Prokaryotes (2006) 6:508–563
DOI: 10.1007/0-387-30746-x_18

CHAPTER 3.3.18

The Genera *Vibrio* and *Photobacterium*

J. J. FARMER III AND F. W. HICKMAN-BRENNER

Introduction

Both *Vibrio* and *Photobacterium* are old genera that were described in the 1800s. The genus name *Vibrio* was coined by Pacini in 1854 during his studies on cholera, and it is one of the oldest names for a bacterial genus. Pacini also named the cholera bacillus, which eventually became *V. cholerae*, the type species for the genus *Vibrio*. Much of the history and literature on *Vibrio* came from medical microbiology and concerned cholera and other similar vibrios that early bacteriologists had difficulty in differentiating from the true cholera bacillus. In contrast, the genus name *Photobacterium* was coined by Beijerinck (188a) who was interested in environmental microbiology. Most of the literature on *Photobacterium* and on the marine species of *Vibrio* has come from environmental microbiologists, particularly those studying bacteria found in the sea. Unfortunately, there has been a poor interchange of ideas, information, and bacterial cultures between these two disciplines, which has led to considerable confusion in the literature. The methods for isolation and identification used by the two groups of workers are quite different and add further confusion. Several of the *Vibrio* species are very important in human or animal disease, and many of the species of *Vibrio* and *Photobacterium* are widely distributed in the environment. Many papers review various aspects of the two genera including: animal diseases (Anderson and Conroy, 1970; Baross et al., 1978; Sinderman, 1970); basic biology (Baumann and Baumann, 1977); enzyme structure and function (Bang et al., 1978b; Baumann and Baumann, 1973, 1978; Crawford, 1975; Gee et al., 1975); cellular structure or composition (Eberhard and Rouser, 1971); ecology and distribution (Golten and Scheffers, 1975; Harrell et al., 1976); human infections (Blake et al., 1979; Ryan, 1976; Wachsmuth et al., 1980; World Health Organization Scientific Working Group, 1980); physiology and metabolism (Doudoroff, 1942b; Eagon and Wang, 1962; Gauthier, 1976; Humm, 1946; Ingraham, 1962; Payne et al., 1961; Richter, 1928; Stanier, 1941); nucleic acid structure, function, or relatedness (Baumann and Baumann, 1976; Schiewe et al., 1977); nutrition (Doudoroff, 1942a); symbiotic relationships (Reichelt et al., 1977; Ruby and Morin, 1978); and taxonomy (Baumann et al., 1971a; Baumann et al., 1984; Fitzgerald, 1977; Hendrie et al., 1970; Reichelt and Baumann, 1975; Shewan and Véron, 1974; Singleton and Skerman, 1973).

History

The Concept of "Marine Vibrios"; Requirement for Na^+ and Other Ions (Baumann and Baumann, 1981)

The first comprehensive study of bacteria indigenous to the oceans was done by Bernhard Fischer (1894) of the University of Kiel. His major conclusions have been confirmed and extended by numerous investigators who have shown that the majority of the heterotrophic bacteria in the open oceans are Gram-negative, straight or curved rods or spirals that are usually motile by means of flagella (Baumann et al., 1971a, 1972; MacLeod, 1965, 1968; Pfister and Burkholder, 1965; Sieburth, 1979). Most of these organisms are eubacteria and have rigid cell walls. Fischer (1894) made the important observation that the highest viable counts (plate counting method) were obtained when seawater or 3% NaCl was included in the nutrient medium. This finding was subsequently interpreted to be primarily an osmotic phenomenon, since many marine bacteria were found to lyse in dilute media (Harvey, 1915; Pratt, 1974). However, Richter (1928) clearly demonstrated a specific Na^+ requirement for the growth of a marine luminous strain and, in addition, showed that complex media were not suitable for establishing this requirement since they contain substantial levels of inorganic ions (for reviews dealing with the early literature, see Larsen, 1962; MacLeod, 1965, 1968). Since these results were overlooked, and complex media continued to be used, considerable controversy persisted concerning the presence and stability

This chapter was taken unchanged from the second edition.

of this character. MacLeod independently reestablished the early findings of Richter and developed synthetic media for testing the presence of a Na^+ requirement (MacLeod, 1968). The extensive application of these methods has since established that all or most Gram-negative marine bacteria have a specific requirement for Na^+ (Baumann et al., 1971a, 1971b, 1972, 1973; Hidaka and Sakai, 1968; MacLeod, 1965, 1968). Furthermore, the work of MacLeod (1968) and others (reviewed by Pratt, 1974) has indicated the genetic stability of this requirement. Using 31 different marine isolates, including seven species of facultative anaerobes (with GC contents of 39–48 mol%), 12 species of nonfermentative marine organisms (with GC contents in their DNAs of 30–68 mol%), and one strain of a marine host-independent bellovibrio, Reichelt and Baumann (1974) studied the effect of NaCl concentration on growth rate and cell yield in media containing 50 mM Mg^{++} and 10 mM Ca^{++} (marine medium) and 2 mM Mg^{++} and 0.55 mM Ca^{++} (terrestrial medium). The optimum growth rates and cell yields in the marine medium ranged from 70 to 300 mM NaCl, while the optima in the terrestrial medium ranged from 100 to 460 mM. In many strains, the higher concentrations of Mg^{++} and Ca^{++} present in the marine medium reduced the amount of NaCl required for optimal growth rate and yield and decreased the generation time. Some strains did not grow unless the medium contained the higher Mg^{++} and Ca^{++} concentrations, indicating that the addition of 3% NaCl to a terrestrial medium will not make it suitable for the cultivation of many common marine bacteria. The extent to which the Na^+ requirement may be partially reduced by other ions differs considerably among marine isolates (MacLeod, 1965, 1968; Pratt, 1974). In the case of *Alteromonas haloplanktis* (the organism used in the extensive studies of MacLeod and his collaborators), Li^+ has little or no sparing effect, while in the case of *Vibrio parahaemolyticus*, this ion is able to considerably reduce the concentration of Na^+ required (Morishita and Takada, 1976).

Detailed studies of the physiological basis of the Na^+ requirement have so far been restricted to a strain of the species *Alteromonas haloplanktis*. In this organism Na^+ is essential for: 1) the function of all the examined permease systems which include those involved in the uptake of amino acids, tricarboxylic acid cycle intermediates, galactose, orthophosphate, and K^+ (Thompson and MacLeod, 1973); and 2) maintenance of the integrity of the cell wall (Forsberg et al., 1970). Conclusion (1) has been extended to the marine species *Vibrio fischeri* (Drapeau et al., 1966) and to *Pseudomonas doudoroffii* which requires 75mM Na^+ for the optimal rate of uptake of D-fructose (Baumann and Baumann, 1981). Conclusion (2) has also been extended to an *Alteromonas espejiana* strain, the host of the lipid-containing, marine bacteriophage PM-2 (Baumann and Baumann, 1981). In a recent survey of the effect of Na^+ on the integrity of the cell wall, it was found that in the absence of this ion a weakening of the outer membrane occurred in 14 of 20 marine strains examined. These observations suggest that the requirement for Na^+ by marine bacteria is a complex, multigenic trait which would not be readily lost by mutation. In contrast to the marine bacteria and the extreme halophiles which require over 3 M Na^+ (Larsen, 1962), the growth of most Gram-negative terrestrial organisms does not appear to be Na^+ dependent. Where a requirement has been demonstrated it has generally been found to be considerably lower than that observed in marine bacteria and may only be present under certain conditions of cultivation (Kodama and Tanaguchi, 1976; Reichelt and Baumann, 1974). An interesting exception are the rumen bacteria, which live in an environment having a relatively high concentration of this ion (Caldwell and Hudson, 1974; Reichelt and Baumann, 1974). The level of the Na^+ requirement and its stability imply that marine bacteria would not be able to colonize most terrestrial habitats. Conversely, there is considerable evidence that Gram-negative terrestrial bacteria do not survive in the marine environment (Jannasch, 1968; Moebus, 1972). These observations suggest an ecological separation of Gram-negative marine and terrestrial organisms as a consequence of specific adaptions to their respective habitats. In the case of marine bacteria, this attribute was probably acquired as a result of physiological adaptations to life in an environment having a relatively constant ionic composition. In this context, it is curious that many marine bacteria appear to grow better at 50–75% seawater than at 100% concentration (Gundersen, 1976) and that the optimal concentration of Na^+ (70–300 mM) for the growth of a number of marine isolates is considerably lower than the Na^+ concentration in seawater (450–480 mM) (Reichelt and Baumann, 1974). No obvious correlation has been observed between the source of isolation of the strains and the amount of Na^+ necessary for optimal growth.

Another fundamental question is whether there are bacterial species unique to the marine environment. Stanier (1941) succinctly formulated this problem and proposed an experimental solution, the essence of which was a comparison of the bacterial flora with similar biological functions (e.g., mineralization of simple organic compounds) in both marine and terrestrial habitats. The application of this approach has been facilitated by the existence of relatively

specific enrichment methods for the isolation of terrestrial pseudomonads as well as by the extensive phenotypic characterization of these organisms, which allows the ready identification of species (Stanier et al., 1966). In utilizing enrichment methods for the isolation of marine bacteria, it is essential to use seawater samples obtained aseptically at locations where contamination by terrestrial organisms is minimal or absent. Using these precautions, it could readily be demonstrated that when a marine inoculum was used in enrichment cultures selective for certain species of terrestrial pseudomonads, the resulting flora consisted of facultative anaerobes and nonfermentative organisms which were different from terrestrial species (Baumann et al., 1971a, 1971b, 1972, 1973; Reichelt and Baumann, 1973). These results established that the mineralization of simple organic compounds in the ocean is done by a bacterial flora that is different from those with this role in terrestrial habitats. This may not apply to estuarine habitats, since these environments are complex with respect to the diversity of habitats, salinity, availability of nutrients, and contamination by terrestrial organisms. The designation of a species as "estuarine" has no real conceptual meaning since it is not yet known whether there are any species indigenous to estuaries or other coastal habitats. Since the open oceans contain relatively low concentrations of bacteria relative to those found directly off shore, the inability to detect a particular species in the open ocean may simply be due to the limitation of sample size.

About 50% of the oceans (by area) reach depths where the pressures range from 380 to 1,100 atmospheres (ZoBell, 1963). Consequently, an important question has been the possible existence of bacteria which are specifically adapted to life at high hydrostatic pressures and which would be inhibited or killed by exposure to one atmosphere. The resolution of this problem has been undertaken by H. Jannasch and his collaborators who have constructed special samplers which allow the cultivation of marine microorganisms at the hydrostatic pressures at which they were sampled without introducing any decompression steps (Jannasch and Wirsen, 1977; Jannasch et al., 1976). Although detailed investigations of this problem are few, the initial results strongly suggest that if microorganisms specifically adapted to high pressures do exist, their numbers constitute a relatively small proportion of the total metabolically active, heterotrophic, bacterial flora of the deep oceans.

Over 90% of the marine environment (by volume) has a temperature below 5°C (ZoBell, 1963). Consequently, it is also of considerable interest to know whether the psychrophilic isolates from the ocean differ from previously characterized mesophiles only in their relation to temperature or whether they actually constitute different species. An answer to this question should be readily obtained by application of the various methods used for the characterization of marine bacteria.

One practical consequence of the marine nature of most of the species of *Vibrio* and *Photobacterium* is shown in Table 1, which indicates that biochemical test results used for routine identification vary depending on the Na^+ content of the media. The addition of NaCl usually results in an increase in the number of positive reactions and in the rapidity of the positive results.

History—Medical Aspects

The cholera bacillus, *Vibrio cholerae*, was first cultured by Koch in 1882 during an epidemic of cholera in India and Egypt. This led to an intensive study of the vibrios by many workers with the main goal of differentiating the "nonpathogenic" environmental and marine vibrios from the true cholera bacillus. No other pathogenic species of *Vibrio* were documented until 1950, when Fujino isolated *V. parahaemolyticus* from an outbreak of severe food poisoning in Osaka, Japan (see Fujino et al., 1974). Recently, a number of other species have been described and their role in causing human disease has been postulated or shown.

Nomenclature and Classification

In the last two decades the vibrios and photobacteria have changed from a poorly characterized heterogeneous group of organisms to several well-understood natural groups. This has been due to the transfer of "nonfermentative vibrios," "åerobic vibrios," and "microaerophilic vibrios" to other genera such as *Campylobacter (Vibrio fetus)*, *Wolinella (Vibrio succinogenes)*, *Pseudomonas*, and *Alteromonas* (Baumann and Baumann, 1981). The species currently recognized in *Vibrio* and *Photobacterium* are listed in Table 2. The genera *Vibrio* and *Photobacterium* are classified in the family Vibrionaceae along with two other genera, *Aeromonas* and *Plesiomonas*. Fig. 1 in Chapter 156 gives the gc content of the genera of vibrionaceae. It can be used as a first step in determining the relatedness of genera. *Vibrio* is closely related to *Photobacterium*, which is expected because of their phenotypic similarities, but these genera are more distantly related to *Aeromonas* and to the family enterobacteriaceae (Fox et al., 1980). These relationships were shown by nucleic acid hybridization experiments that are summarized in Fig. 1

Table 1. The effect of media (especially Na+ content) and test conditions on biochemical test results for some species of the genus *Vibrio*.

	Percentage positive for:[a]									
Test or property	*V. cholerae*	*V. mimicus*	*V. metschnikovii*	*V. hollisae*	*V. damsela*	*V. fluvialis*	*V. furnissii*	*V. alginolyticus*	*V. parahaemolyticus*	*V. vulnificus*
Indole production										
Peptone water	86	94	22	30	0	0	0	14	35	39
Peptone water + 1% NaCl	86	98	26	93	0	0	0	24	80	65
Heart infusion + 1% NaCl	97	95	17	97	0	13	11	42	89	94
Methyl red										
Standard	25	14	17	NG	NG	0	0	NG	NG	NG
Standard + 1% NaCl	99	99	96	0	100	96	100	77	78	79
Voges-Proskauer										
Standard	74	2	26	NG	NG	0	0	NG	NG	NG
Standard + 1% NaCl	73	1	24	0	33	0	0	8	0	0
Standard + 1% NaCl; Barrit method	93	9	96	0	95	0	0	83	0	0
Arginine dihydrolase										
Moeller's	0	0	0	0	81	52	0	0	0	0
Moeller's + 1% NaCl	0	0	59	0	95	93	100	0	0	0
Lysine decarboxylase										
Moeller's	99+	99	13	0	0	0	0	29	63	85
Moeller's + 1% NaCl	99+	100	36	0	52	0	0	99	100	99
Ornithine decarboxylase										
Moeller's	99+	99	0	0	0	0	0	3	71	47
Moeller's + 1% NaCl	99+	99	0	0	0	0	0	53	89	53
Gelatin hydrolysis										
Standard	52	60	65	0	5	41	56	45	66	55
Standard + 1% NaCl	62	63	38	0	6	85	86	76	89	75
Esculin hydroxysis										
Standard	0	0	4	0	0	0	0	0	0	0
Standard + 1% NaCl	2	0	59	0	0	8	0	2	1	39
Nitrate→Nitrite										
Standard	99+	99+	0	NG	NG	59	33	NG	0	2
Standard + 1% NaCl	99+	100	0	100	100	100	100	100	100	100

[a]The number indicates the percentage positive after 48 h of incubation at 36°C; NG, most cultures do not grow in the medium.

Table 2. The species of *Vibrio* and *Photobacterium* and their location in human clinical specimens.

	Occurrence in human clinical specimens:[a]	
	Intestinal	Extraintestinal
Vibrio alginolyticus	+	++
V. carchariae	–	+
V. cholerae		
Serogroup O1	++++	+
Serogroup non;nNO1	++	++
V. cincinnatiensis	–	+
V. damsela	–	++
V. fluvialis	++	–
V. furnissii	++	–
V. hollisae	++	–
V. metschnikovii	–	+
V. mimicus	++	+
V. parahaemolyticus	++++	+
V. vulnificus	+	+++
Species that do not occur in human clinical specimens[b]		
Genus *Vibrio*	*V. marinus*	*V. proteolyticus*
V. aesturianus	*V. mediterranei*	*V. salmonicida*
V. anguillarum	*V. natriegens*	*V. splendidus*
V. campbellii	*V. nereis*	*V. tubiashii*
V. costicola	*V. nigripulchritudo*	Genus
V. diazotrophicus		*Photobacterium*
V. fischeri	*V. ordalii*	*P. angustum*
V. gazogenes	*V. orientalis*	*P. leiognathi*
V. harveyi	*V. pelagius*	*P. phosphoreum*
V. logei	*V. orientalis*	

[a]The symbols +, ++, +++, and ++++ give the relative frequency of each organism in specimens.

[b]Theoretically any of these species could be found in feces after the ingestion of seafood or water that contains them. Based on published data, this must be a rare event.

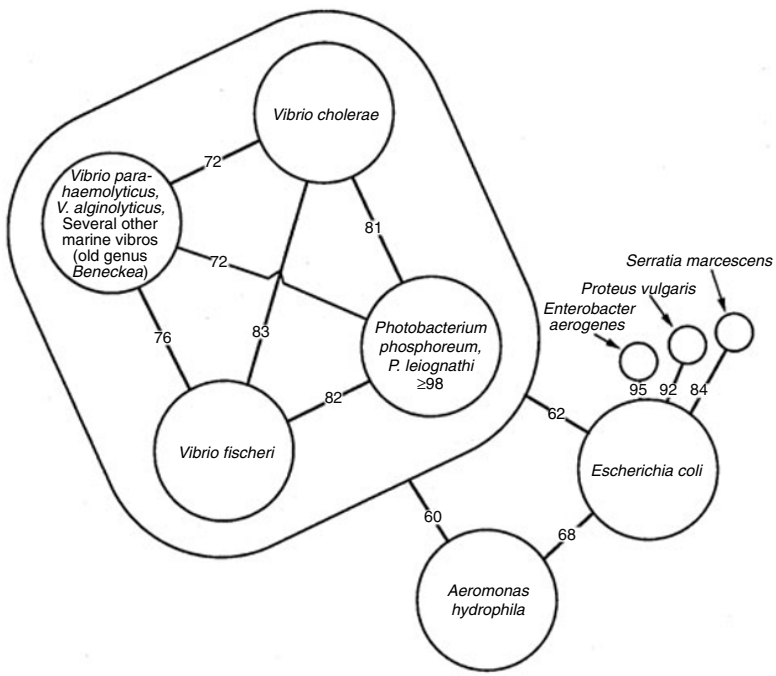

Fig. 1. Relatedness of the species of *Vibrio* and *Photobacterium* to each other and to other organisms in the families Enterobacteriaceae and Vibrionaceae based on the DNA-RNA hybridization studies of Baumann and Baumann and their coworkers. Numerical values represent average percent homologies. (Redrawn from Baumann and Baumann, 1981.)

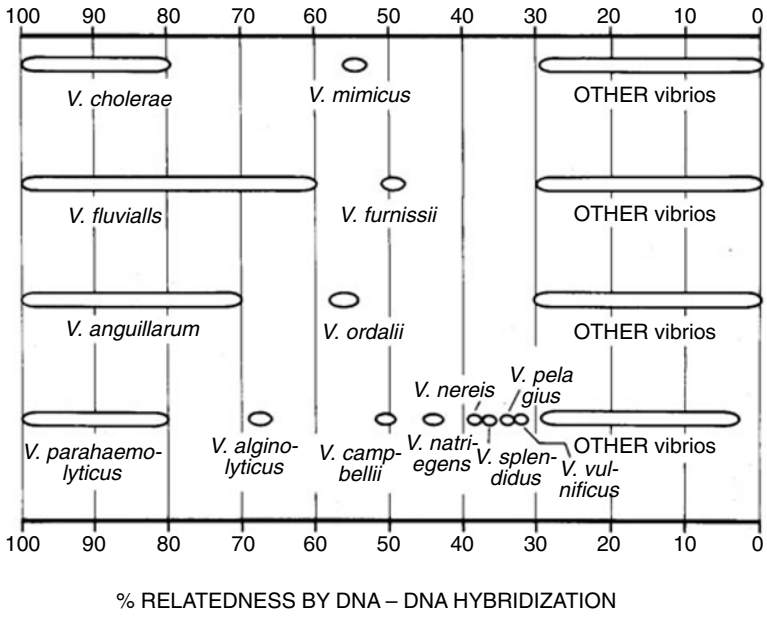

Fig. 2. Related species in the genus *Vibrio* based on DNA-DNA hybridization. (Redrawn from our own data that was summarized by reference is not an exact matchBrenner et al., 1983.)

(Baumann and Baumann, 1981). More distant relatives include the purple photosynthetic bacterium *Chromatium* and the nonfermentative genera *Pseudomonas* and *Acinetobacter* (see Fig. 3 in The Family Vibrionaceae in this Volume). Also Table 4 in The Family Vibrionaceae in this Volume lists some properties that differentiate the four genera in the family Vibrionaceae. Most of the *Vibrio* species are not closely related to each other in a phylogenetic sense (Baumann and Baumann, 1981; Baumann and Schubert, 1984; Brenner et al., 1983a). Thus, *Vibrio* is a heterogeneous genus, in a manner similar to the genus *Pseudomonas*. This is clear from DNA-DNA hybridization experiments that have been confirmed in several laboratories (Anderson and Ordal, 1975) and are shown in Figs. 2 and 3. Since *V. cholerae* is the type species for the genus, the genus definition must be built around it. *V. cholerae* is closely related to *V. mimicus*, but not closely related to the other vibrios (Fig. 2). However, another major evolutionary line includes several of the wellknown *Vibrio* species: *V. parahaemolyticus* is closely related to *V. alginolyticus* and greater than 30% related to several other species (Figs. 2 and 3). Most of the other species do not have close relatives, but there are a few exceptions: *V. fluvialis* with *V. furnissii*, *V. anguillarum* with *V. ordalii*, *V. splendidus* biogroup 1 with *V. splendidus* biogroup 2, *V. pelagius* biogroup 1 with *V. pelagius* biogroup 2, and *P. leiognathi* with *P. angustum* (Fig. 3). Even within a well-defined species there can be considerable divergence. For example, *V. gazogenes* contains three to four DNA hybridization groups (Fig. 4) (Farmer et al., 1988) that are almost identical in their phenotypic properties. Similar genetic comparisons based on a large number of strains have been done for only a few *Vibrio* species. The conclusions for relatedness in *Vibrio* based on nucleic acid hybridization generally agree with those based on protein structure (see Fig. 5.20 of Baumann et al., 1984). However, recent data based on RNA sequences have not been in agreement, and have led to some proposals that need to be confirmed in other laboratories and with different methods before they can be seriously considered.

Habitats

The species of *Vibrio* and *Photobacterium* are widely distributed in the marine environment, and a few species infect marine animals, particularly if they are stressed. In addition, 12 of the *Vibrio* species cause intestinal or extraintestinal human infections (Blake et al., 1979; Colwell, 1984) or have been isolated from human clinical specimens. *Photobacterium* species have been isolated from human clinical specimens.

Infections of the Intestinal Tract—Cholera and Other Diarrheas

Five of the *Vibrio* species appear to cause diarrhea in humans. *V. cholerae* is well known as the cause of cholera (Barua, 1970; Finkelstein, 1973; Pollitzer, 1959), which is a distinct clinical entity in its most severe form, but in its milder form may be hard to distinguish from other watery diarrheas. *V. parahaemolyticus* is a well-documented cause of acute gastroenteritis (Fujino et al., 1974; Miwatani and Takeda, 1976). More recently, *V.*

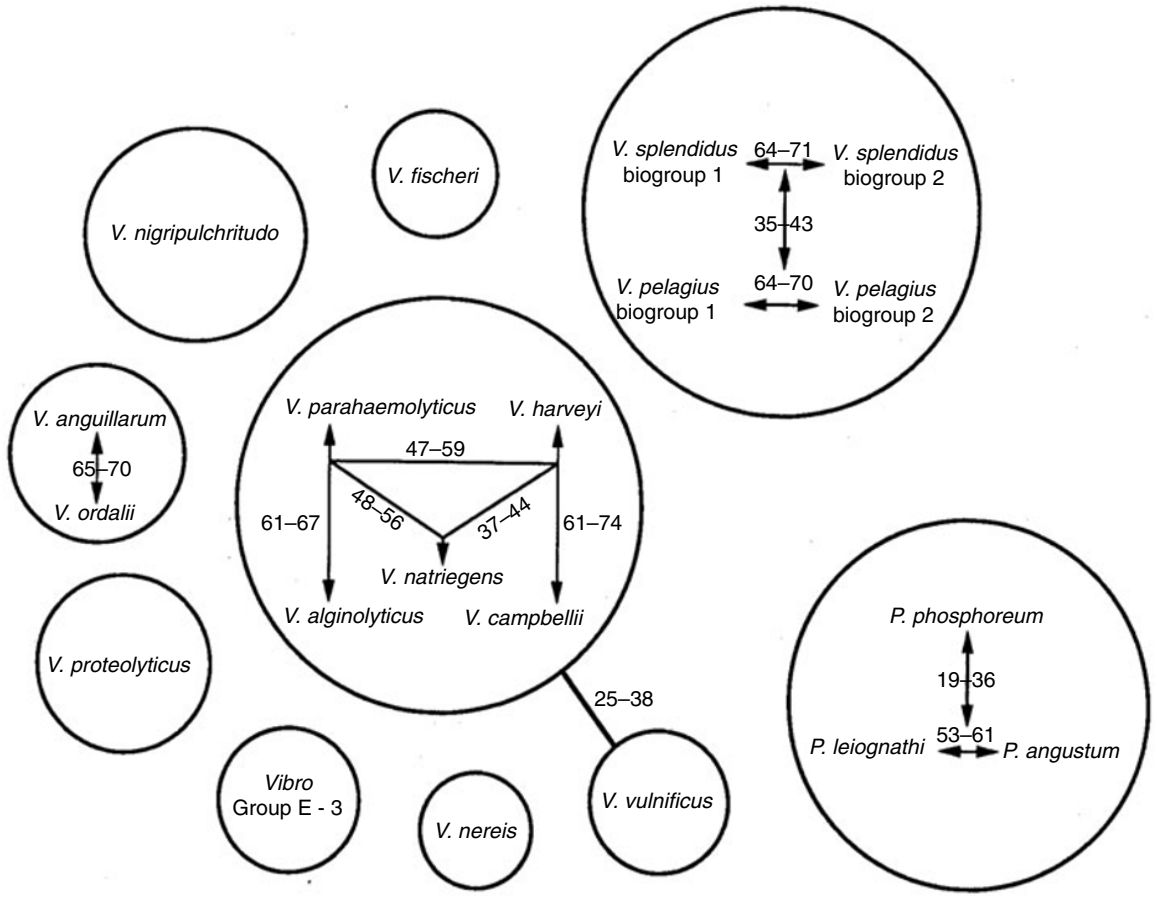

Fig. 3. Related species in the genera *Vibrio* and *Photobacterium* based on DNA-DNA hybridization; strains within each species and biogroup are related by DNA homologies of over 80%. Numbers indicate the range of DNA homology values between different organisms. Circles that are not connected are related by 30% or less (circle size has no meaning). (Redrawn from Fig. 44 of Baumann and Baumann, 1981.)

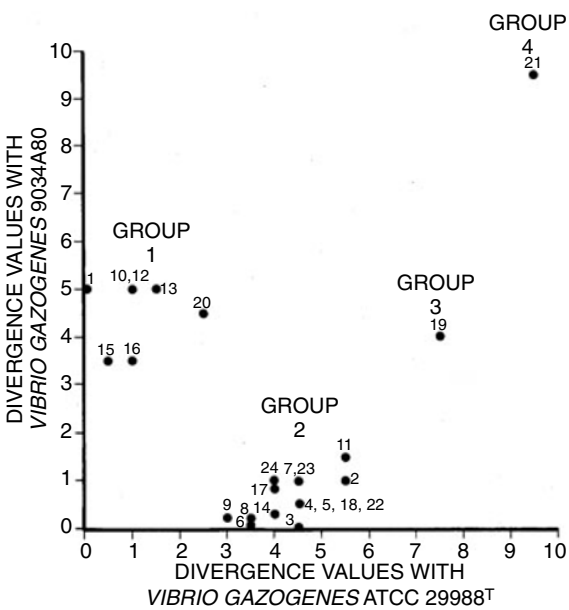

Fig. 4. Subgroups in the species *Vibrio gazogenes* based on divergence values in DNA-DNA hybridization experiments (these values reflect unmatched DNA sequences).

fluvialis (Huq et al., 1980; Lee et al., 1981), *V. hollisae* (Hickman et al., 1982; Morris et al., 1982), and *V. mimicus* (Davis et al., 1981) have been implicated as causes of diarrhea. *V. furnissii* (Brenner et al., 1983b) has been isolated from a few individuals with diarrhea, but there is no evidence that it can actually cause diarrhea.

