CURRENT MICROBIOLOGY, Vol. 4 (1980), pp. 127–132

Current Microbiology
An International Journal

Reevaluation of the Taxonomy of *Vibrio, Beneckea,* and *Photobacterium:* Abolition of the Genus *Beneckea*

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Abstract. As a result of studies on the evolution of glutamine synthetase and superoxide dismutase, the genus *Beneckea* has been abolished and its constituent species, along with *Photobacterium fischeri* and *P. logei*, assigned to the genus *Vibrio*. The definitions of *Vibrio* and *Photobacterium* have been modified accordingly.

In 1971, two of the authors (P.B., L.B.) were faced with the problem of determining the generic assignment for a number of species of Gram-negative, facultatively anaerobic, marine bacteria, most of which were able to decompose chitin [8]. All of these organisms had single sheathed polar flagella when grown in liquid medium; in addition, some species possessed the unusual property of making additional unsheathed peritrichous flagella when grown on solid media [6]. Although the range of the G+C contents in the DNAs of these organisms (45–48 mol%) was similar to that of strains of *Vibrio cholerae* (the type species of *Vibrio*), which also had single sheathed polar flagella, the assignment of marine isolates to *Vibrio* was inadvisable since it was thought that *V. cholerae* was an intestinal and freshwater inhabitant and, therefore, occupied a different ecological niche. This ecological distinction was reflected in the fact that *V. cholerae* could grow in a minimal medium lacking added Na^+ (although optimal growth required the addition of 5–15 mM Na^+), while representative strains of several of the marine species required Na^+ concentrations ranging from 100 to 300 mM for optimal growth [25]. A survey of the literature indicated that an existing genus, *Beneckea*, shared a number of the properties characteristic of our marine isolates [12]. *Beneckea* was defined by Campbell to consist of Gram-negative, peritrichously flagellated (determined on cells grown on solid medium [8]), rod-shaped, chitin-decomposing, facultative anaerobes obtained primarily from marine sources. Since our phenotypic characterization did not provide us with a basis for separating either the chitin-utilizing species from nonutilizers or the polarly flagellated species from those with the ability to synthesize peritrichous flagella, we redefined the genus *Beneckea* to accommodate these species [6,8].

The assignment of our species to *Beneckea* rather than *Vibrio* has been criticized on both substantive and nomenclatural grounds. The substantive criticism has been that *V. cholerae* and species of *Beneckea* had sufficient similarity in phenotype and DNA homology to warrant their placement into a single genus; the nomenclatural criticism has been that the type strain of the type species of *Beneckea* (*B. labra*) was no longer available and the genus, therefore, had no nomenclatural validity. With respect to the substantive issues, it is clear that a numerical analysis of data derived from a phenotypic characterization is, in many cases, only of marginal use for generic assignments [6,18]. We also were, and still are, unwilling to assign species to genera on the basis of low DNA homology values (e.g., 11–28% homology between *V. cholerae* and species of *Beneckea*) since the accuracy and significance of such low homologies is not clear [6,27]. Our decision, therefore, was to clarify the substantive issue, namely, the relationships among *V. cholerae*, *Beneckea*, and *Photobacterium*, before resolving the nomenclatural problems.

Our aim has been to study the sequence homology between informational molecules, which would allow the establishment of evolutionary relationships upon which to base our taxonomic conclusions [30]. We have measured ribosomal RNA (rRNA) homology [3] as well as the amino acid sequence divergence of glutamine synthetase (GS) [2,4], superoxide dismutase (SOD) [2], and alkaline phosphatase, using

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0343-8651/80/0004-0127 $01.20

Table 1. List of species of *Vibrio* and *Photobacterium* together with their synonyms, type strains, and selected properties.

