vibrio harveyi*, *Vibrio fischeri*, *Photobacterium phosphoreum*, and *Photobacterium leiognathi*. Using these methods, Ruby and Nealson (32) showed seasonal population variations in the species composition of planktonic luminous bacteria off the coast of southern California, and Shilo and Yetinson (33, 36) demonstrated similar variations for waters of the Mediterranean Sea and the Gulf of Elat. Ruby et al. (28) showed that different species were found at different depths in the Sargasso Sea, while other workers have identified luminous bacteria as saprophytes, gut symbionts, and light organ symbionts in a variety of environments (4–9, 13, 14, 18, 20–22, 24, 28–32).

The above-cited studies were all subject to similar methodological limitations, which result from the fact that some other luminous marine vibrios (*Vibrio orientalis*, *Vibrio splendidus*, and *Vibrio vulnificus*) in the *V. harveyi* group are sufficiently similar to *V. harveyi* that they cannot be distinguished from *V. harveyi* by the rapid taxonomic methods commonly used (17). Thus, many of the organisms identified by rapid taxonomic methods (17, 25) as *V. harveyi* may in fact be closely related *Vibrio* species and, if subtle interactions or variations occur among these closely related species within the *V. harveyi* group, they may have been missed.

One possible way to improve the situation would be to develop methods for the rapid identification of these species based on molecular techniques, such as the hybridization probe technique. Wimpee et al. (34) recently described such methods for distinguishing among the four major groups of luminous bacteria. They prepared hybridization probes directed against the *luxA* gene and used these probes to identify three major groups (*V. fischeri*, *P. phosphoreum*, and *P. leiognathi*) by high-stringency hybridization analysis. Interestingly, the *V. harveyi* probe reported by this group was unable to distinguish among three closely related vibrios, *V. harveyi*, *V. orientalis*, and *V. vulnificus* (34). Thus, while the ease and speed of analysis were improved, the ability to distinguish among closely related *Vibrio* species remained a problem. No other species-specific probes for luminous bacteria have been reported, although a *luxA* probe has been used to identify *lux*-like genes in nonluminous *Vibrio cholerae* strains (23).

In this report, we describe the use of *luxA* probes to identify bacteria isolated from the Gulf of Arabia. These studies resulted in the identification of *V. splendidus* biotype A or biovar 1 as a member of the planktonic luminous community and in the development of a new *luxA* probe specific for luminous *V. splendidus*.

MATERIALS AND METHODS

Sampling site and collection. Samples were collected in southern Kuwait, near the inlet from the Gulf of Arabia near the town of Al Khiran (Fig. 1). Sampling was done by hand with sterile tubes held with their openings facing into the tidal flow. All samples were taken as the tide was entering the lagoon or inlet. The general location from which each sample was taken is not specified, because all samples were planktonic and presumably represented nearshore gulf water samples.

Samples were plated onto nutrient seawater agar plates (3 ml of glycerol, 1 g of yeast extract, 3 g of peptone, and 15 g of agar per liter of 75% seawater) at a variety of dilutions.

* Corresponding author.