Extraintestinal Infections

Strains of *Vibrio* have been isolated frequently from certain extraintestinal infections but are rarely isolated from others. Table 3 summarizes the sources of cultures studied at the *Vibrio* Reference Laboratory, Centers for Disease Control (CDC) Atlanta, Georgia. *Vibrio* species are often isolated from blood, arm and leg wounds, infected eyes and ears, and from gallbladders removed at surgery. They are rarely reported from patients with meningitis or pneumonia or from infections of the reproductive organs or urinary tract (Tison and Kelly, 1984). Within the genus *Vibrio* there is a division among the species which cause intestinal infection and those

Table 3. Sources of *Vibrio* isolates.

	V. cholerae		*V.*	*V.*	*V.*	*V.*	*V.*	*V.*	*V.*	*V.*	*V.*	*V.*	*V.*
	O1	Non-O1	*mimicus*	*metschnikovii*	*cincinnatiensis*	*hollisae*	*damsela*	*fluvialis*	*furnissii*	*alginolyticus*	*parahaemolyticus*	*vulnificus*	*carchariae*
Human													
Feces or intestine	118	94	39	0	2	30	0	15	16	4	114	6	0
Spinal fluid	0	1	0	0	1	0	0	0	0	0	0	2	0
Blood	1	42	0	1	0	2	0	0	0	0	2	63	0
Wound													
Hand or arm	0	2	0	0	0	0	1	0	0	1	3	12	0
Foot or leg	1	9	1	0	1	0	9	0	0	12	7	10	1
Other or unknown	1	3	1	0	0	0	0	0	1	9	1	1	0
Ear	0	24	6	0	1	0	0	0	0	18	0	0	0
Eye	0	0	0	0	0	0	0	0	0	2	0	0	0
Gall bladder	1	1	1	0	0	0	0	0	0	1	0	0	0
Urine	0	4	0	2	0	0	0	0	0	0	0	1	0
Respiratory tract	0	10	0	0	0	0	0	0	0	6	0	1	0
Other or unknown	4	12	5	4	1	1	0	0	0	6	0	8	0
Nonhuman													
Animals, nonmarine													
Primate	0	1	0	0	0	0	0	0	0	0	0	0	0
Pet or farm	0	6	0	0	4	0	0	0	1	0	0	0	0
Other	0	1	0	0	2	0	1	0	1	0	0	0	0
Animals, marine													
Fish	0	7	0	0	0	0	7	0	0	2	1	0	0
Oyster	8	29	9	0	0	0	1	5	0	0	11	4	0
Clam	0	5	0	0	0	0	0	0	0	2	0	0	0
Shrimp	1	5	1	0	0	0	0	0	0	0	5	0	0
Crab	2	0	0	1	0	0	0	0	0	3	1	0	0
Bird	0	0	1	2	0	0	0	0	0	0	0	0	0
Other	0	1	0	2	0	0	0	0	0	0	2	0	1
Water													
Unspecified	38	80	9	3	0	0	0	1	0	1	1	3	0
Ocean or estuary	0	4	3	0	0	0	0	0	0	3	1	9	0
Lake or stream	0	3	2	0	2	0	0	0	1	0	0	0	0
Sewage	28	20	0	3	0	0	1	0	0	0	0	1	0
Food	0	18	1	3	0	0	0	0	0	1	4	0	0
Culture collections	0	0	0	0	0	0	0	0	0	0	2	0	0
Other or unknown	43	18	13	2	0	1	2	9	3	3	7	3	0
Total	246	400	92	23	14	34	22	30	23	74	162	124	2

Studied at the *Vibrio* Reference Laboratory, Enteric Bacteriology Section, CDC.

Table 4. Growth of *Vibrio* cultures on TCBS agar.

Organism	Colony appearance[a] on TCBS (%)		Growth-plating efficiency
	Green	Yellow	
V. cholerae	0[a]	100[a]	Good
V. mimicus	100	0	Good
V. metschnikovii	0	100	May be reduced
V. hollisae	100	0	Very poor
V. damsela	95	5	Reduced at 36°C
V. fluvialis	0	100	Good
V. furnissii	0	100	Good
V. alginolyticus	0	100	Good
V. parahaemolyticus	99	1	Good
V. vulnificus	90[b]	10[b]	Good
V. carchariae	0	100	Good
V. cincinnatiensis	0	100	Very poor
"Marine vibrios"	Variable	Variable	Variable

[a]Percentage of strains that produce green colonies and yellow colonies, respectively.
[b]The original report describing this species gave the percentage positive for sucrose fermentation as 3%. At the CDC *Vibrio* Laboratory, about 15% of the strains have been sucrose positive.

which cause extraintestinal infections (Table 2); however, this division is not absolute. The pandemic strain of *V. cholerae* (serogroup O1, cholera-toxin$^+$) seems to be well-adapted to the human intestinal tract, and it is seldom found at other sites (Table 3). This is in contrast to other serotypes of this species *V. cholerae* non-O1) which occur at a variety of other sites. Similarly, *V. parahaemolyticus* also occurs mainly in gastroenteritis. Most of the reports of wound infections and septicemia due to this organism were really due to *V. vulnificus*, which had not been described at that time. *V. vulnificus* is an important cause of (often fatal) septicemia and wound infections (Blake et al., 1979, 1980b; Hollis et al., 1976). *V. damsela* (Love et al., 1981) also appears to cause human wound infections (Morris et al., 1982). *V. alginolyticus* (Blake et al., 1979) has been isolated from several types of soft tissue infections. *V. metschnikovii* is usually an environmental organism (Lee et al., 1978a), but has been isolated from a case of peritonitis in a patient with an inflamed gallbladder (Jean-Jacques et al., 1981), and from a few other specimens. Most of the infections caused by the marine species of *Vibrio* are associated with exposure to seawater.

The Marine Environment Since detailed taxonomic studies allowing precise identification of the species of *Vibrio* and *Photobacterium* isolated from this ecological niche are just beginning, generalizations concerning habitats and distribution of most species cannot be made. Baumann and Baumann (1981) (see Table 2 in their review) summarized the information available that appeared to be based on sound taxonomic criteria. They found that strains from geographically diverse locations, which have been assigned to the same species on the basis of phenotypic similarities, were found to have in vitro DNA-DNA homologies greater than 80%. Most of the remaining species of marine enterobacteria and the nonfermentative marine eubacteria were isolated from the open ocean 10–35 miles off the coast of Oahu, Hawaii, at depths ranging from surface waters to 1,300 (Baumann and Baumann, 1981). Most of the sites had little or no obvious terrestrial contamination. Several of these *Vibrio* species have not been reported from other locations.

There are good data on the habitats of the bioluminescent bacteria, some of which are able to enter into symbiotic association with marine animals. A number of studies have appeared dealing with the ecology of luminous bacteria in seawater off the coast of San Diego, California (Ruby and Nealson, 1978), the Eastern Mediterranean, and the Gulf of Elat (Shilo and Yetinson, 1979; Yetinson and Shilo, 1979), as well as from two locations in the open ocean in the North Atlantic and over the Puerto Rico Trench (Ruby et al., 1980). The results of these studies show a species-specific pattern influenced by season, depth, geographical locale, and salinity. These investigations are important because they represent ecological studies in which marine bacteria could be identified to the species level. Shilo and Yetinson (1979) were able to observe a correlation between the ecology of the luminous organisms and their physiological attributes.

Simple Methods to Determine the NaCl Content of Environmental Water Since many of the species of *Vibrio* and *Photobacterium* are

found in salt and estuary water, it is often desirable to quickly estimate salinity in order to decide where to sample, especially for inland waters. Two simple methods are given below that are particularly useful in the field. A quantitative method can be used when the specimen is returned to the laboratory.

Method 1—Silver Nitrate ($AgNO_3$) Method

This method takes advantage of the fact that NaCl is usually the main salt that contributes Na^+ in environmental water samples. Ag^+ reacts with Cl^- to form a dense white precipitate.

Dispense 1.0 ml of 1% silver nitrate solution (see below) into a disposable 13 · 100 mm test tube. Take a 0.1-ml sample of the liquid whose Na^+ content is desired and add it to the tube. A white precipitate will form almost immediately. The amount of precipitate will be proportional to the amount of NaCl present in the sample. An alternative method is to add a drop of the silver nitrate solution to the water and observe the intensity of the white precipitate formed.

This method actually measures the Cl^- content of the liquid, but for bacteriological media and water samples taken near the coast, this is close to the concentration of NaCl.

Silver nitrate solution,	*1%*
Silver nitrate ($AgNO_3$)	10 g
Water	1 liter

Dissolve the $AgNO_3$ in the water. Store in a brown bottle (or in a clear glass bottle that must be kept in the dark). In the presence of light, a brown-black precipitate will form. It is sometimes convenient to dispense 1-ml volumes into a rack of screw-cap tubes and store them in the dark until sampling is done.

Method 2—Dissolved Solids Meter

There are number of inexpensive pocket meters that measure conductivity or total dissolved solids and provide a convenient way to quickly determine the approximate Na^+ content of media and water samples.

Insert the pocket meter into the liquid sample and obtain a reading. Check this against a calibration curve based on different NaCl concentrations, and read the concentration of dissolved ions, which should approximate the concentration of NaCl.

Isolation

Much has been written about the isolation of both *Vibrio* and *Photobacterium* species. Two different sets of methods have been used, depending on whether the specimen (sample) was from a human clinical specimen or from the marine environment. Although species of *Vibrio* and *Photobacterium* will often grow on both kinds of media, the low Na^+ content (0.5 to 0.85%) of some media will prevent growth of some marine species. Only limited data exist on the growth of the species isolated from human diseases on many of the media traditionally used by marine microbiologists. In spite of these differences, several media are useful for all types of research with these genera.

Artificial Seawater Recipes

It is often desirable to have a medium with all of the inorganic constituents of seawater, but without organic compounds and toxic contaminants. Several artificial seawaters are useful for this purpose.

Artificial Seawater #1—MacLeod's Formula

This artificial seawater is very easy to prepare. Its formula does not contain several of the ingredients found in other formulas for artificial seawater (some of these are trace metals that are probably present in the distilled water added). One advantage of this formula is that it does not form a precipitate when it is autoclaved. This medium should be satisfactory for most work with *Vibrio* and *Photobacterium*.

Artificial Seawater #1—MacLeod's Formula (CDC Medium 1425)

NaCl	23.38 g (0.4 Molar)
$MgSO_4 \cdot 7H_2O$	24.65 g (0.1 Molar)
KCl	1.49 g (0.02 Molar)
$CaCl_2 \cdot 2H_2O$	2.94 g (0.02 Molar)
Water	1 liter

Dissolve the ingredients in the water; a crystal-clear solution with a pH of about 6.6 will result. Dispense into screw-cap bottles or tubes. Autoclave at 121°C for 15 min. The final solution should be colorless and crystal clear. Store at room temperature.

Artificial Seawater #2—"Instant Ocean"

This is an artificial seawater that is prepared from a commercial packaged mix. It contains all of the ingredients found in seawater. Instant Ocean is available from Aquarium Systems, 33208 Lakeland Blvd., Eastlake, Ohio 44094, but it can also be found in many pet stores, particularly those that stock supplies for marine aquariums. It comes in several sizes, including a 1.6-lb package.

Artificial Seawater #2—Instant Ocean

Instant Ocean	40 g
Distilled water	1 liter
pH about 8.6.	

We find that this mix contains a variable amount of water that has been absorbed from the air. Forty grams of Instant Ocean per liter should give approximately normal oceanic salinity; more precise work may require a different amount that can be calculated based on "dry weight" or specific gravity.

Add the Instant Ocean to the water. Stir on a magnetic mixer for 15 to 20 min until all the salt dissolves. A colorless solution will result (sometimes with a slight amount of turbidity). If a sterile solution is needed, dispense and autoclave at 121°C for 15 minutes. Store at room temperature.

There are also several good general plating media for isolation of *Vibrio* and *Photobacterium*

(see below). Marine agar is a nonselective medium, and essentially all vibrios will grow on it. TCBS agar is a very selective medium, and many vibrios strains will grow on it. Most other bacteria are inhibited.

Marine Agar

Marine agar is a useful medium for the isolation and growth of organisms whose natural habitat is marine or other environments with a high content of Na^+. It is particularly useful for the species of *Vibrio* and *Photobacterium* that require Na^+ in amounts higher than the usual content of bacteriological media, which is 0.5 to 1.0%.

Marine Agar

The formula used by Difco Laboratories (Catalog no. 0979) is given below; it is available as a dehydrated powder.

Peptone (Bacto)	5 g
Yeast extract	1 g
Ferric citrate	0.1 g
Sodium chloride	19.45 g
Magnesium chloride	8.8 g
Sodium sulfate	3.24 g
Calcium chloride	1.8 g
Potassium chloride	0.55 g
Sodium bicarbonate	0.16 g
Potassium bromide	80 mg
Strontium chloride	34 mg
Boric acid	22 mg
Sodium silicate	4 mg
Sodium fluoride	2.4 mg
Ammonium nitrate	1.6 mg
Disodium phosphate	8 mg
Water	1 liter
Agar	15 g

Add 55.1 g of Difco marine agar (or the recipe given above) to the distilled water and heat to boiling. pH 7.6 ± 0.2 at 25°C. Dispense into tubes or bottles and autoclave at 121°C for 15 minutes. A brown gelatinous precipitate is produced during autoclaving which settles to the bottom. Cool the flask to 45–50°C and mix the flask contents to disperse the brown precipitate as much as possible. Pour into petri dishes and allow to harden and cool to room temperature. The bottom of the plates will contain some brown precipitate, which should not be confused with bacterial growth.

TCBS (Thiosulfate-Citrate-Bile Salts-Sucrose) Agar

This medium is extremely useful for isolating *Vibrio cholerae* and *V. parahaemolyticus* from diarrheal stool specimens. It is also used as a general isolation-plating medium for vibrios from clinical specimens and from the environment (Table 4, Fig. 5). Some *Vibrio* species do not grow or grow poorly on TCBS agar. Dehydrated TCBS medium is available from BBL, Eiken, Oxoid, and Gibco. These manufacturers may use different peptones (polypeptone by

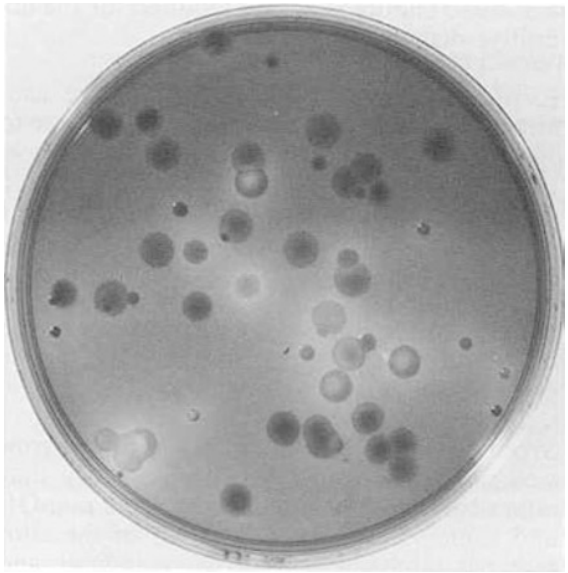

Fig. 5. Growth of vibrios on TCBS agar; 0.01 ml of seawater collected about 10 m from shore was plated and incubated for 3 days at ambient temperature (about 25°C); note the different sizes, shapes, and colors of the colonies.

BBL, Proteose Peptone no. 3 by Difco, Eiken Peptone by Eiken, etc.), and the selectivity of the medium will vary from manufacturer to manufacturer and from lot to lot from the same manufacturer. Quality control should be done on each new lot number.

TCBS Agar

This is the recipe given by BBL for their TCBS agar.

Yeast extract	5 g
Pancreatic digest of casein	5 g
Pancreatic digest of animal tissue	5 g
Sodium citrate	10 g
Sodium thiosulfate	10 g
Oxgall, dehydrated	5 g
Sodium cholate	3 g
Sucrose	20 g
Sodium chloride	10 g
Ferric citrate	1 g
Thymol blue	40 mg
Brom thymol blue	40 mg
Agar	14 g
Water	1 liter

Suspend 88 grams of the dehydrated powder in 1 liter of cold water and heat gently with frequent agitation to boiling to dissolve the agar. pH 8.6 ± 0.2 Do not overheat or autoclave. Cool to 45–50°C and pour into petri dishes.

There are also several good liquid media for isolating or growing vibrios. One recipe is given below.

Alkaline Peptone Water (CDC Medium 1494)

This medium is used for enriching for *Vibrio cholerae* and other *Vibrio* species. Species of

Vibrio grow better than most other organisms at this high pH. They also tend to grow better than other organisms at the aerobic surface of the liquid. Enrichment in alkaline peptone water is usually followed by plating a loopful from the surface onto TCBS agar or onto similar plating medium selective for *Vibrio*. The content of NaCl in alkaline peptone water is not standardized, but is usually 0.5 to 1%. It can be increased to 2% to allow better growth of the marine vibrios. If no NaCl is added, it becomes much more selective for *V. cholerae* and *V. mimicus* (see alkaline peptone water—saltless). The type of peptone used in the medium has also varied depending on several factors including local availability. The formula given below is the one used in our laboratory for many years. Other formulations are probably equally effective, but we have no experience with them.

CDC Medium 1494

Peptone (Bacto)	10 g
Sodium chloride	5 g
Water	994 ml

Sodium hydroxide, 1 N (see below)

Dissolve the peptone and sodium chloride in the water. Insert a pH electrode and add 1N NaOH dropwise until the pH has risen to 8.4; about 6 ml will be required. Dispense and autoclave at 121°C for 15 min. The final medium will be clear and amber colored.

Isolation—Clinical Specimens

Laboratory Routine for *Vibrio* Work

Many of the techniques used in clinical microbiology and enteric bacteriology laboratories work well with the genus *Vibrio*. However, there are some specialized items that are recommended for laboratories that often isolate and identify *Vibrio* species (Barua and Burrows, 1974; Benenson et al., 1964; Hugh and Sakazaki, 1972; Morris et al., 1979; Wachsmuth, 1984; World Health Organization, 1983) and for clinical laboratories that want to increase their capability. Two factors can complicate the isolation and identification of *Vibrio*. Sometimes *Vibrio* cultures will not grow well on the highly selective media used to isolate "enteric pathogens." The other factor is that the halophilic species of *Vibrio* need added NaCl for optimum growth and activity (Baumann and Schubert, 1984), and several common laboratory media have suboptimum amounts of Na^+ (less than 0.5% NaCl). Solutions to these problems are discussed below.

Collection, Transport, and Storage of Specimens

Extraintestinal Specimens The usual procedure for collecting and processing these specimen (blood, wound, tissue, etc.) is followed. There are no special procedures for *Vibrio*.

Stool Specimens Stool specimens should be collected early, preferably within the first 24 hours of illness, and before the patient has received any antimicrobial agents.

Transport Whenever possible, stool or rectal swab specimens should be inoculated on isolation plates with minimal delay. Viability of *Vibrio* species is well maintained at the alkaline pH of typical feces from cholera patients' rice-water stools, but is unpredictable in formed stools. *Vibrios* are very susceptible to desiccation, so specimens must not be allowed to dry.

When there will be a delay in plating a culture (especially when it must be transported by courier), rectal swabs or fecal material should be placed in alkaline peptone water or into Cary and Blair semisolid transport medium, which maintains viability of *Vibrio* cultures for up to 4 weeks. Buffered glycerol-saline, often used in enteric bacteriology, is an unsatisfactory transport medium even for short periods. Tellurite-taurocholate-peptone broth has been extensively used with success as an "enrichment transport" medium at the International Center for Diarrheal Diseases Research in Dhaka, Bangladesh, where specimens collected in the field are generally plated within 12 to 24 hours. In the absence of available suitable transport media, strips of blotting paper may be soaked in liquid stool and inserted into airtight plastic bags. Specimens collected in this way may remain viable for up to 5 weeks.

Storage and Shipment Specimens in transport media may be shipped to the laboratory without refrigeration, but all normal precautions and shipping regulation must be followed.

Overall Plan for Isolation

Table 5 gives four different approaches to the isolation of *Vibrio*. The approach adopted by a particular laboratory will probably depend on the frequency with which *Vibrio* cultures are encountered. The routine use of TCBS medium brings immediate attention to a possible *Vibrio* isolate, but its routine use is not cost effective. In an 18-month study, M. T. Kelly (see Farmer et al., 1985) found that every *Vibrio* isolated from TCBS medium was also detected on other plating media which were screened for oxidase-positive colonies. Other negative factors include the fact that different lots of commercial TCBS medium vary in their selectivity, and some species or strains of *Vibrio* do not grow well on TCBS.

Figure 6 gives an overall plan for using special methods to enhance the *Vibrio* isolation rate. One simple procedure will probably result in a higher isolation rate for *Vibrio*. Hemolytic colonies on sheep blood agar plates can be tested for their oxidase reaction, which will detect strongly hemolytic colonies of *Aeromonas* and some *Vibrio* species. This will also detect some weakly hemolytic *Vibrio* and *Plesiomonas* cultures. Oxidase testing can also be done on non-hemolytic colonies (see below). A latex agglutination test (Fig. 6) can be useful as a rapid screening method for detecting *V. cholerae* but a positive culture is always required for the definitive diagnosis of cholera.

Extraintestinal Specimens These are usually processed with no particular attention to *Vibrio*. The *Vibrio* species of medical importance grow well on blood agar and many also grow on MacConkey agar. On blood agar strains of *V. cholerae* have a characteristic morphology (Fig. 7) and are usually strongly hemolytic (except for the classical biogroup). However, a more thorough search for *Vibrio* isolates can be done with oxidase testing or by including a plate of TCBS medium.

Table 5. Four approaches for the isolation of *Vibrio* cultures from clinical specimens.

1. Use normal procedures and make no special effort to search for *Vibrio*.
2. Use normal procedures and look for oxidase-positive colonies on plating media, especially on blood agar.
3. Incorporate TCBS agar as an extra plate for stool cultures, and also for other likely specimens such as wounds, blood, eye, and ear.
4. Use other special media and procedures to enhance the isolation of *V. cholerae*, *V. parahaemolyticus*, and other *Vibrio* species.

Stool Specimens Cultures of *Vibrio* will grow well on blood agar (Fig. 8) where they may either be: beta-hemolytic (*V. cholerae* non-O1, and some *V. cholerae* O1 strains of the eltor biotype), alpha-hemolytic *V. vulnificus* and many others), or non-hemolytic. They usually grow on MacConkey agar (sometimes with a reduced plating efficiency) and will appear as colorless (lactose-

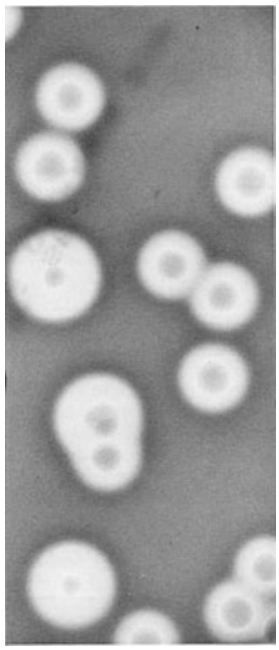

Fig. 7. Strong hemolytic reaction of the Gulf Coast strain of *Vibrio cholerae* on sheep blood agar plates (overnight incubation at 36°C); note that the small colony type has a much larger zone of hemolysis than the large colony type.

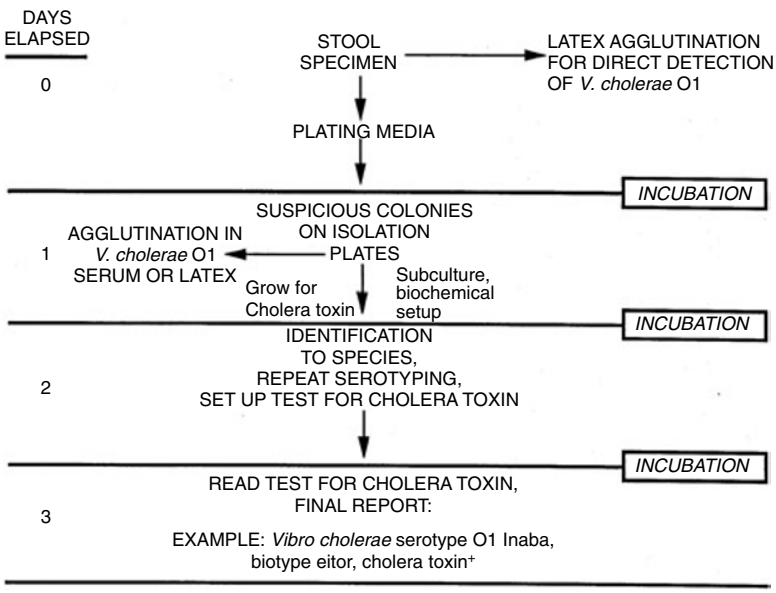

Fig. 6. Overall plan and special methods for the isolation and identification of *Vibrio cholerae* and other *Vibrio* cultures.

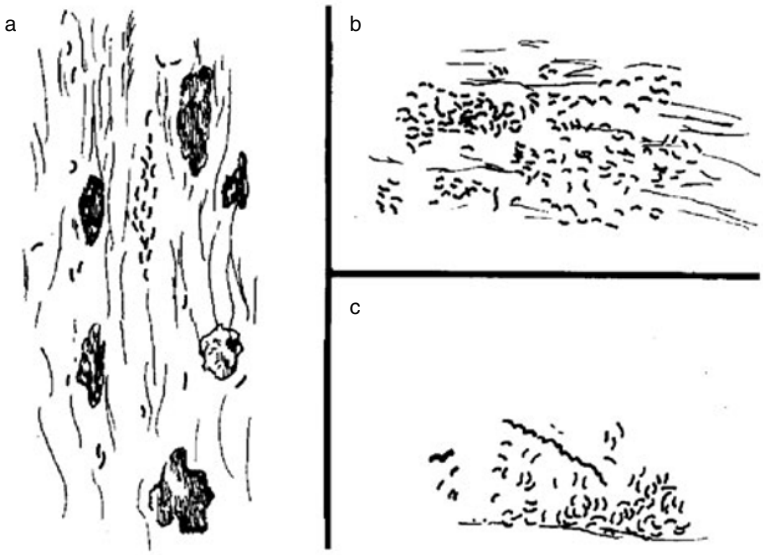

Fig. 8. Variation in the size and shape of *Vibrio cholerae* cells taken directly from cholera patients and laboratory cultures as reported by Robert Koch in 1883; note the curved rods that led Koch to use the name "comma bacillus." (a) Intestinal content of a cholera patient. (b) Rice-water stool of a cholera patient after 2 days incubation on moist clothing. (c) Growth from meat-broth laboratory culture (note long spiral forms). (All redrawn from Koch's original figures as reproduced on p. 100 of Pollitzer, 1959.)

negative) colonies. Oxidase testing can be done on colonies grown on blood agar and on lactose-negative colonies on selective media; however, lactose-positive colonies from selective media can give false-negative oxidase reaction. *Vibrio* cultures often do not grow well on the more selective enteric plating media.

Oxidase Testing of Colonies from Primary Plates

This appears to be a cost-efficient method for the detection of *Vibrio* cultures without having to add an additional plating medium such as TCBS agar.

Oxidase Tests

Method 1. An isolated colony on a blood agar plate is touched and spot tested for oxidase reaction by Kovacs' method. A total of 50 to 10 colonies are tested.

Method 2. Growth from a crowded area is taken up with a cotton swab and smeared onto the filter paper soaked with Kovacs' oxidase reagent.

Method 3. A drop of Kovacs' oxidase reagent is added to an area of the blood agar plate where the colonies are crowded but still separated. The drop should cover 50 to 100 colonies. Oxidase-positive colonies turn purple within 1 minute.