Current designations[a]	Our past designations	Designations in *Bergey's Manual*, 8th ed.	Type strain	Mol% G+C[b] ± SD	Oxidase[c]	Gas from D-glucose	Sheathed polar flagella	Species with strains having peritrichous flagella[d]	PHB accumulation coupled with inability to utilize exogenous β-hydroxybutyrate	Species with luminous strains	Requirement for Na^+[e]	Minimal concn (mM) Na^+ required for optimal growth[e]	References and description
1. *Vibrio fischeri*	*Photobacterium fischeri*	*Vibrio fischeri*[f]	ATCC 7744	38.9 ± 0.4	+	−	+	−	−	+	+	200–280	24
2. *V. logei* comb. nov.	*P. logei*	*V. fischeri*[f]	ATCC 29985	41.2 ± 0.5	+	−	+	−	−	+	+	240–300	1
3. *V. harveyi* comb. nov.	*Beneckea harveyi*	*Lucibacterium harveyi*[g]	ATCC 14126	46.4 ± 0.4	+	−	+	+	−	+	+	320–300	24
4. *V. campbellii* comb. nov.	*B. campbellii*	None	ATCC 25920	46.5 ± 0.4	+	−	+	+	−	−	+	280–320	8
5. *V. parahaemolyticus*	*B. parahaemolytica*	*Vibrio parahaemolyticus* biotype I	ATCC 17802	46.4 ± 0.1	+	−	+	+	−	−	+	160–180	8, 10
6. *V. alginolyticus*	*B. alginolytica*	*V. parahaemolyticus* biotype II	ATCC 17749	46.0 ± 0.4	+	−	+	+	−	−	+	200–260	8, 10
7. *V. natriegens* comb. nov.	*B. natriegens*	None	ATCC 14048	46.3 ± 0.1	+	−	+	−	−	·	+	140–200	8
8. *V. nereis* comb. nov.	*B. nereida*	None	ATCC 25917	46.4 ± 0.3	+	−	+	−	−	·	+	120–180	8
9. *V. vulnificus*[h] comb. nov.	*B. vulnifica*	None	ATCC 27562	47.2 ± 0.3	+	−	+	−	−	−	+	140–200	10, 27
10. *V. proteolyticus* comb. nov.	*B. proteolytica*	*Aeromonas hydrophila* subsp. *proteolytica*[i]	ATCC 15338	50.5	+	−	+	+	·	−	+	120	3
11. *V. splendidus* I, II comb. nov.	*B. splendida* I, II	*Lucibacterium harveyi*[g]	ATCC 33125, NCMB 1	45.6 ± 0.3	+	−	+	+[k]	−	+	+	300–400	27
12. *V. pelagius* I, II comb. nov.	*B. pelagia* I, II	None	ATCC 25916	46.1 ± 0.3	+	−	+	−	−	−	+	260–320	8
13. *V. anguillarum* I, II	*B. anguillara* I, II	*Vibrio anguillarum*	ATCC 19264	45.4	+	−	+	−	−	−	+	60–100	5
14. *V. nigripulchritudo* comb. nov.	*B. nigrapulchrituda*	None	ATCC 27043	46.5 ± 0.3	+	−	+	−	−	−	+	180–240	9
15. *V. fluvialis*[l] I, II			NCTC11327	50.8 ± 0.2	+	+[m]	+	+	−	−	+	20–40	18, 21
16. *V. gazogenes* comb. nov.	*B. gazogenes*[n]	None	ATCC 29988	47.1[o]	−[p]	+	+	−	−	−	+	280	15, 18
17. *V. marinus*		*V. fischeri*[f]	ATCC 15381	42.2	+	−	+	−	−	−	+		18, 24
18. *V. costicola*		*V. costicola*	NCMB 701	50.0 ± 0.0	+	−	+	·	−	−	+	600–700	11, 18
19. *V. cholerae*		*V. cholerae* biotypes *cholerae*, *eltor*, and *albensis*	ATCC 14035	48.2 ± 0.4	+	−	+	−	−	+	−	5–15	11, 18
20. *V. metschnikowii*[q]		*V. cholerae* biotype *proteus*	NCTC 8443	44.8 ± 0.7	−	−	+	−	−	−	−	5–15	18, 20
21. *Photobacterium phosphoreum*	Same	Same	ATCC 11040	41.5 ± 0.2	7%[r]	89%[r]	−	−	+	+	+	160–180	24
22. *P. leiognathi*	Same	*Photobacterium mandapamensis*	ATCC 25521	42.9 ± 0.2	64%	7%	−	−	+	+	+	240–260	26
23. *P. angustum*	Same	None	ATCC 25915	40.9 ± 0.3	+	−	−	−	+	−	+	200–280	27

[a] Includes orthographic corrections.