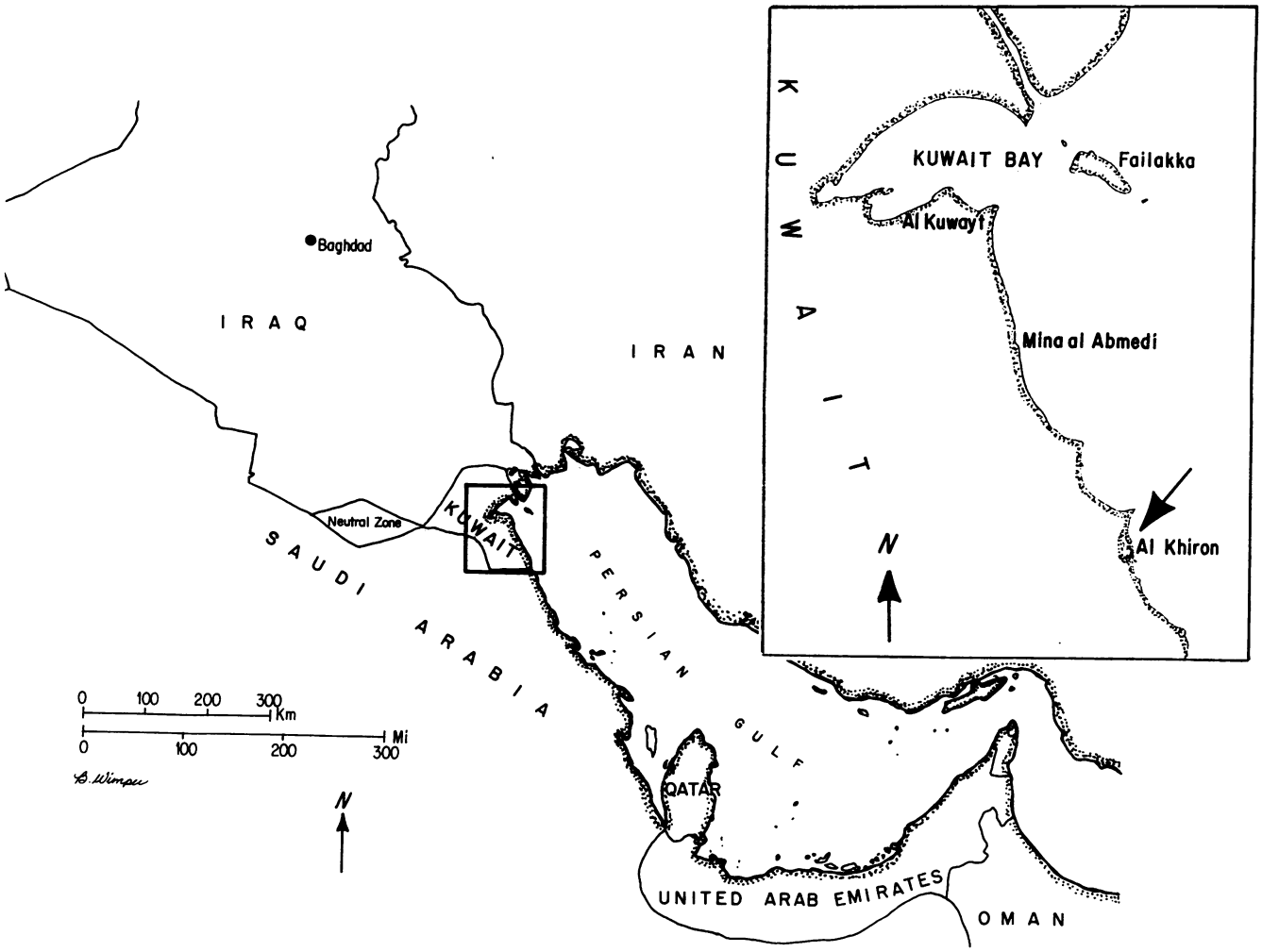

FIG. 1. Map of Kuwait with an inset of the locally sampled area. The collection site at Al Khiran is indicated by the arrow.

Luminous bacteria were identified visually, and colonies were picked with sterile toothpicks, purified to single colonies, and stored as agar slant cultures on nutrient seawater agar medium. For ensuring that no duplicate colonies were obtained, only one luminous isolate was picked from a given plate. Colonies were replated upon return to Wisconsin, checked for bioluminescence and, if luminous, stored for further analysis on nutrient seawater medium at −80°C.

Phenotypic taxonomic analyses. Taxonomic studies were done as described by Nealson (17), who modified the original technique of Reichelt and Baumann (25). In this method (referred to below as the short method), approximately 20 biochemical tests are performed, the kinetics of luciferase turnover are scored (Table 1) and, on the basis of these results, the organisms are placed into one of the four major groups. For more extensive taxonomic studies, we adapted the method of Yang et al. (35) to distinguish more closely related *V. harveyi*-like organisms. The diagnostic characters used are shown in Table 2. Control strains used were *V. harveyi* B392 (25), *V. fischeri* MJ-1 (31), *P. leiognathi* PL721 (27), *P. phosphoreum* NZ11D (29), and *V. splendidus* B397 (25). We had no control strain for *V. orientalis*, so data were taken from Yang et al. (35).