If oxidase-positive colonies are present in methods 2 or 3, individual isolated colonies (not previously touched or exposed to the reagent) from an area of less crowding are tested for their oxidase reactions as described in method 1. Since most specimens will have no oxidase-positive colonies (other than *Pseudomonas aeruginosa*), this method reduces the number of colonies which require individual screening. Oxidase-positive colonies (other than *P. aeruginosa*) detected by the above method are then identified to species. This method has another advantage because it also detects cultures of *Aeromonas* and *Plesimonas*. Oxidase testing should improve the isolation rate, but it may not be very cost effective in some geographical locations because of low yields of *Vibrio*, *Plesiomonas*, and *Aeromonas*.

Specialized Media and Methods for *Vibrio* Isolation

Use of TCBS Agar The advantage of using TCBS agar is that it increases the laboratory worker's awareness of suspect *Vibrio* colonies, and usually results in an increased number of isolates. Many laboratories near oceans or salty areas use TCBS agar as a plating medium for stool and other specimens (Bonner et al., 1983). It is recommended as the single best medium to detect *Vibrio* from human clinical specimens. Table 4 gives some information about the growth of the *Vibrio* species on TCBS agar. This medium is particularly useful for isolating *V. cholerae* or *V. parahaemolyticus* from feces. There is considerable variability in the selectivity of TCBS from different manufacturers and quality control of each new lot is essential.

Special Efforts to Isolate *Vibrio cholerae* and Other *Vibrio* Species from Feces Fig. 6 is a schema adapted from Wachsmuth et al. (Wachsmuth et al., 1980) and Furniss et al. (Furniss et al., 1978) for the isolation of *V. cholerae* from feces. It should also be useful in isolating other *Vibrio* cultures from feces and other specimens. TCBS agar and enrichment in alkaline peptone water select for *Vibrio* species. The main disadvantage of this approach is that these methods are in addition to the usual laboratory routine, and may cause much additional work with a poor yield of specimens positive for *Vibrio*.

Microscopic Examination of Growth In 1883, Koch noted the appearance of small curved rods in the rice-water stools of cholera patients (Fig. 8). These typical curved rods were not seen in feces of patients without cholera. A number of

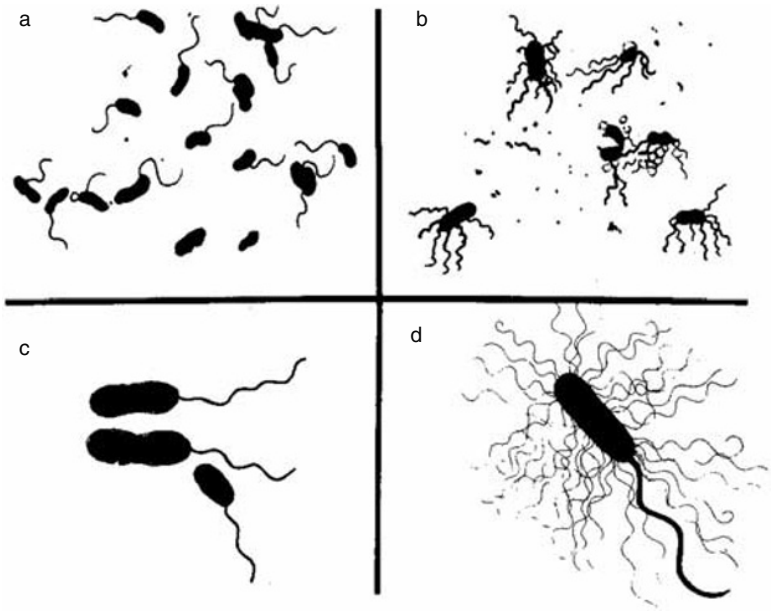

Fig. 9. Cellular morphology of *Vibrio cholerae*, *V. parahaemolyticus*, and *V. alginolyticus*. (a) *V. cholerae* from an 18-h culture on nutrient agar (Van Ermengen cilia stain). (b) Flagella stain of *V. parahaemolyticus*. (c) Electron micrograph of *V. parahaemolyticus*; note single-sheathed polar flagellum. (d) Electron micrograph of *V. alginolyticus* grown on solid media; peritrichous flagella are present; note their different size and shape as compared to the sheathed polar flagellum. (From Farmer et al., 1985.)

other authors have made the same observation and commented on the possible usefulness of this observation. *Vibrio cholerae* has considerable variability in its cellular morphology (Fig. 8), which includes typical curved rods, straight rods, short noncurved rods, and "inovolution forms" (Fig. 8C). Often these can all be seen in the same culture. For this reason, microscopic examination of feces or of cultures does not have a prominent role today. However, the finding of typical curved forms can be used as presumptive evidence for the presence of *Vibrio*. *Vibrio* cultures grown in liquid media have polar flagella, but many strains have peritrichous flagella when grown on solid media (Fig. 9). However, flagella stains are impractical for routine identification.

Isolation—Marine and Environmental Samples

Enrichment Cultures (from Baumann and Baumann, 1981)

Most enrichment cultures are incubated at room temperature (18–22°C), while cultures on petri plates are incubated at ambient temperature or 25°C. These temperatures are primarily a matter of convenience and can be modified (usually reduced, as in the case of the luminous bacteria) to suit the particular needs of the investigator.

Carbon sources are added to enrichments at a concentration of 0.1–0.2% (w/v for solids and v/v for liquids). For the cultivation of amino acid-requiring organisms, basal medium (medium "BM" of Baumann and Baumann, 1981) is supplemented with 1 mg/liter each of L-alanine, L-arginine, L-asparagine, L-asparatate, L-cysteine, L-glutamate, L-glutamine, glycine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-methionine L-phenylalanine, L-proline, L-serine, L-threonine, L-tryptophan, L-tyrosine, and L-valine. The amino acids are filter-sterilized and added to the autoclaved medium.

The carbon—energy source (unless labile or volatile) is added to the double-strength medium BM prior to autoclaving. Filter-sterilized labile or volatile compounds are added to the basal medium with added agar (medium BMA of Baumann and Baumann, 1981), which has been cooled to about 41°C prior to the pouring of plates. Some volatile substrates (geraniol, *n*-hexadecane, naphthalene, and phenol) are not added to the medium but are placed on sterile filter paper in the lids of the inverted petri dishes, then incubated in air-tight containers. It should be stressed that the medium of MacLeod based in artificial seawater (1968) may not be suitable for growing some marine organisms that may require additional mineral components. A number of different artificial seawater formulations have been compiled by Kinne (1976). Although many marine bacteria are not adversely affected by Tris buffer, the compound may prove toxic for some strains.

BM Medium (Basal Medium of Baumann and Baumann, 1981)

Tris HCl (pH 7.5) (see below)	15.8 g (100 mM)*
Ammonium chloride (NH_4Cl)	1 g (19 mM)
Potassium phosphate ($K_2HPO_4 \cdot 3H_2O$)	57 mg (0.33 mM)
Ferrous sulfate ($FeSO_4 \cdot 7H_2O$)	28 mg (0.1 mM)
Water	500 ml

Artificial seawater (MacLeod formula) 500 ml
(see "Isolation," this chapter)

Or use 7.9 grams for 50 mM (Baumann and Baumann, 1981)

BMA Medium (Basal Medium Agar of Baumann and Baumann, 1981)

Prepare 500 ml double-strength BM medium, add the carbon source (if it can withstand autoclaving), autoclave, and cool. If the carbon source is heat labile, filter-sterilize and add aseptically to cooled BM medium. Prepare 500 ml of a double-strength agar solution (40 g refined agar per liter), autoclave, and cool. Mix the BM plus carbon source with the agar solution and pour onto plates.

Isolation from Seawater (Baumann and Baumann, 1981)

It is essential that seawater be collected aseptically using sterile samplers. In general, this presents little difficulty when samples of surface waters are collected. A convenient way of obtaining seawater from different depths is by use of the Niskin butterfly sampler (General Oceanics, Miami, FL, USA). The collected samples can be used either for enrichment cultures or for direct isolation.

Direct Isolation Samples of seawater (5–300 ml) are filtered through 0.22- or 0.45-∝m nitrocellulose filters which are placed on petri plates containing either a complex medium (marine agar or a similar nonselective medium) or BMA medium with 0.1% of the carbon and energy source. The size of the filtered sample will depend on the source of the seawater and the composition of the medium used for direct isolation. After an incubation of 2–10 days at 25°C, colonies are picked and restreaked on homologous media. *V. nigripulchritudo* can be frequently obtained by direct isolation on plates containing BMA with 0.2% lactose, which will also often yield *Alteromonas macleodii*.

Many agar decomposers produced a broad but barely perceptible indentation in the agar surrounding the colony. Consequently, it is advisable to streak a pure culture on BMA medium without an added carbon and energy source and containing appropriate supplements for growth-factor-requiring organisms. Growth on this medium indicates that the isolate is able to utilize agar. Organisms in the marine environment apparently do not utilize Tris buffer as a carbon and energy source, although some organisms appear to utilize this compound as a poor nitrogen source.

Enrichment Cultures For aerobic enrichments, 500 ml of seawater is added to a sterile 2-liter Erlenmeyer flask containing 25 ml of 1 M Tris-HCl (pH 7.5), 0.5 g NH_4Cl, 38 mg $K_2HPO_4 \cdot 3H_2O$, 14 mg $FeSO_4 \cdot 7H_2O$, and 0.5–1.0 g of the carbon and energy source (acidic or basic carbon sources may require readjustment of the pH to 7.5). Depending on the source of the sample and the organic compound used to support growth, both the sample volume and the volume of the concentrated solution may be increased or decreased. The flasks are observed for signs of growth for up to 10 days, and then they are streaked on BMA medium containing 0.1% of the same carbon and energy source as was used in the enrichment. Enrichments containing 0.2% chitin, incubated aerobically for 5–10 days, often contain a blue-black sediment associated with the chitin particles. Such enrichments, when streaked on medium BMA containing 0.2% lactose, usually yield *V. nigripulchritudo*, a chitin-decomposer which forms colonies containing crystals of blue-black pigment (Fig. 10) (Baumann et al., 1971b). *V. alginolyticus* can be readily obtained from aerobic enrichments containing 0.5% yeast extract and 0.5% tryptone (instead of the single organic carbon and energy source). When streaked on marine agar or a similar medium, this organism swarms in a manner similar to that of *Proteus*. Single colonies can be obtained by streaking on either a complex medium containing 4% agar or on minimal medium such as BMA with 0.2% glycerol.

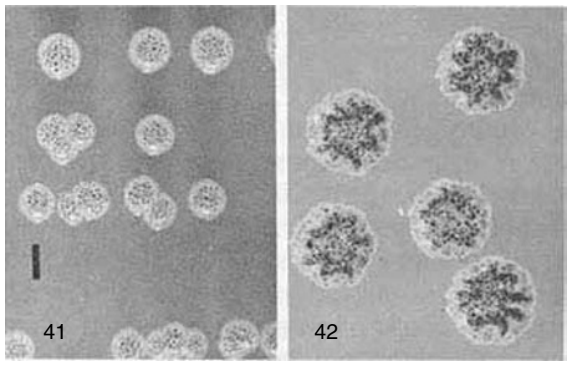

Fig. 10. Colonial morphology of *Vibrio nigripulchritudo*; note the granules of blue-black pigment embedded in the colonies. (From Baumann and Baumann, 1981.)

Isolation from Surfaces and Intestinal Contents of Fish Sterile cotton-tipped applicator sticks are used to swab the gills, mouth, and rectal region as well as other surfaces of the fish. An agar plate is then streaked to give isolated colonies. A 2–3 cm portion of the fish intestine is dissected and placed onto a sterile petri plate. Gentle pressure with sterile forceps or an applicator stick generally forces out some of the intestinal contents, which are streaked on plating medium. In both cases the plates are

incubated for 1–4 days and observed for colonies, which are then purified by streaking on homologous medium. Some marine strains swarm extensively on complex media making it impossible to isolate single colonies. Swarming can be prevented by raising the concentration of agar to 4%.

Isolation of Bioluminescent Bacteria (Baumann and Baumann, 1981)

These organisms are common in the marine environment. The detection of luminescence by a simple visual examination poses a number of problems since the intensity of the emitted light varies greatly with different isolates and is also affected by the cultivation medium as well as by the age of the cells. A satisfactory medium used for the observation of luminescence is LM medium (Baumann and Baumann, 1981). Strains are streaked on this medium and incubated at 15°C and 25°C with periodic examinations at 12–36 h after streaking. In many strains, luminescence is relatively dim and short-lived so that frequent observation (every 3 h) is suggested. It is important to have plates with isolated colonies since, in the case of many dim strains, only the isolated colonies luminesce. Examination for luminous colonies on plates containing LM medium should be done in complete darkness, and the eyes should be dark-adapted for at least 10 min. The picking of luminous colonies is greatly facilitated by the use of sterile toothpicks and a low-intensity light bulb (5–10 watts) connected to a rheostat (Cosenza and Buck, 1966). First, in total darkness, an area of luminescence is observed, and a sterile toothpick is positioned roughly over the site. The current is then switched on and the intensity of the light is gradually increased until the specimen is just barely visible. By fixing one's eyes on the luminous spot and gradually increasing the intensity of the light it becomes apparent which area contains the luminous organisms. The toothpick is quickly touched to this site; a plate of LM medium is inoculated; and the light is quickly turned off. By using this procedure, the investigator's eyes do not have to be repeatedly dark-adapted and, more importantly, it is possible to actually see the area from which the inoculum is picked.

LM Medium (Luminous Medium of Baumann and Baumann, 1981)

Tris HCl (pH 7.5)	7.9 g (50 mM)
Yeast extract	5 g
Tryptone	5 g
Calcium carbonate ($CaCO_3$)	1 g
Agar	20 g
Glycerol	3 g
BM medium	1 liter

Isolation from Seawater In some coastal waters, the concentration of luminous bacteria may be sufficient to allow detection in a 0.1 to 1-ml sample spread onto a plate of LM medium, TCBS medium, or marine agar. With larger volumes of seawater, aliquots of up to 300 ml may be filtered through 0.22 or 0.45-∝m nitrocellulose filters which are subsequently placed onto petri plates containing one or more of these media. Since crowded conditions tend to inhibit luminescence and since nonluminous bacteria greatly outnumber the luminous isolates, it is important that the filter contain a relatively sparse bacterial population. It is not recommended that the soft agar overlay method be used for the enumeration of luminous bacteria since brief exposure to 41°C (the temperature of the molten agar used for the overlay) may kill some strains of *Photobacterium phosphoreum* and *V. logei*.

Isolation from Surfaces and Intestinal Contents Fresh squid and octopus, which have been kept on ice in fish markets, often have luminous spots when examined in the dark; however, luminous spots are rare on fresh fish. The isolation of luminous organisms from such specimens, from the surfaces of marine animals, as well as from the intestinal contents of fish, is done as described above. In general, the intestinal contents of fish have either a relatively large population of luminous bacteria or none (Baumann and Baumann, 1981).

Isolation by Enrichment This procedure is given in Baumann and Baumann (1981) and based on some suggestions of M. Doudoroff. In some cases, visible regions of luminescence can be obtained on fresh fish, squid, or octopus by half-submerging the specimen in a shallow layer of artificial seawater and incubating for 10–18 h at 12–15°C. The luminous sites are touched with sterile toothpicks and inoculated onto plates of LM medium or other media as previously described. This method is relatively specific for *P. phosphoreum*.

Stock Cultures and Methods for Preservation

In the Vibrio Laboratory at CDC, a "permanent frozen stock" is made by suspending growth from a blood agar or marine agar plate in skim milk (10%) which is transferred to a plastic vial and frozen and stored at –70°C. A "working stock" is also prepared. *V. cholerae*, *V. mimicus*, and other nonhalophilic species are grown in screw-cap tubes of working-stock media; halophilic vibrios are grown in marine semisolid medium (marine broth with 0.3% agar added). After the strain has grown well (usually 24 h) a

thin layer of sterile mineral oil is added to cover the top of the agar column to prevent evaporation and drying. We do not transfer these working cultures. The occasional strain that dies is replaced from the permanent frozen stock. Tubes are stored in divided boxes at room temperature in the dark. Do not store stock cultures in the refrigerator because this kills many strains. The only exception is for strains that do not grow well above 20°C; they are stored in the refrigerator. Some isolates of *Vibrio* acquire nutritional requirements after prolonged cultivation and frequent transfer on marine agar. Baumann and Baumann (1981) suggest maintaining strains on medium BMA containing 0.2% glycerol or freeze-drying them.

Most of the strains of *Vibrio* can be freeze-dried and kept at 4°C. With a few exceptions, viable cells could be recovered after 3–5 years of storage. For the preparation of lyophils, the growth from a fresh slant is suspended in about 0.5 ml of a sterile solution consisting of one-quarter-strength artificial sea water #1 (MacLeod's formula), 5 g/liter yeast extract, and 5 g/liter peptone (adjusted to pH 7.5), and transferred into a lyophil tube which is subsequently placed in a mixture of dry ice and acetone and placed under vacuum for 10–12 hrs. The lyophils are reconstituted by suspending the powder in about 0.5 ml of an optimum growth medium and inoculating a solid medium. Growth is generally observed after 1–2 days of incubation at 25°C. For *P. phosphoreum*, a lower temperature (15–18°C) should be used.

Identification—Clinical Isolates

Because of the varying pathogenicity and clinical significance of the different species, *Vibrio* strains isolated from clinical specimens should be identified to species. There are many different approaches to identification (for a review, see Farmer et al., 1981). One common question always needs an immediate answer: "Is the *Vibrio*-like organism isolated from this particular case of cholera-like diarrhea the pandemic strain of *V. cholerae*?" (Fig. 6).

Ruling Out *V. Cholerae* Serogroup O1

It is often important to give a definitive answer to this question as quickly as possible. Numerous sucrose-positive (yellow colonies) on TCBS agar from a patient with rice-water stools or severe watery diarrhea warns of the possible presence of *V. cholerae* serogroup O1 (Fig. 11). Suspicious colonies are subcultured early in the day by heavy inoculation on a nonselective medium such as blood agar or trypticase soy agar which does not contain carbohydrates. After 5–8 h, good growth should be present which can be tested in polyvalent antisera to *V. cholerae* serogroup O1 (Fig. 6). Experienced workers use growth from the primary TCBS plate to do the agglutination. Positive agglutination is presumptive evidence of *V. cholerae* serogroup O1, and the result should be reported immediately to the physician. Biochemical testing is necessary to confirm the identification as *V. cholerae*. Local health authorities should be notified immediately, and the culture, along with information about the case, should be forwarded to the state health laboratory.

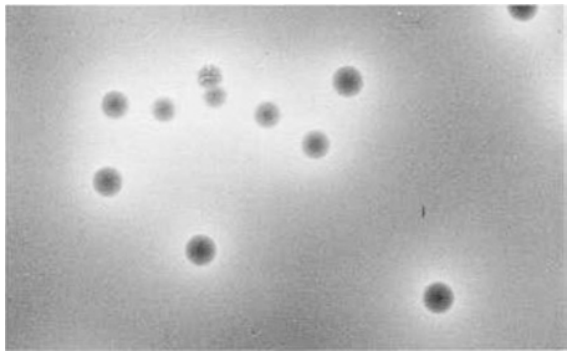

Fig. 11. Colonies of *Vibrio cholerae* on TCBS agar; yellow colonies of 2- to 3-mm diameter are present after overnight incubation at 36°C.

Direct Testing of Liquid Stools for *V. cholerae* O1 Jesudason et al. (1984) in India showed that there was enough O antigen in rice-water stools of cholera patients to agglutinate a reagent consisting of *Staphylococcus aureus* cells coated with antibodies to the O1 antigen. Shaffer et al. (1989) used a commercial reagent (monoclonal antibodies to serofactor "a" of the O1 antigen coated onto latex particles) for the laboratory diagnosis of cholera in the field. If sufficient O antigen is present in the liquid stool, it will cause the latex to clump (Fig. 12); this is a presumptive positive for *V. cholerae* O1. This method is particularly suited for field testing (Fig. 13) in developing countries where laboratory facilities for bacteriological analysis are usually unavailable. False negatives and false positives can occur with these two methods, so a positive culture is always needed for the definitive diagnosis of cholera.

Biochemical Identification

Effect of Media, Reagents, and Na^+ Concentration Most laboratories identify *Vibrio* strains with media and tests designed to identify Enterobacteriaceae. These work well for *V. cholerae* and *V. mimicus* because they have only a low requirement for Na^+, which is fulfilled

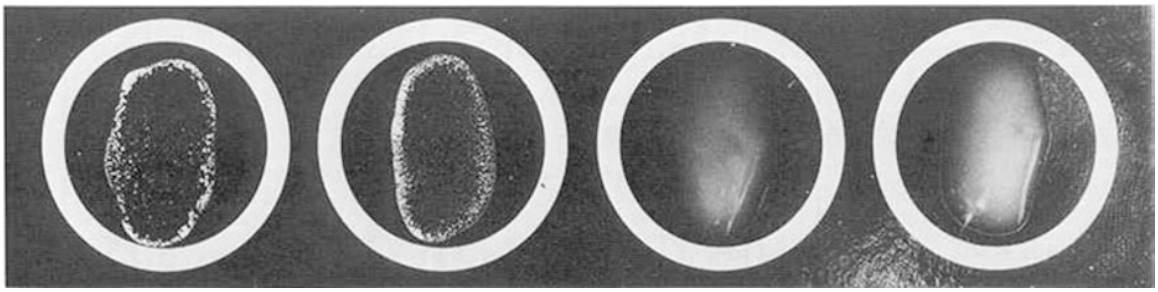

Fig. 12. Identification of *Vibrio cholerae* serotype O1 with a commercial latex reagent; two strong positive reactions are on the left, and two negative ones on the right.

Fig. 13. Laboratory diagnosis of cholera in the field using a commercial latex reagent.

by the amount of NaCl in the medium. However, most of the halophilic vibrios require much more Na^+ for growth and expression of various metabolic pathways. Some of the media for differential biochemical tests do not contain enough NaCl for these halophilic species. In Table 1, the standard enteric test is listed first and the percentage positive for each species is given. The percentage positive for each species is then given for a modified medium with 1% NaCl added. The table also shows that more *Vibrio* species are indole positive when the medium is changed from peptone water to heart infusion broth. More strains are Voges-Proskauer positive when the reagent for detecting acetylmethylcarbinol contains alpha-naphthol (Berrit method). This table illustrates the pronounced effect of media and methods on the results of biochemical tests.

Screening and Tests and Complete Identification Table 6 gives the tests that are most helpful in dividing the 12 species of *Vibrio* that are found in clinical specimens into 6 groups; Table 7 gives the complete biochemical reactions for these 12 species. The vast majority of *Vibrio* cultures isolated from clinical specimens will be easily identified as one of the 12 species listed. Growth in the absence of added Na^+ (growth in nutrient broth with 1% NaCl, but no growth in nutrient broth) is the essential test for differentiating *V. cholerae* and *V. mimicus* from the other 10 species of *Vibrio* and from the marine vibrios. *V. metschnikovii* is easily differentiated because it is oxidase negative and nitrate negative. *V. cincinnatiensis* is easily differentiated because it is inositol positive. *V. hollisae* is negative for arginine dihydrolase and for lysine and ornithine decarboxylases (triple decarboxylase negative), which differentiates it from the remaining *Vibrio* species (Table 6). The remaining seven species are subdivided into the "arginine-dihydrolase-positive group," which includes *V. damsela*, *V. fluvialis*, and *V. furnissii*; and into the "lysine-decarboxylase-positive group," which includes *V. alginolyticus*, *V. parahaemolyticus*, *V. vulnificus*, and *V. carchariae* (Table 6). The tests that are most useful for further differentiating the 12 clinical species are given in Tables 7 to 14. Several of these media and tests are specialized for vibrios and will be described in more detail later.

Computer Identification We have developed several computer programs that have proved useful in the routine identification of *Vibrio* and *Photobacterium* isolates. The programs are based on mathematical analysis of the results of the

Table 6. Key differential tests to divide the 12 *Vibrio* species that are found in clinical specimens into six groups.[a]

	Reactions of the species in:[b]											
	Group 1		Group 2		Group 3		Group 4		Group 5		Group 6	
Test	*V. cholerae*	*V. mimicus*	*V. metschnikovii*	*V. cincinnatiensis*	*V. hollisae*	*V. damsela*	*V. fluvialis*	*V. furnissii*	*V. alginolyticus*	*V. parahaemolyticus*	*V. vulnificus*	*V. carchariae*
Growth in nutrient broth:												
With no NaCl added	+	+	−	−	−	−	−	−	−	−	−	−
With 1% NaCl added	+	+	+	+	+	+	+	+	+	+	+	+
Oxidase			−	+	+	+	+	+	+	+	+	+
Nitrate→Nitrite			−	+	+	+	+	+	+	+	+	+
myo-Inositol fermentation	−	−	V	+	−	−	−	−	−	−	−	−
Arginine dihydrolase					−	+	+	+	−	−	−	−
Lysine decarboxylase					−				+	+	+	+
Ornithine decarboxylase					−							

[a]The boxes indicate the key test results. All data are for reactions within 2 days at 35–37°C, unless otherwise specified.

[b]Symbols: +, most strains (generally about 90 to 100%) positive; V, strain-to-strain variation (generally about 25 to 75% positive); −, most strains negative (generally about 0 to 10% positive).

Table 7. Biochemical test results and other properties of the 12 *Vibrio* species that are found in clinical specimens.

	Percentage positive for:[b]											
Test[a]	*V. cholerae*	*V. mimicus*	*V. metschnikovii*	*V. cincinnatiensis*	*V. hollisae*	*V. damsela*	*V. fluvialis*	*V. furnissii*	*V. alginolyticus*	*V. parahaemolyticus*	*V. vulnificus*	*V. carchariae*
*Indole production (HIB, 1% NaCl)	99	98	20	8	97	0	13	11	85	98	97	100
Methyl red (1% NaCl)	99	99	96	93	0	100	96	100	75	80	80	100
*Voges-Proskauer (1% NaCl; Barritt)	75	9	96	0	0	95	0	0	95	0	0	50
Citrate, Simmons	97	99	75	21	0	0	93	100	1	3	75	0
H_2S on TSI	0	0	0	0	0	0	0	0	0	0	0	0
Urea hydrolysis	0	1	0	0	0	0	0	0	0	15	1	0
Phenylalanine deaminase	0	0	0	0	0	0	0	0	1	1	35	NG
*Arginine, Moeller's (1% NaCl)	0	0	60	0	0	95	93	100	0	0	0	0
*Lysine, Moeller's (1% NaCl)	99	100	35	57	0	50	0	0	99	100	99	100
*Ornithine, Moeller's (1% NaCl)	99	99	0	0	0	0	0	0	50	95	55	0
Motility (36°C)	99	98	74	86	0	25	70	89	99	99	99	0
Gelatin hydrolysis (1% NaCl, 22°C)	90	65	65	0	0	6	85	86	90	95	75	0
KCN test (percentage that grow)	10	2	0	0	0	5	65	89	15	20	1	0
Malonate utilization	1	0	0	0	0	0	0	11	0	0	0	0
*D-Glucose, acid production	100	100	100	100	100	100	100	100	100	100	100	50
*D-Glucose, gas production	0	0	0	0	0	10	0	100	0	0	0	0
Acid production from:												
D-Adonitol	0	0	0	0	0	0	0	0	1	0	0	0
*L-Arabinose	0	1	0	100	97	0	93	100	1	80	0	0
*D-Arabitol	0	0	0	0	0	0	65	89	0	0	0	0
*Cellobiose	8	0	9	100	0	0	30	11	3	5	99	50
Dulcitol	0	0	0	0	0	0	0	0	0	3	0	0
Erythritol	0	0	0	0	0	0	0	0	0	0	0	0
D-Galactose	90	82	45	100	100	90	96	100	20	92	96	0
Glycerol	30	13	100	100	0	0	7	55	80	50	1	0
myo-Inositol	0	0	40	100	0	0	0	0	0	0	0	0
*Lactose	7	21	50	0	0	0	3	0	0	1	85	0
*Maltose	99	99	100	100	0	100	100	100	100	99	100	100
*D-Mannitol	99	99	96	100	0	0	97	100	100	100	45	50
D-Mannose	78	99	100	100	100	100	100	100	99	100	98	50
Melibiose	1	0	0	7	0	0	3	11	1	1	40	0
α-Methyl-D-glucoside	0	0	25	57	0	5	0	0	1	0	0	0

Raffinose	0	0	0	0	0	0	0	11	0	0	0	0
L-Rhamnose	0	0	0	0	0	0	0	45	0	1	0	0
*Salicin	1	0	9	100	0	0	0	0	4	1	95	0
D-Sorbitol	1	0	45	0	0	0	3	0	1	1	0	0
*Sucrose	100	0	100	100	0	5	100	100	99	1	15	50
Trehalose	99	94	100	100	0	86	100	100	100	99	100	50
D-Xylose	0	0	0	43	0	0	0	0	0	0	0	0
Mucate-acid production	1	0	0	0	0	0	0	0	0	0	0	0
Tratrate-Jordan	75	12	35	0	65	0	35	22	95	93	84	50
Esculin hydrolysis	0	0	60	0	0	0	8	0	3	1	40	0
Acetate utilization	92	78	25	14	0	0	70	65	0	1	7	0
*Nitrate→nitrite	99	100	0	100	100	100	100	100	100	100	100	100
*Oxidase	100	100	0	100	100	95	100	100	100	100	100	100
DNase (25°C)	93	55	50	79	0	75	100	100	95	92	50	100
*Lipase (corn oil)	92	17	100	36	0	0	90	89	85	90	92	0
*ONPG Test	94	90	50	86	0	0	40	35	0	5	75	0
Yellow pigment at 25°C	0	0	0	0	0	0	0	0	0	0	0	0
Tyrosine clearing	13	30	5	0	3	0	65	45	70	77	75	0
Growth in nutrient broth:												
*0% NaCl	100	100	0	0	0	0	0	0	0	0	0	0
*1% NaCl	100	100	100	100	99	100	99	99	99	100	99	100
6% NaCl	53	49	78	100	83	95	96	100	100	99	65	100
*8% NaCl	1	0	44	62	0	0	71	78	94	80	0	0
*10% NaCl	0	0	4	0	0	0	4	0	69	2	0	0
12% NaCl	0	0	0	0	0	0	0	0	17	1	0	0
Swarming (marine agar, 25°C)[c]	–	–	–	+	–	–	–	–	+	+	–	100
String test	100	100	100	80	100	80	100	100	91	64	100	100
O129, zone of inhibition[d]	99	95	90	25	40	90	31	0	19	20	98	100
Polymyxin B, % with a zone of inhibition	22	88	100	92	100	85	100	89	63	54	3	100

[a]An * indicates that the test is recommended as part of the routine set for *Vibrio* identification. 1% NaCl in parentheses indicates 1% NaCl has been added to the standard media to enhance growth; HIB, heart infusion broth; TSI, triple sugar iron agar; ONPG, *o*-nitrophenyl-β-D-galactopyranoside.