[b] Mol% G+C determinations performed either by M. Mandel by means of buoyant density measurements in CsCl gradients or by thermal denaturation using as standards strains whose G+C contents were previously determined by CsCl density gradient centrifugation.

[c] Some strains of *Photobacterium* become oxidase positive only after pretreatment with toluene [7, 24].

[d] When grown on solid medium, some strains have unsheathed peritrichous flagella in addition to the sheathed polar flagellum. On liquid medium, only the sheathed polar flagellum is present.

[e] See text for discussion of this trait.

[f] Hendrie, Hodgkiss, and Shewan failed to distinguish *V. fischeri*, *V. logei* (ATCC 15382), and *V. marinus* (ATCC 15381) and included all three species in *V. fischeri* [17].

[g] Hendrie and Shewan included biotype I of *V. splendidus* in *Lucibacterium harveyi* [11,27].

[h] Also known as lactose-positive vibrio [27].

[i] Initially designated *Aeromonas proteolytica* [22].

[j] Roman numeral designates biotype.

[k] Only found in some strains of biotype II.

[l] Designation of Lee et al. [21]; synonymous with Group F and EF-6 [18].

[m] Only positive in strains of biotype II [18,21].

[n] Designation of Harwood [15].

[o] Value from Harwood [15].

[p] Initially erroneously reported to be positive (C. S. Harwood, personal communication), confirmed as negative with a non-prodigiosin-producing mutant.

[q] Designation of Lee, Shread, and Furniss [20].

[r] Percent designates positive strains.

the quantitative microcomplement fixation technique ([30]; S. S. Bang, L. Baumann, P. Baumann, M. J. Woolkalis, manuscripts in preparation). The results of these extensive studies have led us to alter our views and have necessitated the abolition of *Beneckea* and the assignment of species previously in this genus to *Vibrio* along with two species previously in *Photobacterium*, *P. fischeri* and *P. logei*. A summary of these changes, with past synonyms and some properties of the species, is presented in Table 1.

The major conclusion emerging from our investigations is that although the overall congruence between the various approaches used was good (correlation coefficients above 0.9), there were sufficient differences in detail to necessitate subjective judgment. This is illustrated in Table 2, which summarizes the major groupings resulting from studies of rRNA homology and the amino acid sequence similarity of GS and SOD. Crucial to our continued separation of *V. cholerae* and *Beneckea* were the group-

Table 2. Major groupings of species of *Vibrio* and *Photobacterium*.

rRNA homology[a]	*V. fischeri*[b]	*V. harveyi* *V. parahaemolyticus* *V. alginolyticus* *V. natriegens*	*V. nereis* *V. vulnificus* *V. proteolyticus* *V. splendidus*	*V. pelagius* *V. anguillarum* *V. nigripulchritudo*	*V. cholerae*	*P. phosphoreum* *P. leiognathi*
GS amino acid sequence similarity[c]	Closely related species		More distantly related species			
	V. harveyi *V. campbellii* *V. parahaemolyticus* *V. alginolyticus* *V. natriegens* *V. nereis*	*V. vulnificus* *V. proteolyticus* *V. splendidus* *V. pelagius* *V. anguillarum* *V. nigripulchritudo* *V. fluvialis*	*V. fischeri* *V. logei* *V. gazogenes* *V. costicola* *V. cholerae* *V. metschnikovii*		*P. phosphoreum* *P. leiognathi* *P. angustum*	
SOD amino acid sequence similarity[d]	Closely related species		More distantly related species			
	V. fischeri *V. logei* *V. harveyi* *V. campbellii* *V. parahaemolyticus* *V. alginolyticus* *V. natriegens* *V. nereis*	*V. vulnificus* *V. proteolyticus* *V. splendidus* *V. pelagius* *V. anguillarum* *V. nigripulchritudo* *V. fluvialis*	*V. gazogenes* *V. cholerae* *V. metschnikovii*	*V. costicola*	*P. phosphoreum* *P. leiognathi* *P. angustum*	

[a] Data from [3,7].
[b] Asterisk refers to species whose groupings are changed by the various methods used.
[c] Data from [2,4].
[d] Data from [2] and S. S. Bang, L. Baumann, M. J. Woolkalis, and P. Baumann (manuscript in preparation).