Luciferase enzyme turnover kinetics were determined for representative Kuwait strains. The methods used involved cell lysis and examination of a crude cell extract for luciferase activity (in comparison with those in control strains) as described by Nealson (17). Decanal and dodecanal were used separately in the assays, and luciferase kinetics were classified as either slow or fast, depending on the ratio of turnover rates for these two aldehydes.

DNA isolation, *luxA* amplification, radiolabelling, and filter hybridizations were done as described by Wimpee et al. (34). All hybridizations were done at 42°C, and posthybridization washes were done in 0.1× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) at 65°C.

RESULTS

Figure 2 shows the results of screening of the DNA from the 13 Kuwait isolates with the *luxA* probe from *V. harveyi* B392. Nine of the isolates appeared to be in the *V. harveyi* group, while three (K-1, K-11, and K-12) were apparently different organisms. Similar screenings of these isolates were performed with *luxA* probes from *V. fischeri*, *P. leiognathi*, and *P. phosphoreum*, all yielding negative results; in no case were any positive hybridizations between the Kuwait isolates and probes from these species seen (data not shown).

TABLE 1. Taxonomic features of Kuwait luminous isolates[a]

Organism	Growth at:		Production of:		Growth on:												Production of:		Luciferase kinetics
	4°C	35°C	Amylase	Gelatinase	Maltose	Cellobiose	Gluconate	Glucuronate	Mannitol	Proline	Lactate	Pyruvate	Acetate	Propionate	Heptanoate	Tyrosine	PHB	Gas from glucose	
V. harveyi	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	Slow
V. fischeri	(−)	(+)	(−)	−	+	+	(−)	−	+	+	−	(+)	(+)	−	−	−	−	−	Fast
P. leiognathi	−	(−)	−	(−)	−	−	+	−	−	+	+	+	(+)	−	−	−	+	−	Fast
P. phosphoreum	+	−	−	−	+	−	+	−	(−)	(−)	−	−	−	−	−	−	+	+	Fast
V. orientalis	−	+	+	+	+	+	+	−	+	+	+	+	+	−	−	−	+	−	Slow
V. splendidus	−	+	+	+	+	+	(+)	+	+	+	+	+	+	+	+	+	−	−	Slow
K-2 to K-10	−	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	−	−	Slow
K-1, K-11, and K-12	−	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	−	−	Slow

[a] The tests were adapted from those originally described by Reichelt and Baumann (25). Parentheses indicate that a trait showed a small degree of variability. PHB, poly-β-hydroxybutyric acid. Strains were those listed in Materials and Methods. Data for *V. orientalis* were taken from Yang et al. (35). K-2 to K-10 are Kuwait isolates in the *V. harveyi* group; K-1, K-11, and K-12 are Kuwait isolates in the *V. splendidus* group.

With the short method of luminous bacterial taxonomy (17, 25), all of the Kuwait isolates could be placed in the *V. harveyi* group, as shown in Table 1. These results are typical of those obtained in many previous studies done in our laboratory and others, as mentioned above.

Given the apparent inconsistency between the results shown in Fig. 2 and Table 1, we prepared a hybridization probe against strain K-1 by using the primers and polymerase chain reaction amplification methods described in Wimpee et al. (34). When this probe was used to probe the entire collection of DNAs from the luminous bacteria, it hybridized strongly to DNA isolated from *V. splendidus* but showed little or no hybridization to DNA isolated from any other organism (Fig. 3), suggesting that K-1 might belong to the *V. splendidus* group. A *luxA* probe was also made against strain K-3, which was expected to be in the *V. harveyi* group; it hybridized to DNAs from nine of the Kuwait isolates as well as to *V. harveyi* DNA but not to DNA isolated from any other organism (Fig. 4).