[b]The number gives the percentage positive after 48 h of incubation at 36°C (unless other conditions are indicated). Most of the positive reactions occur during the first 24 hours. NG (no growth) means that the organism does not grow, probably because the NaCl concentration is too low.

[c]Symbols: +, most strains (generally about 90 to 100%) positive; –, most strains negative (generally about 0 to 10% positive).

Content of the disk was 10 ∝g.

Table 8. Key characteristics of *Vibrio cholerae* and *V. mimicus* and tests for their differentiation.[a]

Test or property	*V. cholerae*	*V. mimicus*
Frequency of isolation	Very common	Occasional
Properties of both species:		
Oxidase	+	+
Growth in nutrient broth with:		
No added NaCl	+	+
1% NaCl	+	+
Lysine decarboxylase	+	+
Arginine dihydrolase	–	–
Ornithine decarboxylase	+	+
Differentiation of the species:		
Sucrose fermentation	99[b]	0
Lipase (corn oil)	95	10
Voges-Proskauer	95[c]	0
Lactose fermentation (1–2 days)	9	74
Lactose fermentation (3–7 days)	24	56

[a]All data are for reactions within 2 days at 35–37°C unless otherwise specified. Symbols: +, 90 to 100% positive; –, 0 to 10% positive.
[b]Each number gives the percentage of positive reactions after 24 to 48 h of incubation at 35–37°C (unless another temperature or time is indicated). Most of the positive reactions occur during the first 24 h of incubation.
[c]Almost all strains of *V. cholerae* non-O1 and most strains of *V. cholerae* O1 currently isolated are of the eltor biogroup, which is almost always Voges-Proskauer positive; the classical biogroup is Voges-Proskauer negative.

Table 9. Differentiation and properties of the classical and eltor biogroups of *V. cholerae* serogroup O1.[a]

	Biogroup	
Test or property	Classical	Eltor
Frequency of isolation:		
On the Indian subcontinent	Occasional[b]	Common
In the rest of the world	Very rare	Common
Differential test:		
Hemolysis of red blood cells	–	+
Voges-Proskauer	–	+
Inhibition by polymyxin B (50-unit disk)	+	–
Agglutination of chicken red blood cells	–	+
Lysis by bacteriophage:		
Classical IV	+	–
FK	+	–
Eltor 5	–	+

[a]Symbols: +, most strains (generally about 90 to 100%) positive; –, most strains negative (generally about 0 to 10% positive).
[b]The classical biotype reappeared several years ago on the Indian subcontinent and has been found only in some locations.

Table 10. Antigenic serofactors of *Vibrio cholerae* O1: subtypes Ogawa, Inaba, and Hikojima.

Serotype O1 subtype	O factors present in culture	Agglutination in absorbed serum:	
		Ogawa[a]	Inaba[a]
Ogawa	A, B	+	–
Inaba	A, C	–	+
Hikojima[b]	A, B, C	+	+

[a]The specific factor sera are prepared by absorption. For example, an Ogawa antiserum is prepared by injecting an Ogawa culture, and then absorbing the resulting antiserum with an Inaba culture, which removes the antibodies to O antigen factor A, leaving antibodies to O factor B.
[b]Some authorities do not recognize subtype Hikojima, and report these cultures as either Inaba or Ogawa, based on which serum causes the quickest and strongest agglutination.

Table 11. Subdivision of *Vibrio cholerae* below the level of species.

Vibrio cholerae serogroup O1[a]
- Serogroup Inaba
  - Biogroup classical
  - Biogroup eltor
- Serogroup Ogawa
  - Biogroup classical
  - Biogroup eltor

Vibrio cholerae non-O1
- Sakazaki serotyping system: 60 O-antigen groups
- Smith serotyping system: 72 serogroups

[a]A third serogroup called "Hikojima" is recognized by some authors. See note on Table 10.

Table 12. Differentiation of the arginine-positive species—*Vibrio damsela*, *V. fluvialis*, and *V. furnissii*; comparison with *Aeromonas*.[a]

Test or property	*V. damsela*	*V. fluvialis*	*V. furnissii*	*Aeromonas*
Growth in nutrient broth with:				
No added NaCl	–	–	–	+
1% NaCl	+	+	+	+
6% NaCl	+	+	+	–
Voges-Proskauer	+	–	–	V
Citrate, Simmons	–	+	+	V
Fermentation of:				
D-Galacturonic acid	–	+	+	–
L-Arabinose	–	+	+	V
D-Mannitol	–	+	+	+
Sucrose	–	+	+	(+)
Gas production during fermentation	V	–	+	V

[a]All data are for reactions within 2 days at 35–37°C unless otherwise specified. Symbols: +, 90 to 100% positive; (+), 75 to 89.9% positive; V, 25.1 to 74.9% positive; –, 0 to 10% positive.

Table 13. Differentiation of *Vibrio fluvialis* and *V. furnissii*.

	Percentage positive for[a]	
Test or property	*V. fluvialis*	*V. furnissii*
Simple tests		
Gas production during fermentation	0	99
Esculin hydrolysis	72	0
Carbon-source utilization,[b] growth on:		
Citrulline	97	4
D-Glucuronic acid	94	7
Putrescine	31	100
δ-Aminovalerate	0	63
Cellobiose	63	4
Glutaric acid	–	+

[a]Each number gives the percentage positive after 48 h of incubation at 35–37°C (unless other conditions are indicated). Most of the positive reactions occur during the first 24 hours.
[b]Data from Lee et al. 1981 except for glutaric acid, which is from Baumann et al. 1984. These carbon-source utilization tests are usually done in research laboratories rather than in public health or clinical laboratories.

Table 14. Differentiation of the arginine-negative, lysine-positive species *V. alginolyticus*, *V. parahaemolyticus*, *V. vulnificus*, and *V. carchariae*.[a]

Test or property	*V. alginolyticus*	*V. parahaemolyticus*	*V. vulnificus*	*V. carchariae*
Voges-Proskauer	+	–	–	–
Growth in nutrient broth with:				
8% NaCl	+	(+)	–	–
10% NaCl	v	–	–	–
Fermentation of:				
Sucrose	+	–	(–)	+
Salicin	–	–	+	–
Cellobiose	–	–	+	–
Lactose	–	–	(+)	–
L-Arabinose	–	(+)	–	–
Swarming (marine agar, 25°C)	+	+	–	+
Size of zone of inhibition around:				
Colistin	Large	Large	Small	Small
Ampicillin	Small	Small	Large	Small
Carbenicillin	Small	Small	Large	Small

[a]All data are for reactions within 2 days at 35–37°C unless otherwise specified. Symbols: +, 90 to 100% positive; (+), 75 to 89.9% positive; (–), 10.1 to 25% positive; –, 0 to 10% positive.

simple biochemical tests given in Table 15. The biochemical test media are inoculated, read at days 1 and 2 (and up to seven days if desired). Each test result is coded as "+" or "–" for the computer, which then compares the profile of the unknown strain to all the organisms in the data base. The approach is based on the normalized likelihood calculation described by Lapage 1974. Two different programs are used: the first, named "George," is for all species of Enterobacteriaceae and for the species of *Vibrio* that grow well at 35–37°C in media with no added salt (0.5–1.0% NaCl). A second program named "Neptune" was designed specifically to identify the halophilic species of *Vibrio* and *Photobacterium*. The biochemical tests used in this program have marine cations added (Table 15) to give a higher content of Na^+, K^+, and Mg^{++}, and all incubations are done at 25°C since many of the species do not grow well at 35–37°C. Both programs are useful in comparing an unknown to a large data base of species, biogroups, and individual strains. The programs originally were run on a mainframe computer, but we have modified them to run on IBM-compatible microcomputers with DOS. They may be obtained by writing J. J. Farmer.

Bioluminescence Test

This is a useful differential test because a few species are usually bioluminescent (*V. fischeri*, *V. logei*, *V. orientalis*, *V. splendidus* biogroup 1, *Photobacterium phosphorium*, and *P. leiognathi*), in contrast to most of the other species. Some strains of *V. harveyi* are luminescent, as are a scant few strains of *V. cholerae*. None of the members of the Enterobacteriaceae (with the lone exception of *Xenorhabdus luminescens*) and Pasteurellaceae, or of the genera *Aeromonas* and *Plesiomonas* are bioluminescent.

Bioluminescence Test for Vibrios

Inoculate the test strain on a medium or several media that allow good growth, such as marine agar, commercially available *Photobacterium* agar, LM medium, or trypticase soy agar (for organisms that do not require Na^+), and incubate at 25°C. Also inoculate quality-control strains which are "strong" and "weak" for bioluminescence. After incubation for about 8 h and again at 16–20 h, take the plates into a room that can be totally darkened and set them on a bench top. Take in a flashlight, and turn off the overhead light. Observe the weak and strong quality-control cultures for light. Light from the strong positive culture should be visible within a few seconds, but the weak positive culture may take several minutes. Continue to observe the test culture for luminescence and record as positive or negative after 5 and again at 10 minutes. For quality control, include a strong positive such as *Vibrio fischeri* (or a similar reference culture or marine isolate); also include a weak positive such as *Xenorhabdus luminescens* or a weakly luminescent marine strain that takes 3–5 min of dark adaptation to be seen. Test as many cultures as possible for bioluminescence at one time because this test is time consuming to set up. A photographic dark room is a good place to do the observation for light emission.

Carbon Assimilation Tests (Baumann and Baumann, 1981)

The medium described below which is used for assimilation tests was described by McLeod (1968) to study the growth of marine bacteria on different substrates, and was subsequently used by Baumann and Baumann (1981). The carbon sources are added to the basal medium at a final concentration of 0.1 to 1% as described in "Isolation—Marine and Environmental Samples."

Marine Broth

Marine broth is useful for the isolation and cultivation of marine organisms. A dehydrated powder is available from Difco (catalog no. 0791). It uses the following recipe:

Peptone (Bacto)	5 g
Yeast extract	1 g
Ferric citrate	0.1 g
Sodium chloride	19.45 g
Magnesium chloride (dried)	5.9 g
Sodium sulfate	3.24 g
Calcium chloride	1.8 g
Potassium chloride	0.55 g
Sodium bicarbonate	160 mg
Potassium bromide	80 mg
Strontium chloride	34 mg
Boric acid	22 mg
Sodium silicate	4 mg
Sodium fluoride	2.4 mg
Ammonium nitrate	1.6 mg
Disodium phosphate	8 mg
Distilled water	1 liter

Adjust to pH 7.6.

Add 37.4 g of Difco marine broth to the distilled water and heat to boiling. Dispense into tubes or bottles and autoclave at 121°C for 15 minutes. A brown gelatinous precipitate is produced during autoclaving which settles to the bottom.

Marine Semisolid Medium

This is a convenient semisolid medium for maintaining stock cultures of *Vibrio*, *Photobacterium*, or other marine organisms that require added Na^+ and other ions for growth.

Marine broth (Difco 0791)	37.4 g
Agar	4 g
Distilled Water	1 liter

Add the marine broth to the water. Add 4 g of agar, and heat to boiling. Dispense 6 ml into 13 · 100 mm screw cap tubes. Tighten the caps and autoclave at 121°C for 15 minutes.

Marine Cations Supplement 1558

This medium is useful for increasing the salt content of bacteriological media to enhance the growth of marine bacteria. It contains Na^+, K^+, Mg^{++}, and Ca^{++} at 10 times

Table 15. Biochemical reactions of the species of the genera *Vibrio* and *Photobacterium*.[a]

Organism	Oxidase (Kovacs)	Nitrate→nitrite	Indole production	Voges-Proskauer	Lysine decarboxylase (2-day)	Arginine dihydrolase (2-day)	Ornithine decarboxylase (2-day)	Lysine decarboxylase (7-day)	Arginine dihydrolase (7-day)	Ornithine decarboxylase (7-day)	Motility (25°C)	D-Glucose-acid	D-Glucose-gas	Lactose fermentation	Sucrose fermentation	D-Mannitol fermentation	Dulcitol fermentation	Salicin fermentation	Adonitol fermentation	myo-Inositol fermentation	D-Sorbitol fermentation	L-Arabinose fermentation	Raffinose fermentation	L-Rhamnose fermentation	Maltose fermentation
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
Vibrio—clinical species																									
V. alginolyticus	+	+	(+)	+	+	−	V	+	−	V	+	+	−	−	+	+	−	−	−	−	−	−	−	−	+
V. carchariae	+	+	+	−	+	−	+	+	−	+	+	+	−	−	+	+	−	−	−	−	−	−	−	−	+
V. cholerae	+	+	+	+	+	−	+	+	−	+	+	+	−	−	+	+	−	−	−	−	−	−	−	−	+
V. cincinnatiensis	+	+	−	−	(+)	−	−	+	−	−	+	+	−	−	+	+	−	+	−	+	−	+	−	−	+
V. damsela	(+)	+	−	+	V	+	−	V	+	−	+	+	V	−	(−)	−	−	−	−	−	−	−	−	−	+
V. fluvialis	+	+	(+)	−	−	+	−	−	+	−	+	+	−	−	+	+	−	(+)	−	−	−	+	−	−	+
V. furnissii	+	+	−	−	−	+	−	−	+	−	+	+	+	−	+	+	−	−	−	−	−	+	−	−	+
V. hollisae	+	+	+	−	−	−	−	−	−	−	+	+	−	−	−	−	−	−	−	−	−	+	−	−	−
V. metschnikovii	−	−	−	+	+	V	−	+	+	−	V	+	−	(−)	+	(+)	−	(−)	−	V	V	−	−	−	+
V. mimicus	+	+	+	−	+	−	+	+	−	+	+	+	−	−	−	(+)	−	−	−	−	−	−	−	−	+
V. parahaemolyticus	+	+	+	−	+	−	+	+	−	+	+	+	−	−	−	(+)	−	−	.	−	−	+	−	−	+
V. vulnificus	+	+	+	−	+	−	+	+	−	+	+	+	−	−	(−)	(+)	−	−	−	−	−	−	−	−	+
Vibrio—nonclinical																									
V. aestuarianus	+	+	+	−	V	+	−	(+)	+	−	+	+	−	(+)	+	+	−	−	−	−	−	−	−	−	+
V. anguillarum	+	+	(+)	+	−	(+)	−	−	+	−	+	+	−	−	+	+	−	−	−	−	+	(+)	−	−	+
V. campbellii	+	+	(+)	−	+	−	(−)	+	−	(−)	+	+	−	−	−	V	−	−	−	−	−	−	−	−	+
V. costicola	+	V	−	(+)	−	V	−	−	V	−	(+)	+	−	−	V	V	−	−	−	−	−	−	−	−	(+)
V. diazotrophicus	+	+	+	−	−	−	−	−	+	−	+	+	−	V	+	+	−	+	−	−	−	+	−	−	+
V. fischeri	+	(+)	−	−	V	−	−	+	−	−	+	(+)	−	(−)	−	V	−	(+)	−	−	−	−	−	−	+
V. gazogenes	−	−	−	V	−	−	−	−	−	−	+	+	+	(+)	+	+	−	+	−	−	V	+	−	−	+
V. harveyi	+	+	+	−	+	−	(+)	+	−	+	+	+	−	−	+	+	−	−	−	−	−	−	−	−	+
V. logei (15°C)	+	+	−	−	+	−	V	+	−	(+)	+	+	−	(−)	(−)	(+)	−	−	−	−	−	−	−	−	+

Organism	D-Xylose fermentation	Trehalose fermentation	Cellabiose fermentation	α-Methyl-D-glucoside fermentation	Erythritol fermentation	Melibiose fermentation	D-Arabitol fermentation	D-Mannose fermentation	D-Galactose fermentation	D-Galacturanate fermentation	Growth: 0% NaCl	Growth: 0.1% NaCl	Growth: 0.2% NaCl	Growth: 0.3% NaCl	Growth: 0.4% NaCl	Growth: 0.5% NaCl	Growth: 1% NaCl	Growth: 2% NaCl	Growth: 3.5% NaCl	Growth: 6% NaCl	Growth: 8% NaCl	Growth: 10% NaCl	Growth: 12% NaCl	Swarming
	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49
Vibrio—clinical species																								
V. alginolyticus	−	+	+	−	−	(−)	−	+	(−)	−	−	−	−	−	(+)	(+)	+	+	+	+	+	+	+	+
V. carchariae	−	−	+	−	−	−	−	+	+	−	−	−	−	−	−	+	+	+	+	+	+	−	−	+
V. cholerae	−	(+)	−	−	−	−	−	(+)	V	−	+	+	+	+	+	+	+	+	+	(−)	−	−	−	−
V. cincinnatiensis	V	+	+	V	−	−	−	+	(+)	−	−	+	+	+	+	+	+	+	+	+	+	V	−	−
V. damsela	−	V	+	(−)	−	−	−	+	+	−	−	−	−	V	(+)	(+)	+	+	+	+	V	−	−	−
V. fluvialis	−	+	(−)	−	−	−	(+)	(+)	+	+	+	+	+	+	+	+	+	+	+	+	(+)	(+)	−	−
V. furnissii	−	+	−	−	−	−	−	+	(+)	+	+	+	+	+	+	+	+	+	+	+	+	+	(−)	−
V. hollisae	−	−	−	−	−	−	−	+	+	−	−	−	−	−	(−)	+	+	+	+	+	+	(−)	−	−
V. metschnikovii	−	+	−	−	−	−	−	+	V	−	−	(+)	+	+	+	+	+	+	+	+	+	V	−	−
V. mimicus	−	+	−	−	−	−	−	−	+	V	+	+	+	+	+	+	+	+	+	(+)	−	−	−	−
V. parahaemolyticus	−	+	−	−	−	−	−	+	+	−	−	−	−	−	(+)	(+)	+	+	+	+	+	−	−	(+)
V. vulnificus	−	+	+	−	−	−	−	+	+	−	−	V	V	(+)	+	+	+	+	+	+	−	−	−	−
Vibrio—nonclinical																								
V. aestuarianus	−	+	+	−	−	(−)	(−)	+	+	−	−	−	−	+	+	+	+	+	+	+	V	−	−	−
V. anguillarum	−	+	(+)	−	−	−	−	+	V	−	−	−	+	+	+	+	+	+	+	+	−	−	−	−
V. campbellii	−	+	+	−	−	−	−	+	−	−	−	−	−	−	−	+	+	+	+	+	V	−	−	−
V. costicola	−	(+)	(−)	V	−	−	−	(+)	(−)	−	−	−	−	−	−	−	V	(+)	+	+	+	(+)	V	−
V. diazotrophicus	+	+	+	−	−	−	−	(+)	+	+	−	+	+	+	+	+	+	+	+	+	+	V	−	−
V. fischeri	−	−	+	−	−	(+)	−	+	+	−	−	−	−	−	−	−	(+)	+	+	(+)	−	−	−	−
V. gazogenes	+	+	+	−	−	V	−	+	(+)	−	−	−	−	−	−	(−)	(+)	+	+	+	+	+	V	−
V. harveyi	−	+	+	−	−	V	−	+	+	−	−	−	−	−	(−)	(+)	+	+	+	+	(+)	−	−	V
V. logei (15°C)	−	V	+	−	−	−	−	+	+	−	−	−	−	−	−	−	−	+	+	−	−	−	−	−

Table 15. *Continued*

Organism	Oxidase (Kovacs)	Nitrate→nitrite	Indole production	Voges-Proskauer	Lysine decarboxylase (2-day)	Arginine dihydrolase (2-day)	Ornithine decarboxylase (2-day)	Lysine decarboxylase (7-day)	Arginine dihydrolase (7-day)	Ornithine decarboxylase (7-day)	Motility (25°C)	D-Glucose-acid	D-Glucose-gas	Lactose fermentation	Sucrose fermentation	D-Mannitol fermentation	Dulcitol fermentation	Salicin fermentation	Adonitol fermentation	myo-Inositol fermentation	D-Sorbitol fermentation	L-Arabinose fermentation	Raffinose fermentation	L-Rhamnose fermentation	Maltose fermentation	D-Xylose fermentation	Trehalose fermentation	Cellabiose fermentation	α-Methyl-D-glucoside fermentation	Erythritol fermentation	Melibiose fermentation	D-Arabitol fermentation	D-Mannose fermentation	D-Galactose fermentation	D-Galacturanate fermentation	Growth: 0% NaCl	Growth: 0.1% NaCl	Growth: 0.2% NaCl	Growth: 0.3% NaCl	Growth: 0.4% NaCl	Growth: 0.5% NaCl	Growth: 1% NaCl	Growth: 2% NaCl	Growth: 3.5% NaCl	Growth: 6% NaCl	Growth: 8% NaCl	Growth: 10% NaCl	Growth: 12% NaCl	Swarming
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49
V. mediterranei	+	+	+	–	V	V	–	V	+	–	+	+	–	–	+	+	–	–	–	–	+	–	–	–	+	–	+	+	–	–	V	–	+	+	–	–	–	–	–	V	V	+	+	+	+	V	–	–	+
V. marinus (15°C)	+	+	–	–	–	–	–	+	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–
V. natriegens	+	+	–	–	–	–	–	–	–	–	+	+	–	–	+	+	–	+	–	–	–	+	(+)	+	+	–	+	(+)	(+)	–	(+)	+	(+)	V	–	–	–	–	–	–	V	+	+	+	+	+	–	–	–
V. nereis	+	+	V	–	–	(+)	–	–	+	–	+	+	–	–	+	–	–	–	–	–	–	–	–	–	+	–	+	–	–	–	–	–	+	–	–	–	–	–	–	–	–	+	+	+	+	+	(–)	–	–
V. nigripulchritudo	+	+	+	–	–	–	–	–	–	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	+	–	+	+	–	–	–	–	V	V	–	–	–	–	–	–	–	+	+	+	+	–	–	–	–
V. ordalii	+	V	–	–	–	–	–	–	–	–	+	+	–	–	+	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	(+)	+	+	+	+	+	V	–	–	–	–
V. orientalis	+	+	+	–	+	–	–	+	V	–	+	+	–	–	+	+	–	–	–	–	–	–	–	–	+	–	+	+	–	–	–	–	+	–	–	–	–	–	–	–	(–)	+	+	+	+	+	–	–	–
V. pelagius biogroup 1	+	+	–	–	–	–	–	–	–	–	+	+	–	(–)	+	+	–	–	–	–	–	–	–	–	+	–	+	(–)	–	–	V	–	(+)	(+)	–	–	–	–	–	–	(–)	+	+	+	+	+	–	–	–
V. pelagius biogroup 2	+	+	+	–	–	–	–	–	–	–	+	+	–	–	V	+	–	–	–	–	–	–	–	–	+	–	+	–	–	–	–	–	+	V	–	–	–	–	–	–	–	+	+	+	+	–	–	–	–
V. proteolyticus	+	+	+	–	+	+	–	+	+	–	+	+	–	–	–	+	–	–	–	–	+	–	–	–	+	–	+	+	–	–	–	–	+	–	–	–	–	+	+	+	+	+	+	+	+	+	+	+	+
V. salmonicida (15°C)	+	–	–	–	–	–	–	–	–	–	–	V	–	–	–	+	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	(–)	V	–	–	–	–	–
V. splendidus biogroup 1	+	+	+	–	–	(–)	–	–	(+)	–	+	+	–	–	(+)	+	–	–	–	–	–	–	–	–	+	–	+	+	–	–	–	–	+	–	–	–	–	–	–	–	–	(+)	+	+	+	V	–	–	–
V. splendidus biogroup 2	+	+	+	–	–	–	–	–	V	–	+	+	–	–	–	+	–	–	–	–	–	–	–	–	+	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	+	V	V	–	–	–
V. tubiashii	+	+	+	–	–	(+)	–	–	+	–	+	+	–	–	+	+	–	–	–	–	–	–	–	–	+	–	+	(+)	–	–	(–)	–	+	V	–	–	–	–	V	V	V	+	+	+	+	V	–	–	–
V. vulnificus biogroup 2	+	+	–	–	+	–	–	+	–	–	+	+	–	V	–	–	–	–	–	–	–	–	–	–	+	–	+	+	–	–	–	–	V	+	–	–	–	+	+	+	+	+	+	+	+	–	–	–	–
Photobacterium																																																	
P. angustum	V	V	–	V	V	+	–	V	+	–	+	+	–	–	V	–	–	–	–	–	–	–	–	–	V	+	V	–	–	–	–	–	+	–	–	–	–	–	–	–	–	V	+	+	+	–	–	–	–
P. leiognathi	V	(+)	–	(+)	V	(+)	–	V	(+)	–	+	+	V	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	(+)	–	–	–	–	–	–	V	+	+	+	+	–	–	–	–
P. phosphoreum	(–)	(+)	–	+	(+)	+	–	(+)	+	–	V	+	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	+	+	–	–	–	–	–	–	(–)	(+)	+	+	V	–	–	–	–

[a]All data are for reactions that occurred at 1 or 2 days (unless 7 days is specified) in normal enteric media with added marine cations at 25°C (unless 15° is specified). Symbols: +, 90% to 100% positive; (+), 75 to 89.9% positive; V, 25.1 to 74.9% positive; (–), 10.1 to 25% positive; –, 0 to 10% positive.

the in-use concentration. It is our formulation which was modified from the "electrolyte supplement" of Furniss et al. (1978), and it is added in the ratio of one volume of supplement to nine volumes of medium.

Sodium chloride (NaCl)	150 g
Potassium chloride (KCl)	3.7 g
Magnesium chloride ($MgCl_2 \cdot 6H_2O$)	51 g
Calcium chloride ($CaCl_2 \cdot 2H_20$)	7.4 g
Water	912 ml

Dissolve the ingredients in the order listed. All should dissolve readily, and a crystal-clear, colorless solution should result. The volume of the solution will expand to 1 liter after the salts dissolve. Dispense and autoclave at 121°C for 15 minutes. It should remain crystal clear after autoclaving, but a slight amount of fine precipitate may form and settle to the bottom of the container. To use this supplement in other media: aseptically add one volume of marine cations, supplement 1558 to nine volumes of the sterile medium, and mix thoroughly.