ings established by rRNA homology [3] which indicated that (i) *V. fischeri* (*P. fischeri*), (ii) former species of *Beneckea*, (iii) *V. cholerae*, and (iv) *P. phosphoreum* and *P. leiognathi* were approximately equidistant from each other. Since *P. phosphoreum* and *P. leiognathi*, on the basis of structural and physiological properties [6,24] clearly deserved a separate generic status, internal consistency dictated that these four groupings be retained in separate genera. (*V. fischeri* [*P. fischeri*] was left in *Photobacterium* pending further study; see references [4,7] for a discussion of this as well as a confirmation of the results by 16S rRNA oligonucleotide cataloging.) Both the studies of GS and SOD [2,4] differed from the rRNA homology results in indicating that *P. phosphoreum*, *P. leiognathi*, and *P. angustum* were a considerable distance from *V. fischeri* (*P. fischeri*) and species of *Beneckea* (*Vibrio*) (Table 2). The results with GS confirmed the results of rRNA homology in that *V. fischeri* (*P. fischeri*) and *V. cholerae* were on the outskirts of a group of closely related species of *Beneckea* (*Vibrio*). The species *V. logei* (*P. logei*), *V. gazogenes* (*B. gazogenes*), *V. costicola*, and *V. metschnikovii* (which were not included in the rRNA homology studies) were also on the outskirts of this group of closely related species [2,4]. Studies of SOD confirmed most of the results obtained with GS but differed in two major points in that, unlike with GS, (i) *V. fischeri* (*P. fischeri*) and *V. logei* (*P. logei*) were in the group of closely related species of *Beneckea* (*Vibrio*) and (ii) *V. costicola* was quite distant from this group. As is apparent from this discussion and the data summarized in Table 2, there are no consistent internal subdivisions which would allow the formation of distinct genera in the complex of species containing *Vibrio*, *Beneckea*, *V. fischeri* (*P. fischeri*), and *V. logei* (*P. logei*). Therefore, the simplest course of action is to place all of these species into *Vibrio* and to restrict *Photobacterium* to the three closely related species *P. phosphoreum*, *P. leiognathi*, and *P. angustum* (Table 1).

A new group of organisms isolated by Lee et al. [21] (*V. fluvialis*) and included in some of our studies [2,18] is closely related to the marine species yet has a significantly lower Na^+ requirement (20–40 mM). These values, in conjunction with the 60–70 mM optimum determined for *V. anguillarum* [5], span the gap in Na^+ requirement previously observed between *V. cholerae* and the marine species of *Vibrio* [25]. Since our past determinations of the Na^+ concentrations required for optimal growth included only a relatively small sampling of *Vibrio* and *Photobacterium* species [25], we expanded this survey to include a total of three strains (if available) of each species and biotype. The terrestrial medium used differed from that previously described in containing 50 mM Tris buffer and 10 mM glycerol (5 mM D-glucose for *V. costicola*) as the sole or principal carbon and energy source. In the case of strains requiring organic growth factors, 0.05% yeast extract was added

to the medium. The conditions of cultivation and measurement of cell growth have been previously described [25]. The results of the experiments are presented in Table 1. The minimal concentration required for optimal cell yield spanned the range of 5–700 mM, with a continuum from 5 to 400 mM. In Table 1, *V. cholerae* and *V. metschnikovii* are listed as being able to grow in media lacking added Na^+ (contaminating levels below 0.1 mM). The basis for this observation was the fact that all of the strains of *V. cholerae* and *V. metschnikovii* tested (none of which required organic growth factors) could be serially transferred in media containing no added Na^+ (Table 1). It should be noted that while the requirement for Na^+ in marine vibrios has been documented in over 600 strains [1,5,6,18], the lack of a Na^+ requirement by *V. cholerae* and *V. metschnikovii* has been determined for only 12 and 4 strains, respectively.

The following are redefinitions of the genera *Vibrio* and *Photobacterium*. Most of the key references are found in review articles [6,7] or in Table 1; only additional references will be cited. Tables containing phenotypic properties of use for the identification of species are found in references [7] and [27].