With this knowledge in hand, we made a *luxA* probe by using the DNA from *V. splendidus* (ATCC 33369), and this probe was tested against the Kuwait isolates and other organisms in the collection. As shown in Fig. 5, the *V. splendidus* probe hybridized to three Kuwait isolates as well as to *V. splendidus* but to no other organisms, indicating a rather species-specific response; these results are all consistent with K-1, K-11, and K-12 being members of the *V. splendidus* taxonomic group.

Table 2 shows the results of a more extensive phenotypic taxonomic analysis of the Kuwait isolates, modeled after that described by Yang et al. (35). The results are consistent with these bacteria being placed into the *V. splendidus* group, as was suggested from the hybridization probe studies.

DISCUSSION

Other than in one recent report by Makemson et al. (16), which identified *V. harveyi* as the predominant species (18 of 18 isolates) in waters near Bahrain, the Gulf of Arabia has not been investigated with regard to luminous bacterial populations or distribution. Lapota et al. (13) identified *V. harveyi* as a surface bacterium in the Arabian Sea, near the mouth of the Gulf of Arabia. Further east, in the indo-Pacific area, *V. harveyi*, *P. leiognathi*, and *V. fischeri* have been found (5, 6, 8, 9, 27). Even more eastward, Yang et al. (35)

TABLE 2. Additional taxonomic features of Kuwait luminous isolates[a]

Organism	Growth at 40°C	Production of arginine dehydrogenase	Presence of:			Growth on:									
			Swarming	Lateral flagella	Straight rods	D-Alanine	Arginine	α-Ketoglutarole	Spermine	Citrate	Galactose	Sucrose	L-Arabinose	Putrescine	L-Serine
V. harveyi	(+)	−	(+)	+	+	+	+	+	−	+	+	(+)	(−)	−	(+)
V. fischeri	−	−	−	−	−	−	−	−	−	−	+	(−)	−	−	(−)
P. leiognathi	−	−	−	−	−	−	−	−	−	−	+	−	−	−	(−)
P. phosphoreum	−	−	−	−	−	−	−	−	−	−	+	−	−	−	(−)
V. orientalis	−	−	−	−	−	+	ND	−	+	+	+	+	−	+	(+)
V. splendidus	−	+	−	−	−	+	+	+	−	+	+	+	−	−	+
K-2 to K-10	(−)	−	−	+	+	+	+	+	−	+	+	+	−	−	+
K-1, K-11, and K-12	−	+	−	−	−	+	+	+	−	+	+	+	−	−	+

[a] These additional tests were done to distinguish *V. splendidus* from other closely related *Vibrio* species. The tests were chosen on the basis of results presented by Yang et al. (35) and the discussion of species-specific properties by Baumann et al. (3). Data for *V. orientalis* were taken from Yang et al. (35). Other data were obtained in this study by use of the control strains listed in Materials and Methods. Parentheses indicate that the species showed some variability with regard to the given trait. K-2 to K-10 are Kuwait isolates in the *V. harveyi* group; K-1, K-11, and K-12 are Kuwait isolates in the *V. splendidus* group. ND, not determined.