Marine Cations Supplement 1559

This medium is the same as marine cations supplement 1558 except that it lacks the calcium chloride. It contains Na^+, K^+, and Mg^{++} at 10 times the in-use concentration. This avoids precipitates, which are often produced when media containing supplement 1558 are autoclaved.

Sodium chloride (NaCl)	150 g
Potassium chloride (KCl)	3.7 g
Magnesium chloride ($MgCl_2 \cdot 6H_2O$)	51 g
Water	912 ml

Dissolve the ingredients in the order listed. All should dissolve readily, and a crystal-clear, colorless solution should result. The volume of the solution will expand to 1 liter as the salts dissolve. Dispense and autoclave at 121°C for 15 minutes. A crystal clear solution should result after autoclaving, but a slight amount of fine precipitate may form and settle to the bottom of the container.

To use this supplement in other media: aseptically add one volume of marine cation supplement 1559 to nine volumes of the sterile medium, and mix thoroughly.

Susceptibility to the Vibriostatic Compound O129

This test of susceptibility to O129 (2,4-diamino-6,7-diisopropyl-pteridine phosphate) was originally used to differentiate cultures of *Vibrio* (usually susceptible) from *Aeromonas* (very resistant), but the test has differential value in the family Enterobacteriaceae, and can also be used to differentiate cultures of the family Pasteurellaceae from Enterobacteriaceae (Chatelain et al., 1979). Table 16 gives the susceptibility of three families.

Almost all methods for testing O129 susceptibility use paper disks that have been soaked in the compound. Originally the disks had to be prepared in the laboratory (Furniss et al., 1978), but now commercial disks are available (see below). Several different methods have been used to measure O129 susceptibility, but the method used by the Maidstone *Vibrio* Laboratory in England (Furniss et al., 1978) is used in many laboratories.

Commercial O129 disks contain 10 ∝g or 150 ∝g per disk and can be ordered from Oxoid. They should be stored in the refrigerator.

Test method: Grow the organism and dilute the turbidity until it is the same as a McFarland 0.5 standard. Insert a cotton swab into the liquid, then wring out as much as possible on the side of the tube. Streak the swab onto a nutrient agar * or trypticase soy agar (used in our laboratory) plate in three directions. Apply 10- and 150-∝g O129 disks and incubate overnight. Observe for a zone of inhibition and record the zone size to the nearest mm. Interpret as follows: sensitive, a zone around each disk; partially sensitive, a zone around the 150-∝g disk but no zone around the 10-∝g disk; resistant, no zone around either disk.

Table 16. Susceptibility of Enterobacteriaceae, Vibrionaceae, and Pasteurellaceae to the compound O129 (2,4-diamino-6,7-diisopropyl-pteridine phosphate).

Organism	O129 Susceptibility
Family Vibrionaceae	
Vibrio—many species	Very susceptible
Vibrio—some species	Somewhat susceptible
Aeromonas	Very resistant
Family Enterobacteriaceae	
Edwardsiella	Susceptible
Other genera	Resistant
Family Pasteurellaceae	
Pasteurella	Susceptible
Actinobacillus	Susceptible

Salt (Na^+) Requirement and Tolerance Tests

These are useful tests for differentiating species of *Vibrio* and *Photobacterium*. Growth in 0% NaCl is an important characteristic that differentiates *Vibrio cholerae* and *V. mimicus* from the halophilic *Vibrio* species. This test has be on done by many different methods, which helps to explain why published results vary among laboratories. We use the following procedure in our laboratory:

Salt Requirement and Tolerance Test

Grow the organisms on marine agar for 18–24 hours at 36°C (if it does not grow at 36°C, use 25°C; if it does not grow at 25°C, use 15°C). Remove some growth with a cotton swab and suspend it in nutrient broth (no added NaCl) until the turbidity is the same as a MacFarland 0.5 standard. A recipe for nutrient broth + 0.1% NaCl is given below. Immediately inoculate each of the media listed below with 0.02–0.03 ml (one drop from a disposable pasteur pipette). Incubate overnight and read for visible growth.

Salt (Na^+) requirement media:
Nutrient broth + 0% NaCl
Nutrient broth + 0.1% NaCl
Nutrient broth + 0.2% NaCl
Nutrient broth + 0.3% NaCl
Nutrient broth + 0.4% NaCl
Nutrient broth + 0.5% NaCl
Nutrient broth + 1% NaCl
Nutrient broth + 2% NaCl
Marine broth
Salt tolerance media:
Nutrient broth + 6% NaCl

Nutrient broth + 8% NaCl
Nutrient broth + 10% NaCl
Nutrient broth + 12% NaCl

Interpret as follows: If the organism has no Na^+ requirement (or a very small one) it will grow in nutrient broth plus 0% NaCl. If it has a Na^+ requirement, it will not grow in 0% NaCl, but will grow with the added NaCl or in the marine broth. For each organism the minimum and maximum concentration of NaCl can be determined.

Nutrient Broth + 0.1% NaCl

This is a formula for one of the media mentioned above. It is essential to use a commercial formulation for nutrient broth (Difco, BBL, etc.) that does not contain NaCl; some formulas (for example, Oxoid) have NaCl as an ingredient. Nutrient broth with other concentrations of NaCl are prepared similarly.

Nutrient broth (Difco or BBL)	8 g
Sodium chloride solution, 10%	10 ml
Water	990 ml

Dissolve the nutrient broth in the water and add the 10% sodium chloride solution. Dispense 10 ml into screw-cap test tubes, replace the caps and *tighten them* to prevent evaporation and a resulting change in NaCl concentration. Autoclave at 121°C for 15 minutes. Allow the tubes to cool at room temperature, check each cap to be sure it is tight, discard any tubes that have a loose cap or obvious loss of liquid. It is convenient to dispense the media for salt requirement and tolerance into color-coded tubes. This helps to avoid errors as they are being read.

String Test

The string test has some differential value because many *Vibrio* species (particularly *V. cholerae*) are string-test positive, but many other species, including *Aeromonas*, are negative. Sodium deoxycholate is a detergent that lyses Gram-negative organisms. When cells are lysed, DNA is released into the suspending medium, making it very viscous and able to form "strings of DNA" when touched with a loop that is raised from the surface of the liquid.

Grow the organisms on trypticase soy agar (or marine agar for halophilic species). With a loop, remove some of the growth and make a heavy suspension in one drop (0.1 ml) of a 0.5% sodium deoxycholate solution. Every 10 seconds or so, raise the loop to see if a "string of DNA" has been formed. Interpretation:

Positive result: The solution becomes viscous and a string of DNA is obvious within 60 seconds. (Positive string test = *Vibrio cholerae*.)

Negative result: No string of DNA formed within 60 seconds. (Negative string test = *Aeromonas hydrophila*.)

Sodium Deoxycholate Solution, 0.5%

This reagent is used as the lysing solution for the string test.

Deoxycholic acid, sodium salt (Sigma D6750)	5 g
Water	1 liter

Slowly add the white powder to the water as it is being stirred on a magnetic stirrer. A crystal-clear, colorless solution results.

Serotyping

This technique is usually done by reference or research laboratories rather than by clinical laboratories, and some of the typing schemes that have been described are discussed under the specific organisms. One exception is the use of *V. cholerae* O1 antisera, which can be used in clinical laboratories to test a strain of *V. cholerae* to determine whether it is O1-positive or negative. Simonson and Siebeling (1988) described the use of latex agglutination for the routine identification of several of the *Vibrio* species. Antisera are made to the flagella which is usually species specific, absorbed to latex particles or *Staphylococcus aureus* cells, and then used in a simple slide agglutination test.

Antibiotic Susceptibility

The species of *Vibrio* important in clinical microbiology usually grow well on Mueller-Hinton agar, which is used in the disk susceptibility test. Although Mueller-Hinton agar contains no added NaCl, it contains a hydrochloric acid hydrolysate of casein, which apparently has been neutralized with NaOH and has enough NaCl to allow good growth of the halophilic species of *Vibrio*. However, some of the environmental marine vibrios grow poorly on Mueller-Hinton, presumably because of their higher requirement for Na^+. Broth dilution susceptibility tests in Mueller-Hinton broth can be done without modification for most *Vibrio* species because the broth contains sufficient Na^+ (Hollis et al., 1976).

Antibiotic resistance is rare in *Vibrio* compared with Enterobacteriaceae. Table 17 summarizes the results for the strains we have tested. In most cases, resistance appears to be intrinsic to the species rather than acquired through plasmid transfer or through antibiotic exposure. The one exception to this generalization is the antibiotic resistance found in some outbreaks of *V. cholerae*, which have become resistant through the acquisition of R factors. In the United States, strains of *V. cholerae* and other *Vibrio* species have rarely had this type of resistance. Resistance to polymyxin antibiotics (polymyxin B and colistin) can be useful in spotting a culture of the eltor biogroup of *V. cholerae*, or in spotting a culture of *V. vulnificus*. Most other *Vibrio* species are more susceptible.

Identification—Marine And Environmental Isolates

The methods that have been used to identify isolates of *Vibrio* and *Photobacterium* isolated

Table 17. Antibiotic susceptability (Kirby-Bauer disk method) of 1,025 strains of the *Vibrio* species that occur in human clinical specimens.[a]

Antibiotic (zone size range)[b]	Percentage of strains susceptible (number of strains studied)											
	V. cholerae (480)	*V. mimicus* (75)	*V. metschnikovii* (22)	*V. cincinnatiensis* (14)	*V. hollisae* (34)	*V. damsela* (21)	*V. fluvialis* (25)	*V. furnissii* (9)	*V. alginolyticus* (69)	*V. parahaemolyticus* (144)	*V. vulnificus* (130)	*V. carchariae* (2)
Penicillin G (12–21)	2	3	9	0	97	0	0	0	0	0	2	0
Ampicillin (12–13)	87	97	31	36	100	52	32	11	0	12	99	0
Carbenicillin (18–22)	64	8	27	7	100	14	16	0	0	1	54	0
Cephalothin (15–17)	98	100	100	100	100	76	40	0	32	17	65	100
Colistin (9–10)	4	61	91	93	100	76	100	100	25	11	2	0
Tetracycline (15–18)	98	100	73	93	97	86	88	89	94	98	99	100
Sulfadiazine (13–16)	26	17	5	36	56	71	36	11	16	3	28	50
Chloramphenicol (13–17)	99	100	100	100	100	10	88	100	100	100	100	100
Streptomycin (12–14)	60	61	32	86	100	24	84	100	54	17	42	50
Kanamycin (14–17)	92	89	14	79	100	43	88	100	62	37	53	100
Gentamicin (13–14)	98	99	100	100	100	100	100	100	100	97	100	100
Nalidixic acid (14–18)	99	99	100	100	100	100	100	100	97	99	99	100

[a]Studied at the CDC *Vibrio* Laboratory and done on Mueller-Hinton agar (with no added NaCl) at 35–37°C.

[b]The numbers in parentheses give the zone size range for the category "intermediate." For example, "(12–21)" means that resistant strains have 6– to 11–mm zones, strains of intermediate susceptibility have zones that are 12– to 21–mm, and susceptible strains have zones of 22 mm or larger. These particular break points are the ones established in the early 1970s for each antibiotic, and they have been used in our laboratory for over 15 years for taxonomic studies. They may differ slightly from current break points.

from the environment are quite varied because these organisms have been studied in different laboratories, and each has tended to use their own methods. The methods described in this section are taken from Baumann and Baumann (1981) and have proved useful in the research laboratory setting.

Cellular Morphology

Cell Size and Shape When examined during the exponential phase of growth in yeast extract broth (YEB) or other relatively simple media, the cells of most *Vibrio* and *Photobacterium* generally appear to be regular, straight or curved rods (Figs. 8, 9, 14, and 15). Many species give rise to involution forms in early stationary phase, a tendency particularly noticeable in some strains of *Photobacterium*. For this reason, it is important to examine the morphology of marine bacteria in the exponential phase of growth in liquid medium. Many of these involution forms are spherical and have been called "spheroplasts" or "round bodies" (Felter et al., 1969; Levin and Vaughn, 1968). These designations are unfortunate since they imply a regularity in the frequently bizarre shapes observed in old cultures (Felter et al., 1969; Kennedy et al., 1970). In *Desulfovibrio aestuarii* and *Nitrospina gracilis*, the formation of "spheroplasts" and "round bodies" was accompanied by a loss of viability (Levin and Vaughn, 1968; Watson and Waterbury, 1971). Baker and Park (1975) have shown that the formation of these structures in stationary phase cultures of a *Vibrio* species correlates with a decrease in the amount of peptidoglycan and loss of viability. The early stages in the formation of involution forms in marine enterobacteria observed by phase contrast microscopy and electron microscopy (Baumann and Baumann, 1981) resemble the K^+-depleted, plasmolyzed cells of *Alteromonas haloplanktis*. Since the intracellular K^+ content of this organism is involved in the maintenance of turgor and since energy is required for K^+ accumulation and maintenance in the cell (Thompson and MacLeod, 1973), it is possible that one of the early manifestations of cell death is a loss of the ability to maintain a high intracellular K^+ concentration leading to plasmolysis and resulting in structures which subsequently become "spheroplasts," "round bodies," or other involution forms. Colwell (1973) has stated that the formation of "round bodies" is a characteristic of the genus *Vibrio*, but these involution forms are observed in a wide variety of organisms, including strict aerobes of marine origin (Baumann et al., 1972), species of *Photobacterium*, and the nitrifying bacterium *Nitrospina gracilis* (Baumann and Baumann, 1981).

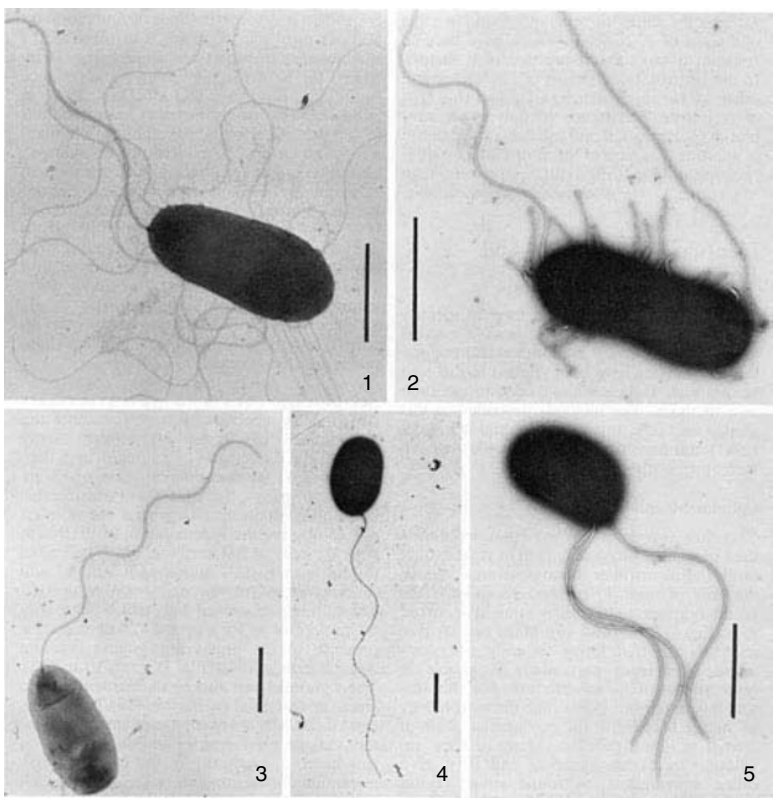

Fig. 14. Different sizes and shapes of *Vibrio* and *Photobacterium* strains seen in electron micrographs. (Parts 1–3 from Allen and Baumann, 1971. Parts 4 and 5 courtesy of R. D. Allen.)

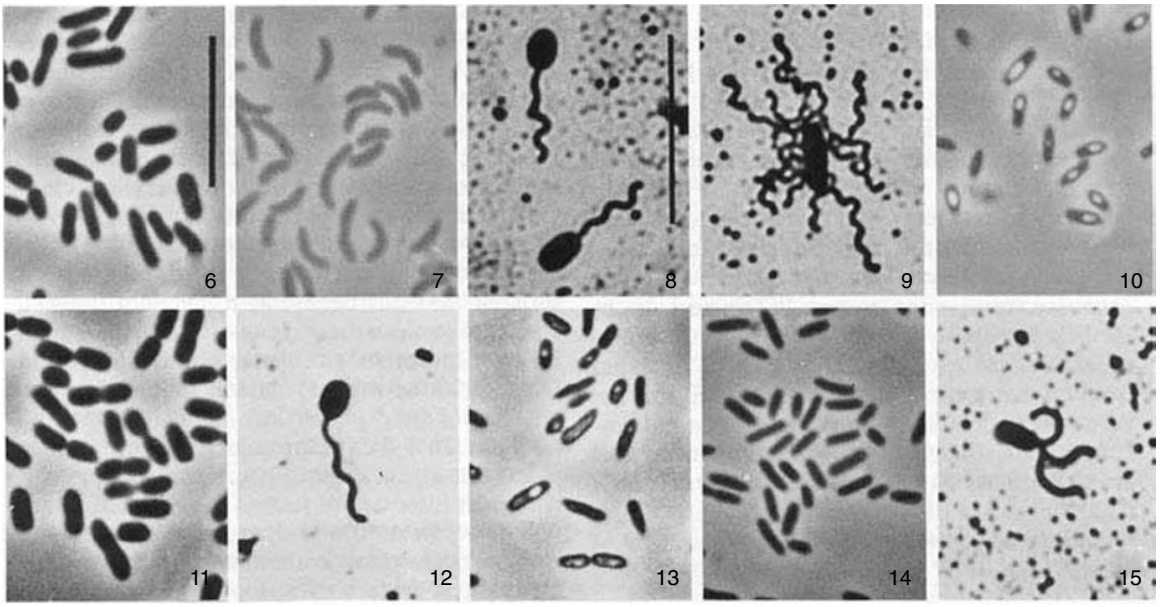

Fig. 15. Different sizes and shapes of *Vibrio* and *Photobacterium* strains seen in phase contrast micrographs and flagella stains. (Parts 6, 7, 10, and 15 from Baumann et al., 1971a; Parts 8 and 9 courtesy of P. Baumann.)

Accumulation of Poly-β-hydroxybutyrate (PHB) Many species of marine eubacteria can accumulate PHB as an intracellular reserve product. In the scheme of Baumann and Baumann (1981) for the differentiation of *Photobacterium* species, this trait was of considerable taxonomic importance. In general, the simplest way to observe the accumulation of PHB is to grow the cells in BM medium which is limited for nitrogen (0.02% ammonium sulfate) and contains excess (0.4%) DL-β-hydroxybutyrate. The culture is examined daily with a phase contrast microscope, for a period of 4 days, for the presence of the bright intracellular granules characteristic of PHB (Fig. 15, parts 10 and 13). These granules can also be stained with Sudan black, as described by Burdon (1946). In some cases difficulties are encountered since the granules may be small and, in addition, some marine bacteria, especially in old cultures, have involution forms containing inclusions which may be confused with PHB. In a research setting, PHB can also be identified chemically by alkaline hypochlorite digestion of the cells followed by the solubilization of the PHB in chloroform and precipitation with acetone (Williamson and Wilkinson, 1958). A quantitative estimation of the extracted PHB can be made by spectrophotometric methods.

Flagella Stains Some strains have tubular projections (Fig. 14), part which are evaginations of the outer membrane of the cell wall (Allen and Baumann, 1971; Baumann et al., 1972) and these might be mistaken for flagella. However, these evaginations are usually readily distinguishable from flagella since they are straight and lack the wave form characteristic of flagella. The flagellins of the polar and peritrichous flagella in the same strain differ in their amino acid composition as well as in their immunological properties (Shinoda et al., 1974a, 1974b, 1976). Genetic studies have shown that the peritrichous flagella are essential for swarming on solid media while the polar flagella are necessary for motility in liquid media (Shinoda and Okamoto, 1977).

Carbon Sources Used For Growth

Baumann and Baumann (1981) indicate that the *Vibrio* and *Photobacterium* ferment only a few carbohydrates. Thus, the fermentation tests that have been extremely useful in the identification of Enterobacteriaceae have been of more limited value for the vibrios. Baumann and Baumann (1981) studied many different carbon compounds and found considerable variation among the species in their utilization pattern. Different species of *Vibrio* and *Photobacterium* vibrios use a range of from 14 to 67 organic compounds as sole sources of carbon and energy; these include pentoses, hexoses, disaccharides, sugar acids, sugar alcohols, 2-carbon to 10-carbon monocarboxylic fatty acids, tricarboxylic acid cycle intermediates, and amino acids. Species utilizing aromatic compounds degrade the intermediate protocatechuate by means of a "meta" cleavage. None of the species utilize cellulose, formate, dicarboxylic acids with 6 to 10 carbon atoms, L-isoleucine, L-valine, L-lysine, L-tryptophan, purines, pyrimidines, or *n*-hexadecane.

Extracellular Enzymes

The production of extracellular chitinase, alginase, amylase, gelatinase, and lipase is determined on the appropriate solid medium inoculated as a spot or streak. A positive reaction is observed as a zone of hydrolysis beyond the limits of growth. Several strains can be tested on one plate, or they can be tested in tubes. Chitinase production is tested on yeast extract agar (YEA) plates overlayed with 10–15 ml of modified YEA containing about 5 g colloidal chitin per liter (prepared as described by Berger and Reynolds, 1958) and 2.5 g yeast extract per liter. To test for alginase activity, colloidal chitin is replaced by 20 g of sodium alginate per liter. An overlay method is not needed to detect the presence of amylase, gelatinase, and lipase. To test for these extracellular enzymes, YEA is supplemented with 2 g starch, 50 g gelatin, or 10 ml polyethylene sorbitan monooleate (Tween-80) per liter, respectively. The hydrolysis of chitin or alginate results in a zone of clearing, whereas lipase activity on Tween-80 plates is detected by the appearance of a precipitate of calcium oleate; all three are observed for a period of 7 days. After 48 h of incubation, the starch plates are flooded with Lugol's iodine solution (Stanier et al., 1966) and the gelatin plates with acidic mercuric chloride in order to visualize the unhydrolyzed polymer.

Vibrio Species of Medical Importance

The following sections give a summary of the information available on each of the 12 species of *Vibrios* that are causes of human disease.

Vibrio cholerae

V. cholerae is by far the most important species in the genus *Vibrio*. It has caused many epidemics of cholera and millions of deaths. Once, it was one of the most feared bacterial pathogens in the world (Pollitzer, 1959).

In the mid-1930s, it was recognized that the vast majority of vibrios isolated from cholera cases agglutinated in a single antiserum (Pollitzer, 1959). Agglutination in this antiserum (later called *V. cholerae* O1 antiserum) became the main criterion for identifying a culture as *V. cholerae*. Organisms that did not agglutinate in this serum were given vernacular names such as "nonagglutinating vibrios," "NAGs," or "noncholera vibrios." These included some organisms known today as *Vibrio cholerae* non-O1, *V. parahaemolyticus*, other *Vibrio* species, and even species of *Aeromonas* or *Plesiomonas*. It was soon recognized that some of the "NAGs" were identical with *V. cholerae* except for their agglutination in O1 antiserum (Sakazaki and Balows, 1981). However, it has only been in the last few years that these have been classified in the species *V. cholerae*. Today it is accepted that there are two main groups of *V. cholerae* strains—serogroups "O1" and "non-O1." It is usually convenient to discuss these separately. *V. cholerae* O1 is usually isolated from cholera cases, where it often produces severe watery diarrhea through the action of cholera toxin. *V. cholerae* non-O1 can cause a cholera-like illness but it can also cause a much wider spectrum of disease (Morris et al., 1981), including extraintestinal infections (Hughes et al., 1978).

Vibrio cholerae Serogroup O1

As discussed above this group of *V. cholerae* strains agglutinate in O1 serum, and include serological subtypes Ogawa and Inaba (and Hikojima) (Donovan and Furniss, 1982), and include two biogroups, classical and eltor.

HISTORY The cholera bacillus was isolated by Koch in 1884, so bacteriological confirmation of cholera was possible only after this date. Cholera, or Asiatic cholera as it was often called in early writings, has probably existed in India since ancient times. It may have been found outside of India before 1800, but not in large epidemics. The spread of Asiatic cholera has come in seven large pandemics. The first pandemic began in 1816–1817; six other pandemics followed beginning respectively in 1829, 1852, 1863, 1881, 1889, and 1961 (Pollitzer, 1959), all except the last caused by the classical biogroup. Although the seventh pandemic of 1961 was caused by the eltor biogroup, the classical biogroup has displaced the eltor biogroup in some parts of the Indian subcontinent.

HABITATS *V. cholerae* O1 is found in the human intestional tract where it can cause a wide spectrum of disease ranging from mild diarrhea to a fatal cholera. The organism is rarely found in other environments unless they have been contaminated with feces from infected individuals. Recent indigenous cases of toxigenic *V. cholerae* O1 infection in the United States and Australia have suggested the possibility of an environmental reservoir.

SYMPTOMS In an area where cholera is endemic, many individuals who ingest *V. cholerae* may have either a mild diarrhea or no symptoms at all (asymptomatic colonization of the intestine with recovery of the organism from formed

stool). This pattern of mild disease was much more common in the seventh (1961) cholera pandemic caused by the eltor biogroup of *V. cholerae*. At the other extreme, some individuals develop an acute diarrhea with constant purging that has been called "cholera gravis." In severe cholera there is massive diarrhea, with large volumes of rice-water stool (clear fluid with flecks of mucus) passed painlessly. The amount of fluid passed can be a liter or more per hour. In 4–6 days this would amount to over twice the body weight. There is usually vomiting and little desire to eat. If untreated, there will be prostration with symptoms of severe dehydration, electrolyte imbalance, painful muscle cramps, watery eyes, loss of skin elasticity and anuria (absence of urine excretion). Death can occur very quickly after onset of symptoms because of the severe dehydration.

Treatment Treatment for the most severe cases of cholera is intravenous therapy with large volumes of a simple balanced salts solution which restores water and electrolyte balance and prevents acidosis. Cases with mild or moderate illness are often treated with oral electrolyte solutions. Tetracycline therapy reduces the period of excretion but is not a substitute for rehydration.

Milder forms of diarrhea due to *V. cholerae* O1 are more difficult to distinguish from other mild diarrheas. They can last from 1–5 days with the passage of several liquid stools per day. Cramping and vomiting can also occur. The role of *V. cholerae* O1 can only be shown by isolating and identifying the causative organism. In patients with the eltor biogroup of *V. cholerae* O1, this milder disease is seven times more common than severe cholera.

Pathogenesis The pathogenesis of diarrhea due to *V. cholerae* O1 is well understood. The organism is ingested and some cells survive the acid pH of the stomach and pass into the small intestine. The organism colonizes the small intestine and begins to grow and produce cholera toxin. Cholera toxin, which has a molecular weight of 84,000, is composed of an A subunit (molecular weight 21,000), A2 subunit (molecular weight 7,000), and five B subunits (molecular weight 10,000 each). The B subunit of intact cholera toxin attaches to a specific receptor, ganglioside Gl, on the cell membrane of cells in the intestine. The A1 subunit of cholera toxin then activates the enzyme adenylate cyclase of the host which then increases the level of cyclic AMP, leading to the hypersecretion of salt and water. The net result is the massive outpouring of liquid stool and resulting dehydration which is typical of cholera.

Sources *V. cholerae* O1 is an intestinal pathogen, and the vast majority of strains come from human feces (Tables 2 and 3). Only a few human isolates are extraintestinal. *V. cholerae* O1 can also be isolated from the environment and from other animals that come in contact with feces of cholera cases. There are occasionally reports of *V. cholerae* O1 from environmental sources that are unlikely to have come from cholera cases. Most of these strains do not produce cholera toxin, and may represent strains of *V. cholerae* from an evolutionary line unrelated to the one that causes pandemic cholera. They are more similar to the *V. cholerae* non-O1 strains in their clinical and public health importance.

Geographical Distribution The World Health Organization (WHO) regularly reports the world-wide distribution of reported cholera cases. In its survey for 1989, 48,403 cases were reported from 35 countries, but some governments probably do not report cases for political and economic reasons. Almost all of the cases were in Africa (35,606) and Asia (12,785).