Genus *Vibrio*

Definition. Cells generally single, straight or curved rods. When grown in liquid media, motile by sheathed polar flagella; monotrichous or multitrichous. On solid media may exhibit mixed flagellation with additional peritrichous unsheathed flagella of a shorter wavelength than the sheathed polar flagella. Gram negative. Do not form endospores or microcysts.

Chemoorganotrophs: facultative anaerobes capable of respiratory and fermentative metabolism using D-glucose as the sole or principal source of carbon and energy. Molecular oxygen is a universal electron acceptor. Do not denitrify or fix molecular nitrogen.

Growth stimulated by Na^+; most species will not grow in the absence of this ion.

The G+C contents in the DNAs of the species range from 38 to 51 mol%.

Type species: *Vibrio cholerae*.

Additional comments. Most species are oxidase positive (*V. gazogenes* and *V. metschnikovii* are oxidase negative). With the exception of *V. anguillarum* biotype II and *V. metschnikovii*, all reduce nitrate to nitrite. All ferment D-glucose, usually without the formation of gas (*V. gazogenes* and *V. fluvialis* biotype II produce gas). Some species (*V. alginolyticus*, *V. proteolyticus*, *V. anguillarum* biotype I, *V. cholerae*) form acetoin and/or diacetyl as well as 2,3-butylene glycol during D-glucose fermentation. Almost all species are able to grow in a minimal medium containing a seawater base with D-glucose and ammonium salts as sole sources of energy, carbon, and nitrogen; *V. anguillarum* biotype II as well as some strains of *V. logei*, *V. cholerae*, *V. metschnikovii*, and *V. costicola* have a requirement for organic growth factors. While growth of all species is stimulated by Na^+, there is considerable variation in the amount of this ion required for optimal growth (Table 1). *V. cholerae* and *V. metschnikovii* will grow in a minimal medium having less than 0.1 mM Na^+ while *V. costicola* will not grow with less than 200 mM. The concentrations of Na^+ required for optimal growth of these three species are 5–15, 5–15, and 600–700 mM, respectively. Growth of some species is improved by supplementing media with seawater levels of Mg^{2+} and Ca^{2+} in addition to Na^+. All grow at 20°C; some grow at 4°C or lower, others at 45°C; none grows at 50°C.

Several species (*V. natriegens*, *V. nereis*, and some strains of *V. nigripulchritudo* and *V. fluvialis*) accumulate poly-β-hydroxybutyrate (PHB) as an intracellular reserve product. Strains of some species (*V. parahaemolyticus*, *V. alginolyticus*, *V. proteolyticus*, *V. harveyi*) swarm on solid complex media. *V. nereis*, *V. proteolyticus*, *V. splendidus* biotype I, *V. anguillarum* biotype I, *V. fluvialis*, and *V. costicola* have a constitutive arginine dihydrolase system (measured as the anaerobic conversion of arginine to ornithine). Luminescence has been found in strains of *V. fischeri*, *V. logei*, *V. harveyi*, *V. splendidus* biotype I, and *V. cholerae* (a single freshwater isolate previously designated *V. albensis*). *V. gazogenes* produces prodigiosin, *V. nigripulchritudo* an insoluble blue-black pigment; *V. fischeri* and *V. logei* have a yellow cell-associated pigment.

Most species have a variety of extracellular hydrolases which may include amylase, gelatinase, lipase, chitinase, and alginase. Different species are able to utilize a total of 12–67 organic compounds as sole or principal sources of carbon and energy; these include pentoses, hexoses, disaccharides, sugar acids, sugar alcohols, C_2-C_{10} monocarboxylic fatty acids, tricarboxylic acid cycle intermediates, and amino acids. None of the species utilizes cellulose, formate, glycolate, C_6-C_{10} dicarboxylic acids, L-isoleucine, L-valine, L-lysine, L-tryptophan, purines, pyrimidines, or *n*-hexadecane. *V. natriegens* and strains of *V. fluvialis* and *V. harveyi* may utilize benzoate, *p*-hydroxybenzoate, or quinate via a pathway which involves degradation of the intermediate protocatechuate by means of a *meta* cleavage.