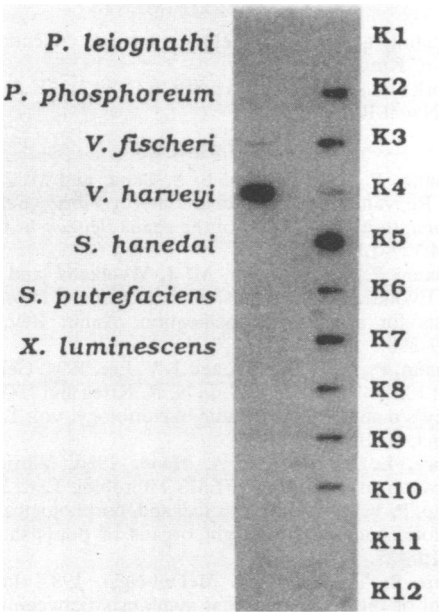

FIG. 2. Hybridization of a *V. harveyi luxA* probe to DNA from various luminous species, including 12 Kuwait isolates (K-1 through K-12). DNA from nonluminous *S. putrefaciens* was included on the filter as a negative control. Five micrograms of DNA from each sample was loaded onto a slot blot apparatus (Bethesda Research Laboratories, Inc.), bound to a Nytran filter (Schleicher & Schuell, Inc.), and probed with a polymerase chain reaction-amplified, ^{32}P-labelled *luxA* probe from *V. harveyi* as described previously (34). The filter was washed at 65°C in 0.1× SSC–0.1% sodium dodecyl sulfate and autoradiographed overnight on Kodak X-Omat AR film. *X. luminescens* = *Xenorhabdus luminescens*.

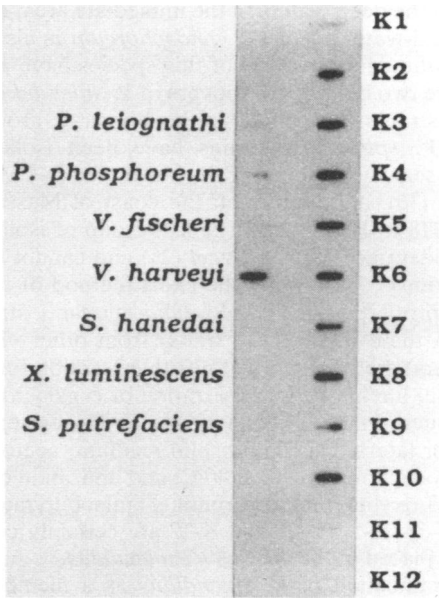

FIG. 4. Hybridization of a K-3 *luxA* probe to DNAs from various luminous species, including 12 Kuwait isolates (K-1 through K-12). DNA from nonluminous *S. putrefaciens* was included on the filter as a negative control. Methods are described in the legend to Fig. 2.

isolated *P. phosphoreum*, *P. leiognathi*, *V. harveyi*, *V. orientalis*, and *V. splendidus* biotype I from waters off the coast of China. To the west, Shilo and Yetinson (33, 36) reported *V. harveyi*, *V. fischeri*, and *P. leiognathi* in waters of the Gulf of Elat, the Suez Canal, and the Mediterranean

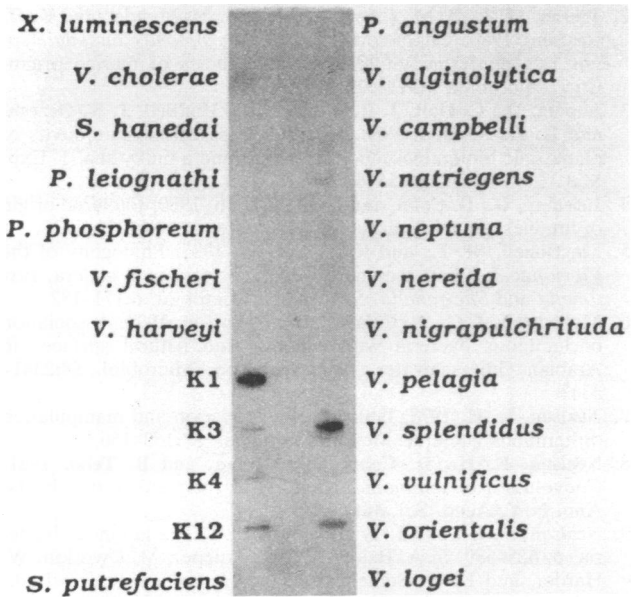

FIG. 3. Hybridization of a K-1 *luxA* probe to a collection of DNAs from various luminous species. DNA from nonluminous *S. putrefaciens* was included on the filter as a negative control. Methods are described in the legend to Fig. 2.