The history of cholera (defined to be the disease caused by toxin-producing *V. cholerae* O1) in the United States is quite interesting (Blake et al., 1980a). The last case of cholera in the United States from a pandemic occurred before 1900. Then, unexpectedly, there was an isolated case from Texas in 1973. This was followed by an outbreak in Louisiana in 1978, two sporadic cases in Texas in 1982, and an outbreak on an oil-drilling platform on a bayou near the Texas coast in 1982, with sporadic cases since. These strains have been thoroughly studied and have the following properties in common: serogroup Inaba (see Table 10), choleratoxin positive, strongly hemolytic, and a unique bacteriophage lysis pattern. When compared to other strains from the seventh pandemic of cholera, the American strains are quite different, suggesting that this is a different clone. Apparently, a similar situation is occurring in Australia, which has its own unique clone(s).

Isolation In rice-water stools from cholera cases, *V. cholerae* is usually present essentially as a pure culture and in very high numbers (10^6 to 10^8 organisms per ml stool). Isolation will present no problem in these cases (Figs. 6, 7, 8, and 11). In formed feces, the organism will probably be present in much lower numbers and normal enteric flora will also be present. Enrichment in alkaline peptone water and plating on a highly selective medium will yield some additional positive cultures in this situation (Balows et al., 1971).

Identification In countries where cholera is common there is no need to do a large number

of biochemical tests to confirm a culture as *V. cholerae*. However, complete biochemical testing should be done in countries where cholera is rare (Tables 6 to 11). The test for the Na^+ requirement differentiates *V. cholerae* from the halophilic *Vibrio* species (Table 6). Sucrose fermentation differentiates *V. cholerae* from *V. mimicus* (Table 8). The decarboxylase pattern of arginine negative, lysine positive, and ornithine positive differentiates (with one exception) *V. cholerae* from the oxidase-positive fermentative species in *Aeromonas* and *Plesiomonas*.

Antibiotic Susceptibility Resistance is rare in *V. cholerae*. Most strains are susceptible to tetracycline, the drug of choice. Occasionally a strain of *V. cholerae* becomes resistant and can spread. This usually happens in cholera-endemic areas where there is more chance for a strain to acquire an antibiotic resistance plasmid. Susceptibility to the polymyxin group of antibiotics is a property used to diffrentiate the classical and eltor biogroups (Table 9).

Vibrio cholerae Non-O1

This group of organisms is strikingly similar to *V. cholerae*, but they do not agglutinate in O1 antiserum. They can cause a cholera-like disease, but are usually isolated from patients with mild diarrhea and from extraintestinal infection and the environment, which is a very important reservoir. These organisms were originally not classified in the species *V. cholerae* but were reported as "nonagglutinating vibrios," "NAGs," or "noncholera vibrios." Now, however, they are classified as *V. cholerae* non-O1, much data are appearing on their ecology (Colwell, 1984) and role in human disease (Hughes et al., 1978; Morris et al., 1981).

Habitats Strains of *V. cholerae* non-O1 have been isolated from patients with severe dehydrating (cholera-like) gastroenteritis. Other strains have been isolated from patients with mild diarrhea. Unlike *V. cholerae* O1, the non-O1 strains have also been found in patients with diarrhea and fever whose feces have contained blood and/or mucus.

Although the pathogenic process is not as well known as is that of *V. cholerae* O1, three possible mechanisms have been postulated: 1) production of cholera (or cholera-like?) toxin; 2) production of a heat-stable enterotoxin (positive in the infant mouse assay used for *Escherichia coli* heat-stable enterotoxin); or 3) invasive disease (positive response to whole cells but not to cell filtrates in the ligated rabbit ileal loop or infant mouse assay). However, some strains of *V. cholerae* non-O1 have no positive response in any of the above assays, so the correlation of the possible virulence factors and the different types of gastroenteritis-invasive diarrhea need further investigation.

Extraintestinal Infections *V. cholerae* non-O1 has a wider spectrum of disease than the *V. cholerae* O1. Non-O1 strains have been isolated from patients with septicemia who have cirrhosis and/or other underlying disease. In our collection (Table 3), there were 42 blood isolates, compared with only one blood isolate of *V. cholerae* O1. The non-O1 strains were also isolated from ears, wounds, respiratory tract, and urine. There were 52 isolates from these sources compared with only two for *V. cholerae* O1 (Table 3).

Geographical Distribution *V. cholerae* non-O1 strains have been isolated worldwide both from patients and the environment. In the United States many are found in sporadic cases of diarrhea. Most of these have occurred after eating raw oysters. In the United States less than 5% of the isolates have been positive for cholera toxin; this is in contrast to the situation in Bangladesh where about one-third of the isolates have been positive (Blake, 1981).

Isolation and Identification The discussion for *V. cholerae* O1 also applies to the non-O1 strain. The essential difference is that the O1 strains agglutinate in antiserum to *V. cholerae* O1 but the non-O1 strains do not. The biochemical reactions of both *V. cholerae* groups are almost identical.

Serotyping Strains of *V. cholerae* non-O1 can be further differentiated by serotyping by one of several systems (Brenner et al., 1982; Smith, 1979). In a system described by Sakazaki and Shimada (1977), antisera are made against heated cells, and the O antigen is determined by agglutination. There are over 60 different O antigens in this schema. In a system described by Smith (Smith, 1979), antisera are made against unheated cells, and the "Smith type" is determined by slide agglutination of living cultures. There are over 72 different types in the Smith schema. Serological typing of non-O1 *V. cholerae* should be done only to answer specific epidemiological questions, and is done by only a few reference laboratories. An international working group has been proposed to study the two systems and make recommendations for standardization.

Vibrio mimicus

Vibrio mimicus is a species of *Vibrio* (Davis et al., 1981) that apparently causes diarrhea, usu-

ally after the consumption of uncooked seafood, particularly raw oysters (Shandera et al., 1983). *V. mimicus* has been isolated from many countries (Canada, Mexico, Philippines, New Zealand, Guam, Bangladesh) which suggests a worldwide distribution in countries situated along an ocean. The isolation of *V. mimicus* from water, oysters, and shrimp, and its distribution in coastal areas indicate that its ecology may be similar to the ecology of *V. cholerae* non-O1.

History There have been a number of reports of *V. cholerae* strains with atypical biochemical reactions. Traditionally they have been reported with designations such as "*V. cholerae*—lysine-decarboxylase negative," "*V. cholerae*—mannitol negative," or "*V. cholerae*—sucrose negative." Davis et al. (1981) studied representative strains from six of these unusual biogroups by both DNA hybridization and phenotypic analysis. Five of the six groups were highly related to *V. cholerae* by DNA hybridization. These had been identified correctly as "atypical strains of *V. cholerae*." However, the group of sucrose-negative strains was only 24–54% related to *V. cholerae*. Based on this low relatedness and on their phenotypic differences, Davis et al. (1981) proposed a new species, *Vibrio mimicus*. The name "mimicus" refers to the fact that the strains "mimic" *V. cholerae*.

Habitats *V. mimicus* has been isolated from diarrhea cases, extraintestinal infections, and the environment. Based on the information submitted with cultures, Davis et al. (1981) suggested that *V. mimicus* may be a new cause of diarrhea, probably linked to eating shellfish. Three strains of *V. mimicus* were positive for heat-stable enterotoxin in the infant mouse assay and five were positive for heat-labile enterotoxin in the Y-1 adrenal cell assay or in the ELISA assay. However, other strains from diarrhea cases were negative in the assays. The enterotoxin data were very similar to those for *V. cholerae* non-O1 strains in that some produced heat-labile or heat-stable enterotoxin, but most strains from patients with diarrhea produce neither toxin.

Isolation and Identification Most of the *V. mimicus* strains have been isolated during a search for *V. cholerae* and other *Vibrio* species. *V. mimicus* grows well on TCBS agar and forms green (sucrose-negative) colonies (Table 4). *V. mimicus* grows well on the usual enteric media and in nutrient broth with no added NaCl. Isolation and identification should pose no special problems.

Biochemical Reactions Tables 6 to 8 give the reactions of *V. mimicus* and compare them with those of *V. cholerae*. These two species are very similar in most of the tests normally needed for identification; however, Table 8 gives reactions which are useful for differentiation. *V. mimicus* is negative for lipase production and sucrose fermentation. *V. cholerae* usually has the opposite pattern, although a few strains are lipase negative. *V. mimicus* resembles the "classical biogroup" of *V. cholerae* because it is Voges-Proskauer negative and susceptible to polymyxin. The "eltor biogroup" of *V. cholerae* is usually Voges-Proskauer positive and resistant to polymyxin. *V. mimicus* grows in nutrient broth without added NaCl, a reaction which differentiates it, along with *V. cholerae*, from the halophilic *Vibrio* species that require higher NaCl concentration for growth.

Vibrio parahaemolyticus

V. parahaemolyticus has been known as a cause of acute gastroenteritis since 1950 (Zen-Yoji et al., 1965). Foodborne outbreaks and sporadic cases occur worldwide and are usually associated with the consumption of contaminated seafood. Two books (Fujino et al., 1974; Miwatani and Takeda, 1976) and a review (Joseph et al., 1982) summarize much of the information known about this organism.

History On 20 and 21 October 1950, there was an outbreak of food poisoning in Osaka, Japan, involving 272 patients with acute gastroenteritis and 20 deaths. This led to an extensive investigation and eventually an organism was isolated which was shown to be the etiological agent. The fascinating account of this discovery can be found in Miwatani and Takeda (1976, p. 1–5). The organism was studied and named as a new species, *Pasteurella parahaemolytica*. Its halophilic nature was not discovered until 1955 (Miwatani and Takeda, 1976), when it was reclassified as a halophilic *Vibrio* species, *V. parahaemolyticus*.

Symptoms *V. parahaemolyticus* causes gastroenteritis with nausea, vomiting, abdominal cramps, low-grade fever, and chills (Barker and Gangarosa, 1974). The diarrhea is usually watery but can sometimes be bloody. The disease is usually mild and self-limiting but can be fatal (a 7% fatality rate was reported in the first outbreak). There is a good correlation between pathogenicity and a positive Kanagawa test (a test which measures the ability of the strain to produce a hemolysin for human red blood cells when grown on a special medium; Ljungh and Wadstrom, 1983). About 96% of the *V. parahaemolyticus* strains from well-documented cases of human gastroenteritis are Kanagawa positive,

but only about 1% of the strains isolated from the environment are positive. Although there is excellent correlation between this hemolysin and human disease, the mechanisms of pathogenesis are still unclear, since other toxins or virulence factors may be involved. Rehydration is usually the only treatment needed, but in some severe cases the patient will require hospital admission. Antimicrobial therapy may be beneficial, and tetracycline appears to be the drug of choice.

Outbreaks of gastroenteritis due to *V. parahaemolyticus* occur worldwide, but are not common in the United States. In Japan, *V. parahaemolyticus* is an extremely important diarrheal agent, and causes 50–70% of the cases of foodborne enteritis (Sakazaki and Balows, 1981). All of the outbreaks were associated with seafood either directly or indirectly. Direct infection comes from the ingestion of raw fish or shellfish which are contaminated with the organism. This mechanism of transmission is apparently quite common in Japan because of the national custom of eating raw fish (Sakazaki and Balows, 1981). Contamination after cooking apparently is the mechanism of indirect infections.

Isolation Much has been written about the isolation of *V. parahaemolyticus*. The fact that it was not detected as an important cause of gastroenteritis until 1950 indicates that isolation with the usual enteric media can pose problems. Oxidase testing of colonies on a nonselective medium such as blood agar should provide a high isolation rate. A plate of TCBS medium (Table 4) can be included in those geographical areas where *V. parahaemolyticus* is most common. The isolation methods designed for *V. cholerae* are also efficient for *V. parahaemolyticus*. *V. parahaemolyticus* grows well on TCBS agar as green colonies 2 to 3 mm in diameter.

Identification *V. parahaemolyticus* is one of the halophilic *Vibrio* species (Baumann and Baumann, 1973; Twedt et al., 1969) belonging in the lysine-positive, arginine-negative group (Table 6). It is negative for the Voges-Proskauer test and for fermentation of lactose and salicin. These and other tests are useful in differentiation (see Tables 6, 7, and 14).

Urea-Positive Strains Initially, all strains of *V. parahaemolyticus* were reported to be negative for urea hydrolysis. Sakazaki and Balows reported that none of their 2,354 strains were urea positive (Sakazaki and Balows, 1981). However, in the last decade we have received many strains which were otherwise typical of *V. parahaemolyticus* but were urea-positive. These urea-positive strains were confirmed by DNA hybridization as *V. parahaemolyticus* (Brenner et al., 1983a). In our laboratory the number of urea-positive strains has increased significantly: 1977, 17% urea-positive; 1978, 14% positive; 1979, 36% positive; 1980, 12% positive; 1981, 47% positive; 1982, 73% positive; and 1983, 83% positive, etc. This sample is biased because laboratories refer atypical strains rather than typical ones. However, in recent years, 50% or more of *V. parahaemolyticus* strains from California and from outbreaks investigated by CDC have also been urea-positive. These samples are less biased, and show that the increase in urea-positive strains is real.

Serotyping A serological typing schema for *V. parahaemolyticus* includes 11 numbered O antigens and 55 numbered K antigens. The O antigen is first determined by slide agglutination with an autoclaved antigen. The K antigen is then determined by slide agglutination with an unheated suspension. A complete set of O and K antisera (individuals and pools) is produced commercially by Toshiba Kagaku Kogyo Co., Inc., Maruishi Bldg. 2, 1-Chrome Kanda-Kajicho, Chiyode-KU, Tokyo, Japan. It may still be available in the United States from Nichimen Company, Inc., 1185 Avenue of the Americas, New York, NY 10036. Serotyping of *V. parahaemolyticus* should be done in reference laboratories and only to answer specific epidemiological questions.

Antibiotic Susceptibility *V. parahaemolyticus* is usually resistant to ampicillin and carbenicillin, but susceptible to colistin (Table 17). These properties are shared with *V. alginolyticus*, but *V. vulnificus* has the opposite susceptibilities (Bonner et al., 1983) (Table 15).

Vibrio hollisae

V. hollisae is a halophilic species (Hickman et al., 1982) that is associated with diarrhea following consumption of raw seafood.

History *V. hollisae* was named as a new species in 1982 (Hickman et al., 1982). Two laboratories had independently studied this organism under the vernacular names "EF13" and "Enteric Group 42." DNA hybridization studies indicated that these strains were highly related and distinct from other named *Vibrio* species, so Hickman et al. (1982) named this organism *Vibrio hollisae*.

Habitats *V. hollisae* probably causes human diarrhea. Its association with diarrhea is very strong, but additional evidence is needed to further document its causal role and pathogenesis. Fifteen of the original sixteen strains were from feces, and many of the patients had diarrhea

(Hickman et al., 1982). Morris et al. (1982) described the clinical and epidemiological features of 11 diarrhea cases that were culture positive for *V. hollisae*. They also reported one isolate from blood. It was from a patient with hepatic cirrhosis, hepatic encephalopathy, bronchopneumonia, and sepsis due to the yeast *Cryptococcus*. The patient was comatose and died two days after hospital admission.

Isolation Laboratory strains of *V. hollisae* do not grow on TCBS agar (Table 4) or MacConkey agar (2 days, 36°C). Since these two media are frequently used for the isolation of *Vibrio* and Enterobacteriaceae from stools, it is possible that *V. hollisae* is often missed, even though it may be the predominant organism. *V. hollisae* grew well on sheep blood agar (36°C), so it can be detected by the use of the oxidase reagent as previously discussed.

Identification *V. hollisae* does not grow in nutrient broth without added NaCl, thus it is one of the halophilic *Vibrio* species. Tables 6 and 7 give its biochemical reactions, and only a few reactions need comment. *V. hollisae* is indole positive when tested in heart infusion broth with 1% NaCl added (Table 1), but only 38% positive in peptone water with 0.5% NaCl (the usual content). Moeller's lysine, arginine, and ornithine are all negative, which is a distinguishing characteristic (Table 6). Motility in *V. hollisae* is very slow. None of the strains were motile in semisolid medium after 48 h incubation at 36°C, but 88% were motile after 7 days. This is an unusual characteristic for the genus *Vibrio*. *V. hollisae* ferments only D-glucose, L-arabinose, D-galactose and D-mannose; this is a characteristic fermentation pattern among *Vibrio* species. Recently we confirmed a urea-positive strain of *V. hollisae*, the first one we have seen with this unusual property.

Antibiotic Susceptibility Strains of *V. hollisae* have a very characteristic antibiogram. There are very large zones around all antibiotics tested, including penicillin (Table 17).

Vibrio fluvialis

V. fluvialis appears to cause sporadic cases of diarrhea worldwide (Lee et al., 1981; Nishibuchi and Seidler, 1983; Tacket et al., 1982), and it has been implicated in outbreaks of diarrhea in Bangladesh (Huq et al., 1980).

History Lee et al. (1981) gave the name *Vibrio fluvialis* to a group of halophilic vibrios that had been previously been known as "Group F vibrios" (Lee et al., 1978b) and as "Group EF6." These organisms had been isolated from a number of environmental sources throughout the world and from humans with diarrhea. Lee et al. (1981) defined two biogroups in this species which correlated with source of isolation and certain biochemical tests. *V. fluvialis* biogroup I did not produce gas during fermentation (it was "aerogenic") and was isolated from human diarrhea as well as the environment. *V. fluvialis* biogroup II produced gas during fermentation (it was "aerogenic") and was isolated from the environment, but not from humans with diarrhea. Brenner et al. (1983b) later used DNA-DNA hybridization to show that strains of *V. fluvialis* biogroup II were related to strains of *V. fluvialis* biogroup I, but they were sufficiently different to be a new species that they named *V. furnissii*.

Habitats The sources of early clinical isolates of *V. fluvialis* showed a marked association with diarrhea (Lee et al., 1981), and further studies have strengthened its causative role. One difficulty in assessing the clinical symptoms and epidemiology of *V. fluvialis* has been the presence of other possible pathogens. Most of the reports have come from geographical areas where several possible pathogens are often present in feces.

Gastroenteritis caused by *V. fluvialis* is usually described as "cholera-like." Patients typically have watery diarrhea with vomiting (97%), abdominal pain (75%), moderate to severe dehydration (67%), and often fever (35%). Usually infants, children, and young adults are affected. Frank blood is found in a small percentage of stool samples, but red or white blood cells are found in most cases (75%).

The pathogenesis of *V. fluvialis* diarrhea is not completely known. Assays for heat-labile and heat-stable enterotoxin (LT and ST) and invasiveness have generally been negative. About 20% of strains give a positive rabbit ileal loop test for enterotoxin. Recent studies have provided additional evidence for enterotoxin or for "enterotoxin-like" molecules (Lockwood et al., 1982; Nishibuchi and Seidler, 1983).

Isolation *V. fluvialis* grows on TCBS agar as 2- to 3-mm yellow colonies (Table 4). Although it requires Na^+ for growth, the requirement is much lower than some of the more halophilic *Vibrio* species, which makes isolation and identification easier. Nishibuchi et al. (1983) recently described a selective broth for isolating this species from water.

Identification The most striking aspect of *V. fluvialis* identification is the possible confusion with *Aeromonas*, since both species are arginine-dihydrolase positive. Many cultures sent to us

labeled "possible *V. fluvialis*" turn out to be *Aeromonas*. The converse is also true. A simple test to differentiate these two organisms is growth in nutrient broth with 0% and 1% NaCl. *V. fluvialis* is a halophilic vibrio and will not grow without the added NaCl whereas strains of *Aeromonas* grow in both media (Table 12). *V. fluvialis* is generally susceptible to antibiotics (Table 17).

Vibrio furnissii

This species was formerly known as "*V. fluvialis* biovar II," "*V. fluvialis* aerogenic," or "*V. fluvialis* gas$^+$." It is now recognized as a separate species, *V. furnissii* (Brenner et al., 1983b).

Habitats This species has been isolated from human clinical specimens and the environment. The vast majority of the original isolates of *V. fluvialis* (which included *V. furnissii* from patients with diarrhea) did not produce gas during fermentation. Thus, they are *V. fluvialis*.

Further investigation indicated that *V. furnissii* had been isolated from two outbreaks of acute gastroenteritis among American tourists returning from the Orient in 1969. Other pathogens or possible pathogens were also isolated (*V. parahaemolyticus*, *V. cholerae* non-O1, *V. fluvialis*, *Salmonella*, and *Plesiomonas*), so the causal role of *V. furnissii* was very doubtful. There have been no documented cases of diarrhea caused by this organism. Thus we have not included it with the other *Vibrio* species as a probable cause of diarrhea. However, microbiologists should be alert to its presence and for evidence which might show that it has a causal role in diarrhea. *V. furnissii* is apparently rare in human clinical specimens. Table 3 indicates that human stool was the most common source (16 isolates), but one isolate was from a wound. Lee et al. (1981) mentions that *V. furnissii* (listed as *V. fluvialis* biovar II) is widespread in the aquatic environment and is more common in estuaries.

Isolation and Identification Table 7 indicates that *V. furnissii* resembles *V. fluvialis* very closely. The two species are so close phenotypically that only a few tests are useful in differentiating them (Tables 12 and 13). Gas production will be the key differential test in most clinical laboratories (Table 13). *V. furnissii* can also be confused with *Aeromonas* if its halophilic nature is not recognized.

Vibrio vulnificus

V. vulnificus has been recognized as a distinct species of *Vibrio* since 1976 (Baumann and Schubert, 1984). It causes wound infections and life-threatening septicemia (Blake et al., 1979). In the last few years, there has been intensive study of this organism (Baumann and Schubert, 1984; Blake, 1981; Colwell, 1984; Sakazaki and Balows, 1981; Tison and Kelly, 1984).

History Hollis et al. (1976) described a salt-requiring organism that appeared to be different from other *Vibrio* species. It was similar to *V. parahaemolyticus* and *V. alginolyticus* in many of its biochemical reactions, but the new organism fermented lactose. The vernacular name "lactose-positive *Vibrio*" was given to this organism, and in the literature it has been referred to as "L$^+$ *Vibrio*" and "Lac$^+$ *Vibrio*." The organism was studied in several laboratories (Clark and Steigerwalt, 1977), and given the scientific name *Beneckea vulnifica* by Reichelt et al. (1976). Classification of the organism in the genus *Beneckea* was not widely accepted, so it was subsequently classified in the genus *Vibrio* (Farmer, 1979). Today, it is almost universally known as *V. vulnificus*.

Habitats *V. vulnificus* is widely distributed in the marine environment, where some strains come in contact with humans and cause infections. This species has been associated primarily with two disease syndromes, primary septicemia and wound infection (Blake et al., 1979). Primary septicemia is a very serious infection with a fatality rate of about 50%. Most patients with primary septicemia due to *V. vulnificus* have pre-existing liver disease (Blake et al., 1979); but some were healthy individuals (Tison and Kelly, 1984). In most cases the disease begins several days after the patient has eaten raw oysters. Cultures of blood and skin lesions are usually positive for *V. vulnificus*. *V. vulnificus* also causes severe wound infections, usually after trauma and exposure to marine animals or the marine environment (Blake et al., 1979). The mortality rate is not nearly as high as in primary septicemia cases (about 7% compared to about 50%). Other infections from which *V. vulnificus* has been isolated include pneumonia in a drowning victim and endometritis which developed in a woman after exposure to seawater (Tison and Kelly, 1984).

Pathogenesis Animal studies have shown that *V. vulnificus* can cause severe local infections with gross edema leading to tissue necrosis and death. Iron availability appears to be important in pathogenesis (Tison and Kelly, 1984). A toxin produced by *V. vulnificus* has been demonstrated, although its role is still unclear.

Sources Table 3 gives the sources of our isolates. The vast majority were from blood or wound. Unusual sources included one from

urine and five from stool. *V. vulnificus* is also frequently isolated from the marine environment which is not apparent from our collection that is weighted toward human clinical specimens.

Isolation *V. vulnificus* grows well on blood agar and TCBS agar. Although most strains of *V. vulnificus* are sucrose-negative (only 3% were positive in the original report of Hollis et al., 1976), the number of sucrose-positive strains in our collection has increased markedly over the years. Thus, on TCBS agar, *V. vulnificus* can be either green (most) or yellow (some) (Table 4). This fact has not always been considered in studies looking for it in the environment. Most isolations of *V. vulnificus* in clinical laboratories will be made on blood agar, since blood and wounds are the usual sources. Commercial blood culture bottles usually support growth of *V. vulnificus*, however the Na^+ content is probably suboptimal, which may yield some cells with aberrant sizes and shapes. *V. vulnificus* does not survive well on media with an NaCl content of only 0.5% NaCl. Often cultures that have been shipped to us on ordinary enteric media are nonviable.

Identification *V. vulnificus* is a halophilic *Vibrio* species. It is lysine positive, and arginine negative, and is in the same group as *V. parahaemolyticus* and *V. alginolyticus* (Tables 6, 7, and 14). It is differentiated by lactose fermentation, the ONPG test, salicin and cellobiose fermentation and several other tests (Table 4). The antibiogram (disk diffusion) is also helpful in differentiating these three species (Table 14). *V. vulnificus* has either no zone or a small zone around the colistin disk, but large zones around ampicillin and carbenicillin. *V. parahaemolyticus* and *V. aginolyticus* have the opposite pattern. Although *V. vulnificus* is susceptible to most antibiotics used against Gram-negative bacteria, animal studies indicate that tetracycline may be most effective in vivo (Tison and Kelly, 1984).

Vibrio damsela

V. damsela is a *Vibrio* species that appears to cause human wound infections. It is found in the marine environment and causes skin lesions on certain marine fish.

History *V. damsela* was described by Love et al. (1981). The original isolates were from skin lesions on damselfish on the California coast. Koch's postulates were fulfilled when it was documented that this organism alone caused the fish lesions. Strains of *V. damsela* were then compared to our collection of unidentified *Vibrio* strains from human clinical specimens, which revealed that *V. damsela* had occurred in human infections (Love et al., 1981). The strains from marine fish, human clinical specimens, and the environment were studied, and *V. damsela* was proposed as a new species.

Habitats *V. damsela* has been isolated from the marine environment, marine fish, and human clinical specimens. Morris et al. (1982) reviewed the case histories of six patients with wound infections whose isolates had been sent to our laboratories from 1971 to 1981. All had been acquired in coastal areas. *V. damsela* appears to cause human wound infections, but its causal role needs to be further documented. The sources of our *V. damsela* isolates are given in Table 3. All of the clinical isolates were from wounds, and 9 of the 10 were leg or foot wounds. Strains were also from marine animals, particularly marine fish. A number of fish from Dakar, Senegal, also yielded this organism. Others were from sewage, oysters, and a wound on a raccoon.

Isolation *V. damsela* grows on blood agar and other noninhibitory media, but the Na^+ content is less than optimum. Strains grow on TCBS agar as 2–3 mm green or yellow colonies (Table 4). Love et al. (1981) noted that the damselfish isolate grew much better on TCBS at 25°C than 36°C. There have been few details on the isolation of this organism from human clinical specimens.

Identification The complete biochemical reactions of *V. damsela* are given in Table 7. It is a typical halophilic *Vibrio* in many respects. Strains do not grow in nutrient broth without added NaCl, which indicates its requirement for Na^+. *V. damsela* is arginine positive, Voges-Proskauer positive and has a characteristic fermentation pattern, positive for D-glucose, D-mannose, and usually for D-galactose and trehalose. The characteristics which differentiate it from other *Vibrio* species are given in Tables 6, 7, and 12.

Antibiotic Susceptibility *V. damsela* is generally susceptible to antibiotics, but less so than many other *Vibrio* species (Table 16).

Vibrio alginolyticus

V. alginolyticus is very common in the marine environment (Stephen et al., 1978) and also occurs in human clinical specimens.

History *Vibrio parahaemolyticus* was the first of the halophilic *Vibrio* species to be studied in detail. An organism similar to, but different from, *V. parahaemolyticus* was often isolated from the marine environment, but not from patients with gastroenteritis. This organism was

originally called "biotype 2 of *V. parahaemolyticus*" (Sakazaki, 1968). However, a number of studies have shown it to be a distinct species, now universally known as *V. alginolyticus* (Sakazaki and Balows, 1981; Sakazaki et al., 1963).

Habitat Many papers describe the isolation of *V. alginolyticus* from soft tissue infections (Pien et al., 1977; Rubin and Tilton, 1975). Wound infections and ear infections are usually mentioned, with eye infections mentioned much less frequently. The data show the definite association of *V. alginolyticus* with infection at these sites, however the etiological role and pathogenesis of this organism have not been thoroughly shown. However, most authors list *V. alginolyticus* as a pathogenic *Vibrio* species, particularly of wound and ear infections. Antibiotic treatment has been used in most cases and surgical debridement has been used in some.