Species of *Vibrio* (including luminous isolates) are common in marine and estuarine environments and can be readily isolated from the surfaces and intestinal contents of marine animals [6,7,16]. Of the marine luminous vibrios, only *V. fischeri* appears to be capable of entering into a symbiotic association as demonstrated by its isolation from the luminous organs of teleost fishes and squid [16]. *V. cholerae* and *V. metschnikovii*, which have a low Na^+ requirement for optimal growth, have been isolated from fresh water as well as estuarine environments, a niche which they may share with some of the species having moderate Na^+ requirements (Table 1) [19,20,21,29]. *V. cholerae*, *V. parahaemolyticus*, *V. vulnificus*, *V. fluvialis*, and possibly *V. alginolyticus* are potential human pathogens. *V. anguillarum* is a pathogen of fish and eels, and *V. logei* is a possible pathogen of marine crabs.

All the species of the genus *Vibrio* have an outstanding morphological feature, namely, the presence of sheathed polar flagella. This property is not unique to species of *Vibrio* since it has also been found in marine and terrestrial strains of *Bdellovibrio* [7,28], *Pseudomonas stizolobii* [14], and *Ectothiorhodospira halophila* [23], all of which are readily distinguishable from *Vibrio* on the basis of their physiology and/or parasitic associations. *V. fischeri* and *V. logei* are morphologically different from the remaining species of *Vibrio* (Table 1); both have a yellow cell-associated pigment and, generally, more than four sheathed polar flagella. The remaining species of *Vibrio* usually have single polar flagella, although rare cells may have up to three polar flagella. The appearance of the latter (as observed by electron microscopy) differs from *V. fischeri*

and *V. logei* in that all the flagella are inserted in close proximity to each other while those of *V. fischeri* and *V. logei* are inserted over a broader area of the cell apex.

As can be expected from the ecological and nutritional diversity of the 20 species included in *Vibrio*, this genus is considerably more heterogeneous than typical genera of the family Enterobacteriaceae [2,6]. Immunological studies of GS indicate that the internal diversity of *Vibrio* is somewhat greater than that between *Escherichia coli* and *Serratia*, while studies of rRNA homology indicate that it is similar to that between *E. coli* and *Proteus*. The internal diversity of *Vibrio*, in comparison to genera within the Enterobacteriaceae, should not, however, be construed as an argument for future fragmentation of this genus. The present tendency is to circumscribe genera in the Enterobacteriaceae on the basis of DNA homology; this results in excessive generic splintering, without necessarily adding significant informational content to the generic definition. Therefore, the internal diversity of *Vibrio* can quite comfortably be accommodated by a single genus.

Relation of *Vibrio* to *Lucibacterium*. In the eighth edition of *Bergey's Manual*, the marine species *V. harveyi* is assigned to a newly created genus, *Lucibacterium* [11]. The primary feature distinguishing the genera *Lucibacterium* and *Vibrio* (as defined in *Bergey's Manual*) is flagellation—the former being peritrichous and the latter polar. Although our work on the shift from polar flagellation in liquid medium to peritrichous flagellation on solid medium in species of marine vibrios is cited, the authors of *Lucibacterium* failed to investigate this property in *V. harveyi* and based their definition of this genus on the flagellation of cells grown on solid medium. Subsequent investigation has shown that *V. harveyi* resembles other species of *Vibrio* in undergoing a shift in flagellation. In addition, in vitro DNA/DNA hybridization has shown a high level of homology between *V. harveyi*, *V. campbellii*, *V. parahaemolyticus*, and *V. alginolyticus*, necessitating their assignment to a single genus [27].

Genus *Photobacterium*

Definition. Cells generally single, straight rods. Motile by 1–3 unsheathed polar flagella; some nonmotile. Gram negative. Do not form endospores or microcysts.

Chemoorganotrophs: facultative anaerobes capable of respiratory and fermentative metabolism, using D-glucose as the sole or principal source of carbon and energy. Molecular oxygen is a universal electron acceptor. Do not denitrify or fix molecular nitrogen.

Growth is dependent on the presence of Na^+.

All are able to accumulate PHB as an intracellular reserve product but are not able to utilize the exogenous monomer, β-hydroxybutyrate.

The G+C contents in the DNAs of the species range from 40 to 44 mol%.

Type species: *Photobacterium phosphoreum*.