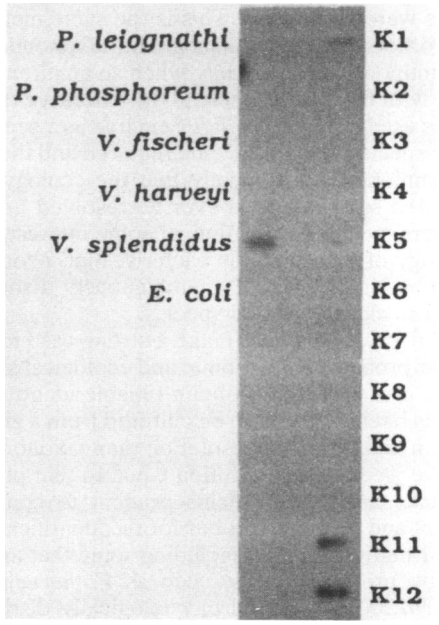

FIG. 5. Hybridization of a *V. splendidus luxA* probe to DNAs from various luminous species, including 12 Kuwait isolates (K-1 through K-12). DNA from nonluminous *S. putrefaciens* was included on the filter as a negative control. Methods are described in the legend to Fig. 2.

Sea. Given the deep waters in the immediate area, it is likely that the cold-water species *P. phosphoreum* is also present (19, 28), although no reports of this species have appeared.

There are two biotypes or biovars of *V. splendidus*, and all luminous strains are found in the biovar 1 group. Such luminous *V. splendidus* strains have been isolated from temperate seawater, including the North Sea (NCMB-1), the China Sea (35), and waters off the coast of Massachusetts (strains B378 to B380) (26). The last group of isolates were originally identified as *V. harveyi* (25) and cannot be distinguished from *V. harveyi* by the short method of taxonomy (17). The properties of *V. splendidus* biovar 1 strains that distinguish them (by 3 to 10 traits) from other vibrios (or their biovars) are curved rods; positive reaction for arginine dihydrolase; luminescence; utilization of D-galactose, cellobiose, D-glucuronate, α-ketoglutarate, and L-serine; negative reaction for lateral flagella on solid medium; acetoin and/or diacetyl production; growth at 40°C; and utilization of L-arabinose, β-hydroxybutyrate, D-sorbitol, γ-aminobutyrate, and putrescine (1–3). K-1, K-11, and K-12 are certainly compatible with being placed in the species *V. splendidus*.

The identification of *V. splendidus* as a member of the planktonic population in the waters of the Gulf of Arabia deserves some discussion. In the one previous report of luminous bacterial isolates from the area of the Gulf of Arabia, even though all of the isolates were identified as *V. harveyi*, it is possible that they were closely related *Vibrio* species. No taxonomic data were presented, but it was stated that several taxonomic tests were performed, including those in the API 20E (Analytab Products, Plainview, N.Y.) identification system, to establish the identity of the isolates. It is not clear that these methods would distinguish between *V. harveyi* and *V. splendidus*, but the placement of the isolates in the *V. harveyi* group seems unequivocal. When the recommended short method for taxonomic identification was used (Table 1), we also concluded that all of our isolates were *V. harveyi*. This is the same method used by most workers for the ecological and taxonomic identification of luminous bacteria. Only when an apparent discrepancy arose with the *luxA* probe and the extensive taxonomic method was used did *V. splendidus* emerge as a separate and identifiable species. Given the time required and the expense of such techniques, it is unlikely that the ecology of these closely related species would ever be resolved unless new methods were available. It is thus of some interest, in terms of the ecology of the luminous bacteria, that a combination of *luxA* probes is capable of unambiguously distinguishing between these closely related species.

The results presented here make a strong case for the use of molecular probes for taxonomic and ecological studies. In a few days, it is possible to obtain reliable identification of the luminous bacteria that can be cultured from a given area, identification that has more resolution than taxonomic methods that take weeks and resolution equal to that of methods that may take months. It seems prudent to continue the development and testing of probes for the identification of all of the major marine species, including some that are not yet separable by probe methods, such as *V. harveyi* and *V. orientalis* (34, 35), as well as physiologically distinct luminous species, such as *Shewanella hanedai* (12, 15). Such methods should be of great value in the presence of temporal and spatial variations, for which standard taxonomic approaches may be prohibitive because of the labor-intensive techniques involved.