Table 3 gives the sources of the 74 isolates of *V. alginolyticus* in our collection. Wounds and ears are the most common sources. Interestingly, there were 12 isolates from foot or leg wounds, but only one from hand or arm wounds. *V. alginolyticus* is also widely distributed in the marine environment (Furniss et al., 1978; Sakazaki and Balows, 1981). Most clinical isolates probably come from patients exposed to the marine environment, particularly after trauma.

Isolation and Identification

V. alginolyticus is a marine *Vibrio* and requires added NaCl for optimum growth. However, it grows well on many of the enteric media. It is sucrose-positive and occurs as 2–3 mm yellow colonies on TCBS agar (Table 4). It is easy to isolate from saltwater samples. A small volume is spread on a marine agar plate, which is then incubated at 25°C. After 18–24 of incubation, the plate is observed for colonies that swarm in a way similar to *Proteus*. A colony is restreaked onto marine agar and to TCBS agar (to prevent swarming) for purity and then identified. Many of the swarming colonies will be *V. alginolyticus* (de Boer et al., 1975a, 1975b).

Table 7 lists the properties of this species. It is lysine-positive and arginine-negative, which puts it into the group with *V. parahaemolyticus* and *V. vulnificus* (Table 6). *V. alginolyticus* is usually Voges-Proskauer positive and grows in nutrient broth with 8 and 10% NaCl, which differentiates it from the other two species (Table 4).

Antibiotic Susceptibility *V. alginolyticus* is usually resistant to ampicillin and carbenicillin but susceptible to colistin (Table 17). This pattern is similar to *V. parahaemolyticus*, but *V. vulnificus* has the opposite reactions (Table 14).

Vibrio metschnikovii

V. metschnikovii is a species in the genus *Vibrio* that has been frequently isolated from fresh, brackish, and marine waters, but rarely from human clinical specimens (Lee et al., 1978a).

History In 1884, Gamalria reported the isolation of a new organism, *Vibrio metschnikovii*, from a fowl that had died of a "cholera-like" disease (Lee et al., 1978a). Little was written about the organism until Lee et al. (1978a) began isolating similar organisms on TCBS agar from marine and freshwater environments in the United Kingdom. These strains were oxidase-negative and did not reduce nitrate to nitrite, but otherwise were quite typical halophilic *Vibrio* species. They proposed that the strains be classified as *V.metschnikovii* and wrote an amended description of this organism (Lee et al., 1978a).

Habitats Lee et al. (1978a) described 40 strains of *V. metschnikovii* but none were from human clinical specimens; however, Jean-Jacques et al. (1981) reported the first clinically significant isolate of this newly redefined species. An 82-year-old woman with peritonitis and an inflamed gallbladder yielded *V. metschnikovii* from a positive blood culture. The isolate was considered clinically significant.

Table 3 gives the sources of the isolates in our collection. An unusual finding was the two strains from urine. One was from the midstream urine of an 80-year-old woman in Canada. The other was from a routine urine culture of a 65-year-old man with chronic alcoholism, poorly controlled diabetes, and incontinence and urinary frequency. Another interesting isolate was from a wound: A 64-year-old female with peripheral vascular disease and on renal dialysis developed an ulcer around a bunion on her foot, which was treated by a wedge resection, followed by a foot amputation and finally a below-the-knee amputation. The wound yielded *V. metschnikovii* along with five other possible pathogens. The patient went fishing frequently, which may have been her source of *V. metschnikovii*. Further study is needed to determine the clinical significance of *V. metschnikovii* from sources such as urine and wound.

Nonhuman sources include rivers, sewage, cockles, shrimp, lobster, crab, and fowl (Lee et al., 1981; and our own data). These data suggest that *V. metschnikovii* is widely distributed in the environment and that humans may occasionally become colonized or possibly infected from these sources.

Isolation and Identification *V. metschnikovii* grows on TCBS agar as 2–3 mm yellow colonies

(Table 4), and apparently grows well on other laboratory media. It requires Na^+ for growth but in much smaller amounts (5 to 15 mM) than the other Na^+ requiring *Vibrio* species (Baumann and Schubert, 1984). Table 7 gives the characteristics of *V. metschnikovii*. It is very easy to identify because it is oxidase negative, does not reduce nitrate to nitrite, and requires Na^+ for growth (Table 7). These three properties differentiate it from all the other species of Enterobacteriaceae and Vibrionaceae. *V. metschnikovii* is generally susceptible to antibiotics (Table 17).

Vibrio cincinnatiensis

Little is known about this species, which was first reported by Brayton et al. (1986) from a case of bacteremia and meningitis that yielded an unusual isolate of the genus *Vibrio*. Biochemical testing and 5S ribosomal RNA sequencing led to the conclusion that this was a new species, which was named *V. cincinnatiensis*.

Isolation and Identification *V. cincinnatiensis* grows very poorly on TCBS medium (Table 4), but isolates from extraintestinal clinical specimens should grow on one of the normal plating media such as blood agar. *V. cincinnatiensis* requires salt for growth (Table 7), and ferments myo-inositol (Table 6), which should make it easy to identify.

Vibrio carchariae

Grimes et al. (1984) studied two urease-positive halophilic vibrios isolated from a brown shark (*Carcharhinus plumbeus*) that had died in captivity in a large aquarium. One of the organisms (strain 1116a) was identified as *V. damsela*, but the other (strain 1116b) could not be identified. After further phenotypic testing and DNA-DNA hybridization, Grimes et al. (1984) concluded that this was a new species and named it *V. carchariae*. *V. carchariae* has now also been found in human clinical specimens.

Habitats *V. carchariae* has been isolated from other sharks since the original report, but it has also been shown to occur in human clinical specimens. Pavia et al. (1989) described a case in which the organism was isolated from a wound following a shark bite. An 11-year-old girl was attacked by a shark while wading in knee-deep water off the coast of South Carolina. She sustained several deep lacerations to her left calf that became infected after subsequent surgery. A culture from the infected wound yielded an unusual vibrio that was subsequently identified as *V. carchariae*.

Isolation and Identification *V. carchariae* appears to grow on plating media such as chocolate agar and MacConkey agar (Pavia et al., 1989), and grows well on TCBS agar as yellow colonies (Table 4). It is a halophilic vibrio and is similar to *V. parahaemolyticus*, *V. alginolyticus*, and *V. vulnificus* in its biochemical properties, but can be differentiated (Table 14). Strains are resistant to ampicillin, carbenicillin, and colistin (Table 17).

Vibrio Species That Do Not Occur in Human Clinical Specimens

In addition to the 12 *Vibrio* species discussed above that occur in clinical specimens, there are over 20 marine species that apparently do not. Theoretically, these marine vibrios could occur in feces following ingestion of environmental water or uncooked seafood, and we have some evidence that this occurs. Many of the species have few literature citations (see Table 1 in Chapter 156), and this group has not been studied as thoroughly as the human pathogens. Table 15 gives the phenotypic properties of these species based on the methods used in our laboratory. Some of the biochemical test results at 25°C in media with added marine cations differ from those originally published. This may reflect the different methods used since these types of differences are not unusual in "between-laboratory" comparisons. These marine species are briefly described in the following sections.

Vibrio anguillarum

V. anguillarum is a marine vibrio that causes disease in marine fish and other marine animals (Baumann et al., 1978; Sakazaki and Balows, 1981; Smith, 1961). The most important is a septicemic disease in marine fish characterized by a deep necrotizing myositis and subdermal hemorrhages. This disease is particularly important when it occurs at fish farms because the economic losses are world-wide and considerable. The main predisposing factor seems to be a rise in water temperature to 15°C or more (Sakazaki and Balows, 1981). *V. anguillarum* may also cause a necrotic disease of bivalve mollusks' larvae and juveniles (Tubiash et al., 1970).

V. anguillarum grows on many bacteriological media if the NaCl content is 1 to 2%, but most strains do not grow well on TCBS agar (Sakazaki and Balows, 1981). BTB teepol agar, originally described for the isolation of *V. parahaemolyticus*, is a good isolation medium for specimens obtained from diseased fish.

BTB-Teepol Agar (Akiyama et al., 1963)

Beef extract	5 g
Peptone	10 g
Sucrose	10 g
Sodium chloride	20 g
Teepol (Shell Chemical Co.)*	2 ml
Bromthymol blue	80 mg
agar	15 g
water	1 liter

Adjust the ph to 7.8. Dissolve the ingredients by gentle heating. Autoclave at 121°c for 15 min, and pour into plates. *V. anguillarum* grows as moderate-sized yellow colonies (Sakazaki and Balows, 1981), but other vibrios also grow, so complete identification will be required for the colonies picked.

*Sakazaki and Balows (1981) indicate that Turgitol 7 may be substituted.

Identification of *V. anguillarum* may be difficult because strains vary considerably in their phenotypic properties (Table 15). Strain from fish disease with this biochemical pattern are likely to be *V. anguillarum*.

Vibrio ordalii

V. ordalii is a halophilic *Vibrio* species that is very closely related to *Vibrio anguillarum* and was named by Schiewe et al. (1981). Previously, other investigators had studied vibriosis of marine fish and isolated a halophilic vibrio similar to, but less biochemically active (Table 15) than *V. anguillarum*. This organism was referred to as "*Vibrio* sp. 1669 group," "*Vibrio* sp. RT group" *Beneckea anguillara* biotype I, or *Vibrio anguillarum* biotype 2. However, the DNA-DNA hybridization studies of Schiewe et al. (1981) indicated that, although it was closely related to *V. anguillarum*, it was a separate species. *V. ordalii* has been isolated from diseased salmonid fish in Washington, Oregon, British Columbia, and Japan reference is not an exact match (Schwiewe et al., 1981), and from ayu (*Plecoglossus altivelis*) and rockfish fingerlings (*Sebastes schlegeli*) in Japan (Muroga et al., 1986). The GC content of the DNA is 43–44 mol%.

Vibrio aestuarianus

V. aestuarianus is a halophilic *Vibrio* species that was first described by Tison and Seidler (1983) who isolated it from water and shellfish off the coast of Oregon. Strains are arginine-positive, Voges-Proskauer-negative, sucrose-positive, and lactose-positive, and do not grow in media with 0 or 5% NaCl. The GC content of the DNA is 43–44 mol%.

Vibrio campbellii

V. campbellii is a halophilic *Vibrio* species that was first described by Baumann et al. (1971a) who isolated 60 strains from ocean water off the coast of Hawaii. They noted considerable variation in the phenotypic properties of this large group, but included all of them in *V. campbellii*. The GC content of the DNA is 46–48 mol%, and this species is about 65% related by DNA-DNA hybridization to *Vibrio harveyi* (Reichelt et al., 1976). Grimes et al. (1986) isolated 20 strains from seawater during a voyage from Barbados to Puerto Rico to Bermuda, and concluded that *V. campbellii* is also found in the open waters of the Atlantic ocean.

Vibrio costicola

V. costicola was first described by Smith (1938). It is a unique *Vibrio* species because of its large requirement for and tolerance to NaCl. Strains usually will not grow in laboratory media unless the NaCl content is 200–250 mM (1.17 to 1.46% NaCl), which defines *V. costicola* as a moderate halophile. Strains have been isolated from environments with a very high salt content, such as meat-curing brines and solar salterns (Garcia et al., 1987). *V. costicola* has been a popular organism in physiological studies, particularly those comparing different halophilic bacteria or comparing halophilic with nonhalophilic species. The GC content of the DNA is 50 mol%.

Vibrio diazotrophicus

V. diazotrophicus is a halophilic *Vibrio* species that was first described by Guerinot and Patriquin (1981), who isolated nitrogen-fixing vibrios from sea urchins (*Stronglyocentrotus droebachiensis*) in Nova Scotia. This group of three strains plus 10 additional ones was named *Vibrio diazotrophicus* by Guerinot et al. (1982) because it was not highly related to other vibrios by DNA-DNA hybridization. An additional nitrogen-fixing species isolated from a sea urchin (*Tripneustes ventricosus*) in Barbados was only 14% related by DNA-DNA hybridization, so it was excluded from the species. The ability to fix molecular nitrogen seems to be a unique property for this species of *Vibrio*. Other strains have been isolated from ditch water, sediment, and reeds at Chetney Marsh, England; the Humbler River (England), and Chesapeake Bay water. The GC content of the DNA is 46–47 mol%.

Vibrio fischeri

V. fischeri is a halophilic *Vibrio* species that is also bioluminescent. It was first described as *Photobacterium fischerii* by Beijerinck (1989) and later classified in the genus *Vibrio* by Lehmann and Neumann (1896). It was named for Bernhard Fisher who was one of the first to sys-

tematically study bioluninescent bacteria. Strains grow at 30°C but many can not grow at 37°C. Many strains produce yellow pigment. *V. fischeri* has been isolated from many geographical locations and from coastal and open ocean seawater, surfaces and feces of fish and squids, luminous organs (Baumann and Baumann, 1981), and seafood (Furniss et al., 1978). The GC content of the DNA is 39–41 mol%.

Vibrio gazogenes

V. gazogenes is a halophilic *Vibrio* species that produces a characteristic dry red colony on marine agar that makes it easy to isolate and identify. It was first described as *Beneckea gazogenes* by Harwood (1978), but was reclassified in the genus *Vibrio* when the genus *Beneckea* was abolished. Strains produce gas during fermentation but do not reduce nitrate to nitrite, which also proves very useful in identification. *V. gazogenes* has been isolated from salt marshes in Massachusetts (Harwood, 1978), North Carolina, and South Carolina (Farmer et al., 1988) and from a hypersaline basin at Laguna Figueroa, Baja California de Norte, Mexico (Giovannoni and Margulis, 1981). The GC content of the DNA is 47 mol%.

Vibrio harveyi

V. harveyi is a halophilic *Vibrio* species that was first described as *Achromobacter harveyi* by Johnson and Shunk (1936), who named it for E. N. Harvey, a pioneer in the study of bioluminescence. It has been classified in *Lucibacterium* and *Beneckea*, but is now included in *Vibrio*. Many strains are bioluminescent, but nonluminescent strains occur also and can be difficult to distinguish from other vibrios (Furniss et al., 1978), particularly *V. vulnificus* (Yang et al., 1983). *V. harveyi* has been isolated from many geographical locations and from coastal and open ocean seawater, and surfaces and feces of fish and squids (Baumann and Baumann, 1981). Many papers have been published on its physiology and metabolism, and it is often used in bioluminescence studies. The GC content of the DNA is 46–48 mol%.

Vibrio logei

V. logei is a halophilic *Vibrio* species that was first described as *Photobacterium logei* by reference is not an exact matchBang et al. (1978) who studied a group of strains that had been isolated from a variety of marine sources. Strains of *V. logei* are bioluminescent, yellow-pigmented, and grow at 4 and 15°C, but not at 30°C. Many of the isolates of *V. logei* were obtained in the Arctic, and sources include exoskeleton lesions of tanner crabs, fish intestinal contents, scallops, and marine sediments (Baumann and Baumann, 1981). The GC content of the DNA is 40–42 mol%.

Vibrio marinus

V. marinus is a halophilic *Vibrio* species that was first described as *Spirillum marinum* by Russell (1891), who isolated it from samples taken in the Gulf of Naples (Ford, 1927; Colwell and Morita, 1964). Colwell and Morita (1964) described 16 strains of *V. marinus* that were isolated from Puget Sound on the Pacific coast of the United States. However, only one strain (ATCC 15381, from seawater) is still available, and there has been limited work on this species. *V. marinus* is a psychrophilic marine vibrio and grows at 4 and 20°C, but not at 30°C (Baumann et al., 1984). The GC content of the DNA is 42 mol%.

Vibrio mediterranei

V. mediterranei is a halophilic *Vibrio* species that was first described by Pujalte and Garay (1986), who isolated it from plankton, sediments, and seawater in two coastal areas south of Valencia, Spain (Pujalte et al., 1983). There are no outstanding characteristics for *V. mediterranei*, so complete biochemical testing is required to distinguish it from other marine vibrios (Table 4). The GC content of the DNA is 42–43 mol%.

Vibrio natriegens

V. natriegens is a halophilic *Vibrio* species that was first described as *Pseudomonas natriegens* by Payne et al. (1961), who isolated it from salt marsh mud on Sapelo Island off the coast of Georgia. *V. natriegens* uses a wider variety of carbon sources than other marine vibrios (Baumann and Baumann, 1984), which make it easy to isolate and identify. It also has the shortest generation time (9.8 min) of any bacterium (Eagon, 1962), which makes it a popular species to use in teaching exercises and physiological studies. *V. natriegens* has been isolated from coastal seawater in several locations (Baumann and Baumann, 1981; Furniss et al., 1978). The GC content of the DNA is 46–47 mol%.

Vibrio nereis

V. nereis is a halophilic *Vibrio* species that was first described as *Beneckea nereida* by Baumann et al. (1971a), who isolated it from seawater off the coast of Oahu, Hawaii. *V. nereis* accumulates PHB is positive for arginine dihydrolase, and does not grow on TCBS agar (Furniss et al.,

1978), but is otherwise similar to other marine vibrios. Kusuda et al. (1986) isolated *V. nereis* from an outbreak of fatal infections of sea bream *(Acanthopagrus schlegeli)* at hatcheries in western Japan, but did not think this organism was actually infecting the fish. The GC content of the DNA is 46–47 mol%.

Vibrio nigripulchritudo

V. nigripulchritudo is a halophilic *Vibrio* species that was first described as *Beneckea nigrapulchrituda* by Baumann et al. (1971b), who isolated it from seawater off the coast of Oahu, Hawaii. Colonies of *V. nigripulchritudo* produce a distinct blue-black pigment which simplifies its isolation and identification. The GC content of the DNA is 46–47 mol%.

Vibrio orientalis

V. orientalis is a halophilic *Vibrio* species that was first described by Yang et al. (1983), who isolated it from seawater and shrimp off the coast of China. No other isolates have been reported in the literature. All the original strains of *V. orientalis* were reported to be bioluminescent, but in our hands strains (which had been freeze-dried) have been weak or negative. Strains of *V. orientalis* grow at 4, 30, and 35°C, accumulate PHB, and are arginine positive. However, it can easily be confused with other marine vibrios (Table 15). The GC content of the DNA is 45–46 mol%.

Vibrio pelagius

V. pelagius is a halophilic *Vibrio* species that was first described as *Beneckea pelagia* by Baumann et al. (1971a) who isolated it from seawater off the coast of Oahu, Hawaii. Few additional isolations have been reported in the literature; however, Furniss et al. (1978) indicate that *V. pelagius* is one of the most common vibrios isolated around the British coasts. This species is subdivided into two groups based on DNA-DNA hybridization and phenotypic properties, and can easily be confused with other marine vibrios. The GC content of the DNA is 45–47 mol%.

Vibrio proteolyticus

V. proteolyticus is a halophilic *Vibrio* species that was first described as *Aeromonas proteolytica* by Merkel et al. (1964), who isolated it from *Limnoria tripunctata* (a small, wood-boring isopod crustacean) collected from wood pilings at Fort Jackson Marine Biological Laboratory, Charleston, South Carolina. This is the only strain described in the literature. This strain of *V. proteolyticus* swarms on complex marine media, is highly proteolytic and Voges-Proskauer positive, but can easily be confused with other marine vibrios (Table 15). The GC content of the DNA is 50.5 mol%.

Vibrio salmonicida

V. salmonicida is a halophilic *Vibrio* species that causes a serious disease ("Hitra disease" or "cold-water vibriosis") of Atlantic salmon (*Salmo salar*) and rainbow trout (*Salmo gairdneri*) in Norwegian salmonid farms (Egidius et al., 1981). This organism had a number of vernacular names until it was named *V. salmonicida* by reference is not an exact matchEgidus et al. (1986), who showed that it was not closely related to other *Vibrio* species by DNA-DNA hybridization (Wiik and Egidius, 1986). Strain of *V. salmonicida* grow slowly on laboratory media and have a very low growth temperature range of 1–22°C. Its psychrophilic nature, very slow growth rate, and narrow ecological niche are helpful in isolation and identification. The GC content of the DNA is 42 mol%.

Vibrio splendidus

V. splendidus is a halophilic *Vibrio* species that was first described as *Photobacter splendidum* by Beijerinck (1900). This species is subdivided into two groups by DNA-DNA hybridization and phenotypic properties, and can easily be confused with other marine vibrios (Table 15). *V. splendidus* biogroup 1 strains are bioluminescent and positive for arginine dihydrolase, but strains of biogroup 2 are negative for both tests. Strains of *V. splendidus* have been isolated from seawater and fish on the east coast of North America and Denmark (Baumann and Baumann, 1981). The GC content of the DNA is 45–46 mol%.

Vibrio tubiashii

V. tubiashii is a halophilic *Vibrio* species that was first isolated by Tubiash et al. (1965), who was studying bacilliary necrotic disease of larval and juvenile bivalve mollusks. These authors identified the causative bacterium as *Vibrio anguillarum* and deposited three strains (ATCC 19105, 19106, and 19109) in the American Type Culture Collection. However, Hada et al. (1984) studied these three strains and showed that they belonged to a new species they named *V. tubiashii*, in honor of H. S. Tubiash. This species does not have any outstanding phenotypic properties to distinguish it from other marine vibrios, but seems to be isolated only from diseased marine mollusks. It is pathogenic for larvae of three oyster species (Hada et al., 1984), *Crassostrea virginica*, *Crassostrea gigas*, and *Ostrea edulis* (Lodeiros et al.,

1987); larvae of the clam *Mercenaris mercenaria* (Hada et al., 1984); and for the lesser octopus *Eledone cirrhosa* (Bullock et al., 1987). The GC content of the DNA is 43–45 mol%.

The Genus *Photobacterium*

The genus name *Photobacterium* was first used by Beijerinck (1889), and for much of its history the genus has been based on a single characteristic, bioluminescence, the ability to produce light. As time passed, nonfermentative and Gram-positive bioluminescent species were removed from the genus, but only in the decade has it had a more narrow definition based on evolutionary relationships (Baumann and Baumann, 1981, 1984). Today, most authorities recognize only three species in the genus: *P. phosphoreum*, *P. leiognathi*, and *P. angustum*. Because other bioluminescent bacteria have been included in the genus *Photobacterium* for so much of its history, it is very difficult to identify the true species. Table 18 lists the differential and other properties of the three *Photobacterium* species.

History, Nomenclature, and Classification

A general discussion of bacterial bioluminescence is given in The Luminous Bacteria in the second edition. Bacterial bioluminescence dates back to ancient times, but its bacterial nature has been known for only a hundred years (Sieburth, 1979). Aristotle recorded that dead fish could sometimes produce light, and Robert Boyle noted that air was required for light production. However, it was not until the late 1880s that studies were done on bioluminescent bacteria (ZoBell, 1946).

Luminescent bacteria were isolated from a variety of marine samples by several early bacteriologists, and Pflüger (1875) may have been the first of these (ZoBell, 1946). He isolated luminescent bacteria from fish, but did not name any species. Bernard Fischer, for whom *Photobacterium fischeri* (now *Vibrio fischeri*) is named, served as a ship's physician on the voyage of the S. M. S. Molthe from the Baltic Sea to the West Indies and made a number of discoveries in marine microbiology (ZoBell, 1946). He isolated a bioluminescent organism from waters in the West Indies and named it *Photobacterium indicum* (Fischer, 1887).

Over the next hundred years, a large number of bioluminescent bacteria were isolated from different sources, mostly marine. Many of these organisms were given scientific names and classified in the genus *Photobacterium*. *Index Bergeyana* (Buchanan et al., 1966, p. 803–806) lists over 40 different *Photobacterium* species, and the *Supplement to Index Bergeyana* (Gibbons et al., 1981, p. 201–202) lists an additional 10 species. The original descriptions of most of these organisms are sketchy, but some are probably synonyms of the current *Photobacterium* species or of bioluminescent *Vibrio* species (Baumann and Baumann, 1981).

The genus *Photobacterium* as it is currently defined is based on the studies of Reichelt et al. (1976), who used DNA-DNA hybridization to compare several species of *Photobacterium* and *Vibrio* (Baumann and Baumann, 1981). They examined *P. phosphoreum* strain 404 and found that it was 84% related (at a hybridization temperature of 63°C) to the type strain of this species (strain 439 = ATCC 11040) and 92 to 99% related to three other strains of this species (Fig. 16). *P. phosphoreum* strain 404 was 22–36% related to five strains of *P. angustum*, and 24–36% related to five strains of *P. leiognathi*. However, these latter two *Photobacterium* species were more closely related to *P. phosphoreum* than was *V. harveyi* (11–13%) (called *Beneckea harveyi* in the paper) *V. fischeri* (5–12%), *V. cholerae* (0%), and six other *Vibrio species* (0–5%). The close relatedness of the three *Photobacterium* species is the basis of the genus as it is now defined; however, *P. angustum* and *P. leiognathi* are much more closely related to each other than they are to *P. phosphoreum* (Fig. 16).

In their chapter for the first edition of this handbook, Baumann and Bauman (1981) included *Vibrio fischeri* and *V. logei* (Bang et al., 1978a) in the genus *Photobacterium*, but in the Addendum they reclassified both of these species in the genus *Vibrio*. This latter classification was in better agreement with the morphological, phenotypic, and molecular data.

Description of the Genus *Photobacterium*

The following description now applies to the genus: small, plump, rod-shaped bacteria, Gram-negative, motile with one to three unsheathed polar flagella (Fig. 14, part 5; Fig. 15, parts 11 to 15), usually bioluminescent (two of the three species), accumulate large amounts of PHB when grown in a basal medium with glucose as the sole source of carbon (Fig. 15, part 13), do not form PHB when grown in rich media containing peptone, grow in the presence and absence of oxygen, are oxidase-positive (2 of 3 species) and catalase-positive, reduce nitrate to nitrite, grow with glucose as the sole source of carbon and NH_4^+ as the sole source of nitrogen (some strains require amino acids for growth, see Table 18), require Na^+ for growth, metabolism is fermentative rather than oxidative, and one species produces gas during fermentation.

Table 18. Differential and other properties of the three species of *Photobacterium*.[a]

Property or test	*P. phosphorium*	*P. leiognathi*	*P. angustum*
Association with fish family			
Leiognathidae	+	–	–
Apogonidae	+	–	–
Macrouridae	–	+	–
Merluccidae	–	+	–
Opisthoproctidae	–	+	–
Trachichthydae	–	+	–
Moridae	–	+	–
Growth at:			
4°C	96	0	(+)
20°C	+	+	+
25°C	99	100	
30°C	85	100	+
35°C	0	96	+
40°C	0	0	–
Cell morphology			
Straight rods	100	96	
Curved rods	0	4	
Poly-β-hydroxybutyrate accumulation	100	100	
Flagellation pattern			
Number of unsheathed polar flagella	1–3	1–3	
Sheathed polar or peritrichous flagella	–	–	
GC content (from Reichelt and Baumann, 1973)	41.2–41.8	42.8–43.3	
GC content (from Baumann and Baumann, 1984)	41–42	42–44	40–42
Require Na^+ (100 mM) for optimum growth	+	+	+
Oxidase (R1)	7	64	
Bioluminescent	+	+	–
Luciferase kinetics (from Hastings and Nealson, 1981)	Fast	Fast	–
Nitrate reduced to nitrite	93	96	V
Gas production during fermentation of D-glucose	89	7	–
Voges-Proskauer	97	93	
Growth on glucose as the sole source of carbon	58		
Nutritional requirements			
L-Methionine alone	24	0	–
L-Histidine alone	1	0	
L-Arginine alone	0	4	
L-Methionine plus one or two other amino acids	15	0	
Amino acid mixture	5	0	
Vitamins (yeast extract)	1	0	
Extracellular enzymes			
Gelatinase	0	0	(+)
Lipase	0	82	V
Chitinase	97	96	–
Alginase	0	0	–
Amylase	0	0	–
Growth on carbon sources:			
D-Xylose	–	–	+
Maltose	99	0	V
Acetate	0	86	+
DL-Glycerate	+	V	–
DL-Lactate	18	100	
Pyruvate	0	96	+
L-Proline	4	96	–
D-Glucuronate	49	0	

[a]The numbers give the percentage positive for *P. phosphoreum* (74 strains studied) and for *P. leiognathi* (28 strains). Adapted from Reichelt and Baumann (1976).