Additional comments. A number of traditional tests applied in bacterial taxonomy are of little use in differentiating species of *Photobacterium*. For example, the ability to form gas during D-glucose fermentation is present in some but not all strains of *P. phosphoreum* and *P. leiognathi*; similarly, both species contain strains which produce acetoin and/or diacetyl as well as 2,3-butylene glycol (Table 1). The oxidase test, which is a measure of cytochrome of the *c* type, is often negative, becoming positive in some strains following treatment with toluene (Table 1) [7,24]. Differential oxidized/reduced cytochrome spectra have indicated the presence of low levels of cytochrome *c* in representative strains of all species of *Photobacterium* [24,27]. Although some strains may produce alkaline products from arginine, none has a constitutive arginine dihydrolase system as measured by the anaerobic conversion of arginine to ornithine.

Most strains of *P. angustum* and *P. leiognathi* have no organic growth factor requirements; some strains of *P. phosphoreum* may require L-methionine, either alone or in combination with other amino acids. For optimal growth, 160–280 mM Na^+ is required; some species may also require seawater levels of Mg^{2+} and Ca^{2+}. All species grow at 20°C. *P. phosphoreum* grows at 4°C, while none grows at 40°C. Luminescence has been observed in *P. phosphoreum* and *P. leiognathi* but not in *P. angustum*.

None of the species of *Photobacterium* has an extracelluar amylase or alginase; some strains may have an extracellular chitinase, lipase, or gelatinase. The nutritional versatility of all the species is relatively low; only 7–22 organic compounds can be utilized as sole or principal sources of carbon and energy. These include hexoses and a few pentoses, disaccharides, sugar acids, tricarboxylic acid cycle intermediates, and amino acids.

Species of *Photobacterium* are common in seawater as well as the surfaces and intestinal contents of marine animals [7,16]. *P. phosphoreum* and *P. leiognathi* are capable of entering into a symbiotic association with marine animals, being found in the specialized light organs of teleost fishes [16]. The genus *Photobacterium* has the unusual property of accumulating PHB while lacking the ability to utilize the exogenous monomer, β-hydroxybutyrate. Accumulation of this polymer can be readily detected by phase microscopic examination of cultures in early stationary phase grown in a minimal medium containing 0.2% D-glucose [7,24] (supplemented with 0.05–0.1% yeast extract for growth factor–requiring strains).

Differentiation of *Vibrio*, *Photobacterium*, *Aeromonas*, and *Plesiomonas*. Studies of GS and SOD indicate considerable evolutionary divergence between these four genera ([2,4]; S. S. Bang, L. Baumann, M. J. Woolkalis, P. Baumann, manuscript in preparation). There are, however, no adequate, readily determinable traits present in all species which can distinguish *Vibrio*, *Photobacterium*, *Aeromonas*, and *Plesiomonas*. Of use are (i) the presence of sheathed polar flagella in *Vibrio* and the absence of a sheath in polar flagella of *Photobacterium*, *Aeromonas* [13], and *Plesiomonas* (B. L. Beaman, unpublished observations) and (ii) the mol% G+C contents, which are 38–51 for *Vibrio*, 40–44 for *Photobacterium*, 57–63 for *Aeromonas*, and 51 for *Plesiomonas* [11]. An additional possible diagnostic property is the requirement for Na^+ or stimulation by Na^+ characteristic of species of *Vibrio* and *Photobacterium* (Table 1), which is lacking in the relatively few strains of *Aeromonas* and *Plesiomonas* which have been examined. Despite

the paucity of distinguishing traits between these genera, the individual species are readily identifiable so that the identification of strains from environments where some of the organisms may coexist (e.g., *Aeromonas* and some species of *Vibrio*) should present no major difficulties.

ACKNOWLEDGMENTS

We are grateful to J. G. Holt for orthographic corrections and to B. L. Beaman, B. M. Tebo, and K. H. Nealson for the examination of the flagella of *Plesiomonas* and *V. costicola* by means of the electron microscope. The mol% G+C determinations in the DNAs of *V. costicola* NCMB 701 and NRC 37001 by M. Mandel is greatly appreciated. This investigation was supported by NIH research grant AI-15924 from the National Institute of Allergy and Infectious Diseases as well as by the Aquaculture Program, University of California, Agricultural Experiment Station.

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