ACKNOWLEDGMENTS

We gratefully acknowledge help with sample collection by Rehda Al-Hasan of Kuwait University.

This work was supported by a grant to C.W. and K.H.N. from the Office of Naval Research.

REFERENCES

1. **Baumann, P., L. Baumann, S. S. Bang, and M. J. Woolkalis.** 1980. Reevaluation of the taxonomy of *Vibrio*, *Beneckea*, and *Photobacterium*: abolition of the genus *Beneckea*. Curr. Microbiol. **4**:127–132.
2. **Baumann, P., L. Baumann, M. J. Woolkalis, and S. S. Bang.** 1983. Evolutionary relationships in *Vibrio* and *Photobacterium*: a basis for a natural classification. Annu. Rev. Microbiol. **37**:369–398.
3. **Baumann, P., A. L. Furniss, and J. V. Lee.** 1984. Genus I. *Vibrio* Pacini 1854, 411, p. 518–538. *In* N. R. Krieg and J. G. Holt (ed.), Bergey's manual of systematic bacteriology, vol. 1. Williams & Wilkins, Baltimore.
4. **Dilmore, L. A., and M. A. Hood.** 1986. Vibrios of some deep-water invertebrates. FEMS Microbiol. Lett. **35**:221–224.
5. **Dunlap, P. V.** 1984. Physiological and morphological state of the symbiotic bacteria from light organs of ponyfish. Biol. Bull. **167**:410–425.
6. **Dunlap, P. V., and N. J. McFall-Ngai.** 1987. Initiation and control of the bioluminescent symbiosis between *Photobacterium leiognathi* and leiognathid fish. Ann. N.Y. Acad. Sci. **503**:269–283.
7. **Fitzgerald, J. M.** 1977. Classification of luminous bacteria from the light organ of the Australian pinecone fish, *Cleidopus gloriamaris*. Arch. Microbiol. **112**:153–156.
8. **Haneda, Y., and F. I. Tsuji.** 1972. The luminous organs of two species of leiognathid fishes recently found in Ambon, Indonesia. Sci. Rep. Yokosuka City Mus. **19**:7–11.
9. **Haneda, Y., and F. I. Tsuji.** 1976. The luminescent systems of pony-fishes. J. Morphol. **150**:539–552.
10. **Harvey, E. N.** 1952. Bioluminescence. Academic Press, Inc., New York.
11. **Hastings, J. W., and K. H. Nealson.** 1981. The symbiotic luminous bacteria, p. 1322–1345. *In* M. P. Starr, H. Stolp, H. G. Trueper, A. Balows, and H. G. Schlegel (ed.), The prokaryotes. A handbook on habitats, isolation, and identification of bacteria. Springer-Verlag KG, Berlin.
12. **Jensen, M. J., B. M. Tebo, P. Baumann, M. Mandel, and K. H. Nealson.** 1980. Characterization of *Alteromonas hanedai* (sp. nov.), a nonfermentative luminous species of marine origin. Curr. Microbiol. **3**:311–315.
13. **Lapota, D., C. Galt, J. R. Losee, H. D. Huddell, J. K. Orzech, and K. H. Nealson.** 1988. Observations and measurements of planktonic bioluminescence in and around a milky sea. J. Exp. Mar. Biol. Ecol. **119**:55–81.
14. **Leisman, G., D. Cohn, and K. H. Nealson.** 1980. Bacterial origin of luminescence in marine animals. Science **208**:1271–1273.
15. **MacDonell, M. T., and R. R. Colwell.** 1985. Phylogeny of the *Vibrionaceae*, and recommendation for two new genera, *Listonella* and *Shewanella*. Syst. Appl. Microbiol. **6**:171–182.
16. **Makemson, J. C., N. Fulayfil, and P. Basson.** 1992. Association of luminous bacteria with artificial and natural surfaces in Arabian Gulf seawater. Appl. Environ. Microbiol. **58**:2341–2343.
17. **Nealson, K. H.** 1978. Isolation, identification and manipulation of luminous bacteria. Methods Enzymol. **57**:153–166.
18. **Nealson, K. H., D. Cohn, G. Leisman, and B. Tebo.** 1981. Coevolution of luminous bacteria and their eukaryotic hosts. Ann. N.Y. Acad. Sci. **361**:76–91.
19. **Nealson, K. H., and J. W. Hastings.** 1991. The luminous bacteria, p. 625–639. *In* A. Balows, H. G. Trueper, M. Dworkin, W. Harder, and K.-H. Schleifer (ed.), The prokaryotes, 2nd ed., vol. 1. Spring-Verlag, New York.
20. **O'Brien, C. H., and R. K. Sizemore.** 1979. Distribution of the luminous bacterium *Beneckea harveyi* in a semitropical estuarine environment. Appl. Environ. Microbiol. **38**:928–933.
21. **Oliver, J. D., D. M. Roberts, V. K. White, M. A. Dry, and L. M.**

Simpson. 1986. Bioluminescence in a strain of the human pathogenic bacterium *Vibrio vulnificus*. Appl. Environ. Microbiol. **52**:1209–1211.
22. **Orndorff, S. A., and R. R. Colwell.** 1980. Distribution and identification of luminous bacteria from the Sargasso Sea. Appl. Environ. Microbiol. **39**:983–987.
23. **Palmer, L. M., and R. R. Colwell.** 1991. Detection of luciferase gene sequence in nonluminescent *Vibrio cholerae* by colony hybridization and polymerase chain reaction. Appl. Environ. Microbiol. **57**:1286–1293.
24. **Ramesh, A., R. Nandakumar, and V. K. Venugopalan.** 1986. Enteric luminous microflora of pond-cultured milk fish *Chanos chanos* (Forskal). Microb. Ecol. **12**:231–235.
25. **Reichelt, J. L., and P. Baumann.** 1973. Taxonomy of the marine, luminous bacteria. Arch. Mikrobiol. **94**:283–330.
26. **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.
27. **Reichelt, J. L., K. Nealson, and J. W. Hastings.** 1977. The specificity of symbiosis: pony fish and luminescent bacteria. Arch. Microbiol. **112**:157–161.
28. **Ruby, E. G., E. P. Greenberg, and J. W. Hastings.** 1980. Planktonic marine luminous bacteria: species distribution in the water column. Appl. Environ. Microbiol. **39**:302–306.
29. **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.
30. **Ruby, E. G., and J. G. Morin.** 1979. Luminous enteric bacteria of marine fishes: a study of their distribution, densities, and dispersion. Appl. Environ. Microbiol. **38**:406–411.
31. **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.
32. **Ruby, E. G., and K. H. Nealson.** 1978. Seasonal changes in the species composition of luminous bacteria in nearshore seawater. Limnol. Oceanogr. **23**:530–533.
33. **Shilo, M., and T. Yetinson.** 1979. Physiological characteristics underlying the distribution patterns of luminous bacteria in the Mediterranean Sea and the Gulf of Elat. Appl. Environ. Microbiol. **38**:577–584.
34. **Wimpee, C., T.-L. Nadeau, and K. Nealson.** 1991. Development of species-specific hybridization probes for marine luminous bacteria by using in vitro DNA amplification. Appl. Environ. Microbiol. **57**:1319–1324.
35. **Yang, Y., L. P. Yeh, Y. Cao, L. Baumann, P. Baumann, J. S. Tang, and B. Beaman.** 1983. Characterization of marine luminous bacteria isolated off the coast of China and description of *Vibrio orientalis* sp. nov. Curr. Microbiol. **8**:95–100.
36. **Yetinson, T., and M. Shilo.** 1979. Seasonal and geographic distribution of luminous bacteria in the eastern Mediterranean and the Gulf of Elat. Appl. Environ. Microbiol. **37**:1230–1238.