Habitats

Photobacterium and other bioluminescent bacteria are widely distributed in the marine environment (Baumann and Baumann, 1981, 1984), and one must be careful to determine which reports are using the terms *Photobacterium* and bioluminescent bacteria interchangeably. Biolumines-

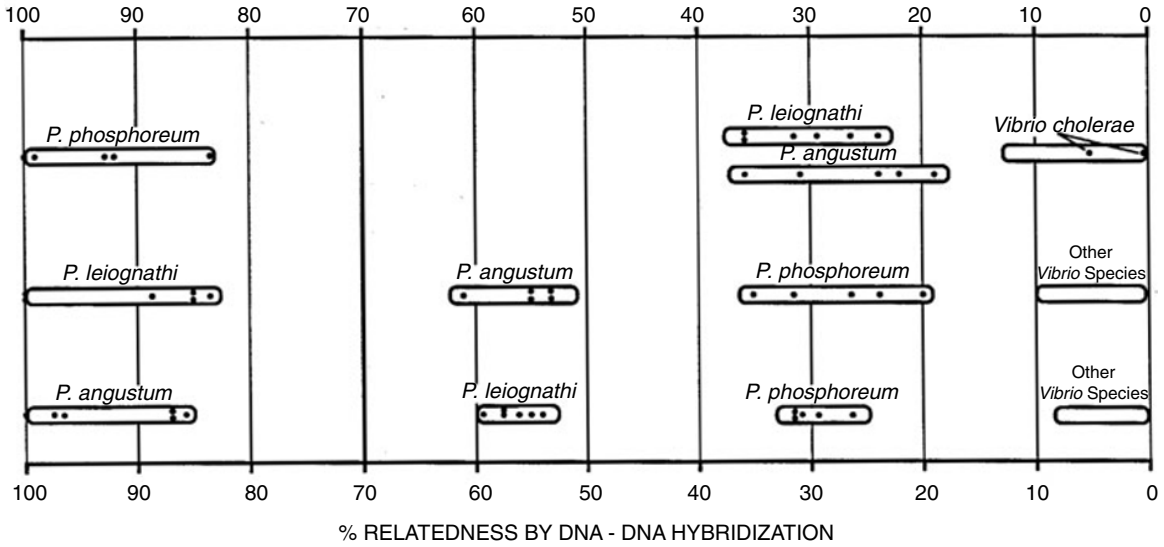

Fig. 16. Relatedness of the three species of *Photobacterium* by DNA-DNA hybridization; note that *P. angustum* and *P. leiognathi* are more closely related to each other than they are to the type species for the genus, *P. phosphoreum*.

cent bacteria are very common in marine water samples, and are presumably shed into the water from more protected ecological niches (Hastings and Nealson, 1977, 1981; see also The Luminous Bacteria in the second edition).

In addition to their common occurrence on fish and other marine animals, luminescent bacteria are found in the light organs (Fig. 17) of many marine animals (Bassot, 1975; Boisvert et al., 1967; Harvey, 1940; Hastings and Nealson, 1977, 1981). Two of the species of *Photobacterium* inhabit the light organs of marine fish. *P. phosphoreum* inhabits the light organs of five fish families (Table 18) that are usually found in deep cold waters. This ecological niche agrees with the fact that this species grows at 4°C, but poorly at 25°C and not at 30 or 37°C. *P. phosphoreum* is clearly a psychrophilic deep-water species. Reichelt and Baumann (1973) found it at depths of 50 to 500 off the coast of Hawaii. *P. leiognathi* inhabits the light organs of two fish families (Table 18) that are usually found in warm, shallow, tropical water (Hastings and Nealson, 1981). This species grows at higher temperatures, (Table 18) which agrees with this warmer habitat. In their review of *Photobacterium*, Baumann and Baumann (1984) mention that the only isolates of *P. angustum* have been from the open ocean off Hawaii. However, both *P. angustum* and *V. logei* have been reported recently from a marine fish-rearing unit (Austin, 1983).

Hastings and Nealson (1981) point out that light emission by bioluminescent bacteria may not directly benefit the bacterium, but may be important to its partner in a mutualistic symbiotic relationship. The symbiotic bioluminescent bacteria provide an excellent example of this relationship where the partner is usually a marine fish. The bacteria are localized in a light organ where they emit light. The review of the symbiotic bioluminescent bacteria by Hastings and Nealson (1981) is highly recommended (see also The Luminous Bacteria in the second edition).

Bioluminescent bacteria are very numerous in the intestinal tract of marine fish and often comprise close to 100% of the aerobic heterotrophic flora. They are also found on the gills, and in lower numbers on the skin (Baumann and Baumann, 1981, 1984).

Isolation

Strains of *Photobacterium* and bioluminescent *Vibrio* species can easily be isolated from seawater and marine animals (Baumann and Baumann, 1981; Hastings and Nealson, 1981). The best sources of *P. phosphoreum* and *P. leiognathi* are the luminous organs of marine fish (Table 18), where they are present in high numbers and are often in pure culture.

Seawater or similar marine media are usually required for growth and optimum light production. Luminescence and respiration are reduced in medium when seawater is diluted by more than 50% with fresh water. Isolation of luminescent vibrios has been discussed previously; there are no specific media to select for *Photobacterium* species at the expense of other vibrios.

Identification

The species of *Photobacterium* are phenotypically very similar to the marine species of *Vibrio* (Table 15; also see Table 4 in The Family

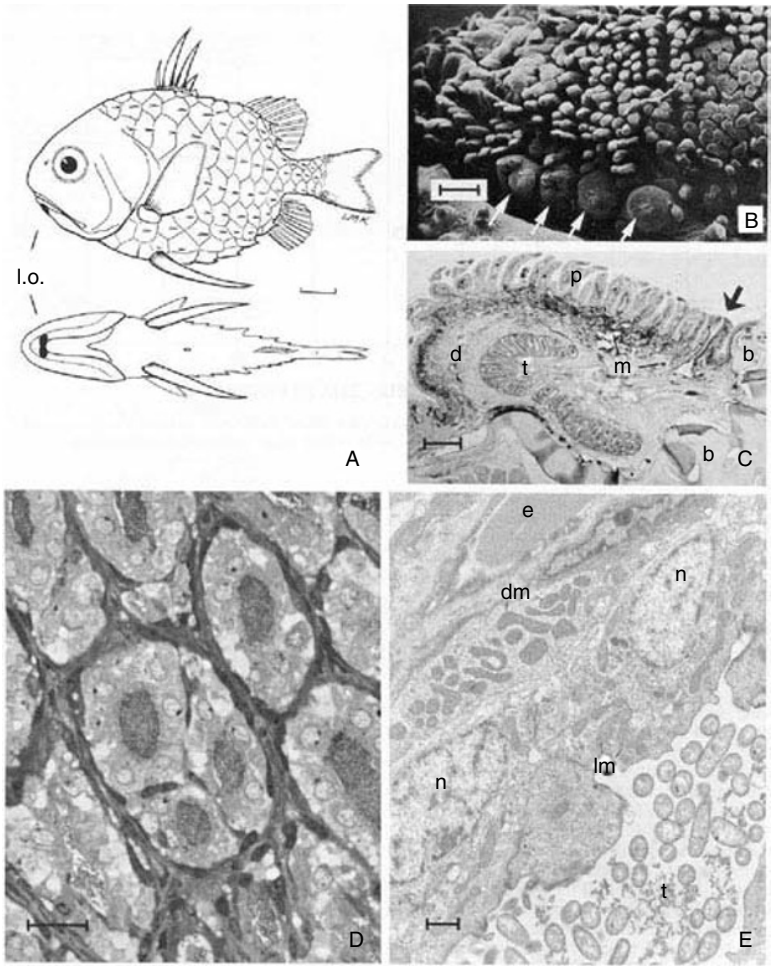

Fig. 17. *Monocentris japonicus.* (A) Line drawing of fish and ventral view of lower jaw, showing location of light organs (l.o.). Bar = 1.0 cm. (B) Scanning electron micrograph of the dorsal surface of the light organ. Numerous dermal papillae can be seen. The emissary ducts from the light organ emerge at the tips of the four large dermal papillae (arrows). Bar = 0.2 mm. (C) Light micrograph of a sagittal section of the lower jaw. m, melanocytes; t, tubules with bacteria; b, mandibular bone; d, dermal layer; p, dermal papillae. Arrow points to emissary duct. Bar = 50 µm. (D) Light micrograph showing the light organ tubules filled with bacteria. Tubules are lined with a single layer of cuboidal epithelial cells that display loose nuclear chromatin and prominent nucleoli supported by connective tissue cells. Blood capillaries are sparse and not readily visible. Bar = 15 µm. (E) Electron micrograph showing the major features of tubule epithelium. Epithelial cells that make up the lining of the tubules have light-staining mitochondria (lm) with fine cristae. Epithelial cells that are further away from the tubule lumen next to the blood capillaries have dark-staining mitochondria (dm) with thick cristae. t, tubule containing luminous bacteria; e, erythrocyte visible in capillary; n, nucleus of tubule epithelium cells. Bar = 1 µm.

Vibrionaceae in this Volume). For this reason it can be difficult to differentiate them (Baumann and Baumann, 1981; Hastings and Nealson, 1981). Samples from the light organs of certain marine fish (Table 18) are likely to yield either *P. phosphoreum* or *P. leiognathi*. Isolates from seawater or decaying organic matter are more difficult to identify to species because they could be one of the *Photobacterium* species or one of the other bioluminescent vibrios. Table 15 gives the biochemical reactions of the three species of *Photobacterium* and the species of *Vibrio* that can be confused with them. Several of the tests require comment.

Biochemical and Other Tests (Baumann and Baumann, 1981; Hastings and Nealson, 1981)

Oxidase The results for this test are variable for the three species of *Photobacterium*, in contrast to most strains of *Vibrio*, *Aeromonas*, and *Plesiomonas*, which are strongly oxidase-positive. Strains of *Photobacterium* apparently contain low levels of cytochrome *c,* the compound that is responsible for a positive oxidase reaction. Strains that are oxidase negative should be retested after treating the cells with toluene (Baumann and Baumann, 1981). Toluene treatment presumably causes cell lysis and liberation of internal proteins. Many oxidase-negative strains of *Photobacterium* are positive with this modification.

Flagella Stains Reichelt et al. (1976) showed that tufts of polar flagella often tended to fold backwards, giving the appearance of peritrichous flagella, especially in Leifson (1960) flagella stains.

Arginine Dihydrolase-Decarboxylase Strains of *Photobacterium* sometimes produce an alkaline reaction in arginine decarboxylase test media, such as Moeller's medium used in clinical microbiology laboratories, even though they lack the enzyme arginine dihydrolase (Baumann and Baumann, 1984). *P. phosphoreum* apparently decarboxylates and deaminates arginine.

Nutritional Requirements These tests may be helpful for identifying *Photobacterium* strains, particularly those that have been maintained in the laboratory. Most freshly isolated strains of *Photobacterium* and other luminescent bacteria do not require vitamins or amino acids (Ruby and Nealson, 1978), but strains that have been maintained in the laboratory may have a requirement (Baumann and Baumann, 1981) (Table 18). Frequent transfers on complex media probably lead to these nutritional defects.

Dark (Nonbioluminescent) Mutants of Bioluminescent Strains Strains may become nonbioluminescent when they are maintained in the laboratory or in culture collections in contrast to the original strains that are brightly luminescent.

Carbon Sources In contrast to most *Vibrio* species that are nutritionally very versatile, the species of *Photobacterium* can use only a few compounds as sources of carbon and energy (Baumann and Baumann, 1981, 1984).

Turnover Kinetics of the Enzyme Luciferase Hastings and Mitchell (1971) showed that the enzyme luciferase differs in its "turnover kinetics" depending on the bioluminescent species from which it is isolated. The luciferase from *V. harveyi* has slow turnover kinetics, in contrast to the fast turnover kinetics of *V. fischeri* and *P. leiognathi*. At one time this difference was used as a characteristic for differentiating the genus *Photobacterium* from the bioluminescent species of *Vibrio* (Hastings and Nealson, 1981).

Acknowledgments. We thank Paul and Linda Baumann who allowed us to reproduce and adapt text, tables, and figures of *Vibrio* and *Photobacterium* from their chapter, *The Marine Gram-Negative Eubacteria: Genera Photobacterium*, *Beneckea*, *Alteromonas*, *Pseudomonas* and *Alcaligenes* (Baumann and Baumann, 1981), in the first edition of *The Prokaryotes*; J. Woodland Hastings and Kenneth H. Nealson for allowing us to reproduce figures from their chapter, *The Symbiotic Luminous Bacteria* (Hastings and Nealson, 1981), in the first edition of *The Prokaryotes*; and the staff and visiting scientists of the Enteric Bacteriology Laboratories at the Centers for Disease Control who did the phenotypic characterization of strains in our collection.

Literature Cited

Akiyama, S., K. Takizawa, H. Ichinoe, S. Enomoto, T. Kobayashi, R. Sakazaki. 1963. Application of teepol to isolation of *Vibrio parahaemolyticus* [In Japanese]. Jpn. J. Bacteriol. 18:255–256.

Allen, R. D., P. Baumann. 1971. Structure and arrangement of flagella in species of the genus *Beneckea* and *Photobacterium fischeri*. J. Bacteriol. 107:295–302.

Anderson, J. I. W., D. A. Conroy. 1970. *Vibrio* disease in marine fishes. 266–272.S. F. Snieszko (ed.) A symposium on diseases of fishes and shell fishes. Special publication no. 5, American Fisheries Society. Washington, DC.

Anderson, R. S., E. J. Ordal. 1975. Deoxyribonucleic acid relationships among marine vibrios. J. Bacteriol. 109: 696–706.

Austin, B. 1983. Bacterial micro flora associated with a coastal marine fish rearing unit. J. Mar. Biol. Assoc. U. K. 63:585–592.

Baker, D. A., R. W. A. Park. 1975. Changes in morphology and cell wall structure that occur during growth of *Vibrio* sp. NCTC 4716 in batch culture. J. Gen. Microbiol. 86: 12–28.

Balows, A., G. H. Herman, W. E. DeWitt. 1971. The isolation and identification of *Vibrio cholerae*—a review. Health. Lab. Sci. 8:167–175.

Bang, S. S., P. Baumann, K. H. Nealson. 1978a. Phenotypic characterization of *Photobacterium logei* (sp. nov.). a species related to *P. fischeri.*Curr. Microbiol. 1:285–288.

Bang, S. S., M. J. Woolkalis, P. Baumann. 1978b. Electrophoretic mobilities of superoxide dismutases from species of *Photobacterium*, *Beneckea*, *Vibrio*, and selected terrestrial enterobacteria. Curr. Microbiol. 1:371–376.

Barker, W. H., Jr., E. J. Gangarosa. 1974. Food poisoning due to *Vibrio parahaemolyticus*. Ann. Rev. Med. 25:75–81.

Baross, J. A., P. A. Tester, R. Y. Morita. 1978. Incidence, microscopy, and etiology of exoskeleton lesions in the tanner crab, *Chionoecetes tanneri*. J. Fisheries Res. Board Canada 35:1141–1149.

Barua, D. 1970. Laboratory diagnosis of cholera cases and carriers. Public Health Pap. 40:47–52.

Barua, D., W. Burrows (ed.). 1974. Cholera. The W. B. Saunders Co. Philadelphia.

Bassot, J.-M. 1975. Les organes lumineux à bactéries symbiotiques de quelques téléostéens *Léiognathides*. Arch. de Zoologie Expérimentale Générale 116:359–373.

Baumann, L., P. Baumann. 1973. Regulation of aspartokinase activity in the genus *Beneckea* and marine, luminous bacteria. Archiv fur Mikrobiologie. 90:171–188.

Baumann, L., P. Baumann. 1976. Study of relationship among marine and terrestrial enterobacteria by means of in vitro DNA-ribosomal RNA hybridization. Microbios Lett. 3:11–20.

Baumann, L., P. Baumann. 1978. Studies of relationship among terrestrial *Pseudomonas*, *Alcaligenes*, and enterobacteria by an immunological comparison of glutamine synthetase. Archiv. Microbiol. 119:25–30.

Baumann, L., P. Baumann, M. Mandel, R. D. Allen. 1972. Taxonomy of aerobic marine eubacteria. J. Bacteriol. 110:402–429.

Baumann, P., S. S. Bang, L. Baumann. 1978. Phenotypic characterization of *Beneckea anguillara* biotypes I and II. Curr. Microbiol. 1:85–88.

Baumann, P., L. Baumann. 1973. Phenotypic characterization of *Beneckea parahaemolytica*: a preliminary report. J. Milk Food Technol. 36:214–219.

Baumann, P., L. Baumann. 1977. Biology of the marine enterobacteria: genera *Beneckea* and *Photobacterium*. Ann. Rev. Microbiol. 31:39–61.

Baumann, P., L. Baumann. 1981. The marine Gram-negative eubacteria: Genera *Photobacterium*, *Beneckea*, *Alteromonas*, *Pseudomonas*, and *Alcaligenes*. 1302–1331.M. P. Starr, H. Stolp, H. G. Trüper, A. Balows, and H. G. Schlegel (ed.) The prokaryotes. Springer-Verlag. Berlin.

Baumann, P., L. Baumann. 1984. Genus II. *Photobacterium* Beijerink 1889, 401[AL]. 539–545. N. R. Krieg and J. G. Holt (ed.) Bergey's manual of systematic bacteriology, vol. 1. Williams and Wilkins. Baltimore.

Baumann, P., L. Baumann, M. Mandel. 1971a. Taxonomy of marine bacteria: the genus *Beneckea*. J. Bacteriol. 107: 268–294.

Baumann, P., L. Baumann, M. Mandel, R. D. Allen. 1971b. Taxonomy of marine bacteria: *Beneckea nigrapulchrituda* sp. n. J. Bacteriol. 108:1380–1383.

Baumann, P., L. Baumann, J. L. Reichelt. 1973. Taxonomy of marine bacteria: *Beneckea parahaemolytica* and *Beneckea alginolytica*. J. Bacteriol. 113:1144–1155.

Baumann, P., A. L. Furniss, J. V. Lee. 1984. Genus I. *Vibrio* Pacini 1854, 411[AL]. 518–538. N. R. Krieg and J. G. Holt (ed.) Bergey's manual of systematic bacteriology, vol. 1. Williams and Wilkins. Baltimore.

Baumann, P., R. H. W. Schubert. 1984. Family II. Vibrionaceae Vernon. 1965, 5245[AL]. 516–517. N. R. Krieg and J. G. Holt (ed.) Bergey's manual of systematic bacteriology, vol. 1. Williams and Wilkins. Baltimore.

Beijerinck, M. W. 1889. *Le Photobacterium luminosum*. Bactérie luminosum de la Mer. Nord. Arch. Neer. Sci. 23:401–427.

Beijerinck, M. W. 1900. On different forms of hereditary variation in microbes. Proc. Acad. Sci. Amst. 3:352–365.

Benenson, A. S., M. R. Islam, W. B. Greenough III. 1964. Rapid identification of *Vibrio cholerae* by darkfield microscopy. Bull. W.H.O. 30:827–831.

Berger, L. R., D. M. Reynolds. 1958. The chitinase system of a strain of *Streptomyces griseus*. Biochim. et Biophy. Acta 29:522–534.

Blake, P. A. 1981. New information on the epidemiology of *Vibrio* infections. 107–117. T. Holme, J. Holmgren, M. H. Merson, and R. Möllby (ed.) Acute enteric infections in children. New prospects for treatment and prevention. Elsevier/North Holland Publishing Co. Amsterdam.

Blake, P. A., D. T. Allegra, J. D. Snyder, T. J. Barrett, L. McFarland, C. T. Caraway, J. C. Feeley, J. P. Craig, J. V. Lee, N. D. Puhr, R. A. Feldman. 1980a. Cholera—a possible endemic focus in the United States. N. Engl. J. Med. 302:305–309.

Blake, P. A., M. H. Merson, R. E. Weaver, D. G. Hollis, P. C. Heublein. 1979. Disease caused by a marine *Vibrio*: clinical characteristics and epidemiology. N. Eng. J. Med. 300:1–5.

Blake, P. A., R. E. Weaver, D. G. Hollis. 1980b. Diseases of humans (other than cholera) caused by vibrios. Ann. Rev. Microbiol. 34:341–367.

Boisvert, H., R. Chatelain, J.-M. Bassot. 1967. Etude d'un *Photobacterium* isole de l'organe lumineux de poissons *Leiognathidae*. Ann. Instit. Pasteur (Paris) 112:520–524.

Bonner, J. R., A. S. Coker, C. R. Berryman, H. M. Pollock. 1983. Spectrum of *Vibrio* infections in a Gulf Coast community. Ann. Intern. Med. 98:464–469.

Brayton, P. R., R. B. Bode, R. R. Colwell, M. T. MacDonell, H. L. Hall, D. J. Grimes, P. A. West, T. N. Bryant. 1986. *Vibrio cincinnatiensis* sp. nov., a new human pathogen. J. Clin. Microbiol. 23:104–108.

Brenner, D. J., B. R. Davis, Y. Kudok, M. Ohashi, R. Sakazaki, T. Shimada, H. L. Smith Jr. 1982. Serological comparison of two collections of *Vibrio cholerae* non O1. J. Clin. Microbiol. 16:319–323.

Brenner, D. J., G. R. Fanning, F. W. Hickman-Brenner, A. G. Steigerwalt, B. R. Davis, J. J. Farmer, III. 1983a. DNA relatedness among Vibrionaceae with emphasis on the *Vibrio* species associated with human infection. Colloq. INSERM (Inst. Nat. Sante Rech. Med.) 114:175–184.

Brenner, D. J., F. W. Hickman-Brenner, J. V. Lee, A. G. Steigerwalt, G. R. Fanning, D. G. Hollis, J. J. Farmer, III, R. E. Weaver, R. J. Seidler. 1983b. *Vibrio furnissii* (formerly aerogenic biogroup of *Vibrio fluvialis*), a new species isolated from human feces and the environment. J. Clin. Microbiol. 18:816–824.

Buchanan, R. E., J. G. Holt, E. F. Lessel. 1966. Index Bergeyana. William and Wilkins Co. Baltimore.

Bullock, A. M., J. L. Polglase, S. E. Phillips. 1987. The wound healing and hemocyte response in the skin of the lesser octopus *Eledone cirrhosa (Mollusca Cephalopoda)* in the presence of *Vibrio tubiashii*. J. Zool. (London). 211:373–386.

Burdon, K. L. 1946. Fatty material in bacteria and fungi revealed by staining dried, fixed slide preparations. J. Bacteriol. 52:665–678.

Caldwell, D. R., R. F. Hudson. 1974. Sodium, an obligate growth requirement for predominant rumen bacteria. Appl. Microbiol. 27:549–552.

Chatelain, R., H. Bercovier, A. Guiyoule, C. Richard, H. H. Mollaret. 1979. Intérêt du composé vibriostatique 0/129 pour différencier les genres *Pasteurella* et *Actinobacillus* de la famille des Enterobacteriaceae. Ann. Microbiol. (Inst. Pasteur) 130A:449–454.

Clark, W. A., A. G. Steigerwalt. 1977. Deoxyribonucleic acid reassociation experiments with a halophilic, lactose-fermenting vibrio isolated from blood cultures. Int. J. Syst. Bacteriol. 27:194–199.

Colwell, R. R. 1965. Proposal of a neotype, ATCC 15381, for *Vibrio marinus* (Russell 1891) Ford 1927 and request for an opinion. Inter. Bull. Bacteriol. Nomen. Taxon. 15:165–175.

Colwell, R. R. 1973. Vibrio and Spirilla. 97–104. A. I. Laskin, and H. A. Lechevalier (ed.) CRC handbook of microbiology, vol. 1. CRC Press. Cleveland, OH.

Colwell, R. R. (ed.). 1984. *Vibrios* in the environment. John Wiley and Sons. New York,

Colwell, R. R., R. Y. Morita. 1964. Reisolation and emendation of description of *Vibrio marinus* (Russell) Ford. Jour. Bact. 88:831–837.

Cosenza, B. J., J. D. Buck. 1966. Simple device for enumeration and isolation of luminescent bacterial colonies. Appl. Microbiol. 14:692.

Crawford, I. P. 1975. Gene rearrangements in the evolution of the tryptophan synthetic pathway. Bacteriol. Rev. 39:87–120.

Davis, B. R., G. R. Fanning, J. M. Madden, A. G. Steigerwalt, H. B. Bradford Jr., H. L. Smith Jr., D. J. Brenner. 1981. Characterization of biochemically atypical *Vibrio cholerae* strains and designation of a new pathogenic species, *Vibrio mimicus*. J. Clin. Microbiol. 14:631–639.

de Boer, W. E., C. Golten, W. A. Scheffers. 1975a. Effects of some physical factors on flagellation and swarming of *Vibrio alginolyticus*. Netherlands J. Sea Res. 9:197–213.

de Boer, W. E., C. Golten, W. A. Scheffers. 1975b. Effects of some chemicals factors on flagellation and swarming of *Vibrio alginolyticus*. Antonie van Leeuwenhoek, J. Serol. Microbiol. 41:385–403.

Donovan, T. J., A. L. Furniss. 1982. Quality of antisera used in the diagnosis of cholera. Lancet 2:866–868.

Doudoroff, M. 1942a. Studies on the luminous bacteria. I. Nutritional requirements of some species with special reference to methionine.J. Bacteriol. 44:451–459.

Doudoroff, M. 1942b. Studies on luminous bacteria. II. Some observations on the anaerobic metabolism of facultatively anaerobic species.. J. Bacteriol. 44:461–467.

Drapeau, G. R., T. I. Matula, R. A. MacLeod. 1966. Nutrition and metabolism of marine bacteria. XV. Relation of Na(+)-activated transport to the Na(+) requirement of a marine pseudomonad for growth. J. Bacteriol. 92:63–71.

Eagon, R. G. 1962. *Pseudomonas natriegens*, a marine bacterium with a generation time of less than 10 minutes. J. Bacteriol. 83:736–737.

Eagon, R. G., C. H. Wang. 1962. Dissimilation of glucose and gluconic acid by *Pseudomonas natriegens*. J. Bacteriol. 83:879–886.

Eberhard, A., G. Rouser. 1971. Quantitative analysis of the phospholipids of some marine bioluminescent bacteria. Lipids 6:410–414.

Egidius, E., K. Andersen, E. Clausen, J. Raa. 1981. Cold-water vibriosis or "Hitra disease" in Norwegian salmonid farming. J. Fish Dis. 4:353–354.

Egidius, E., R. Wiik, K. Andersen, K. A. Hoff, B. Hjeltnes. 1986. *Vibrio salmonicida* sp. nov., a new fish pathogen. Int. J. Syst. Bacteriol. 36:518–520.

Farmer, J. J., III. 1979. *Vibrio ("Beneckea") vulnificus*, the bacterium associated with sepsis, septicemia, and the sea. Lancet 2:903.

Farmer, J. J., III. 1980. Revival of the name *Vibrio vulnificus*. Int. J. Syst. Bacteriol. 30:656.

Farmer, J. J., III, B. R. Davis, F. W. Hickman-Brenner, A. McWhorter, G. P. Huntley-Carter, M. A. Asbury, C. Riddle, H. G. Wathen-Grady, C. Elias, G. R. Fanning, A. G. Steigerwalt, C. M. O'Hara, G. K. Morris, P. B. Smith, D. J. Brenner. 1984. Biochemical identification of new species and biogroups of Enterobacteriaceae isolated from clinical specimens. J. Clin. Microbiol. 21:46–76.

Farmer, J. J., III, F. W. Hickman-Brenner, G. R. Fanning, C. M. Gordon, D. J. Brenner. 1988. Characterization of *Vibrio metschnikovii* and *V. gazogenes* by DNA-DNA hybridization and phenotype. J. Clin. Microbiol. 26:1993–2000.

Farmer, J. J., III, F. W. Hickman-Brenner, M. T. Kelly. 1985. *Vibrio*. 282–301. E. H. Lennette, A. Balows, W. J. Hausler, Jr., and H. J. Shadomy (ed.) Manual of clinical microbiology, 4th ed. American Society for Microbiology. Washington, DC.

Farmer, J. J., III, J. G. Wells, W. Terranova, M. L. Cohen, C. M. West. 1981. Enterobacteriaceae. 341–392. A. Balows, and W. J. Hausler (ed.) Diagnostic procedures for bacterial, mycotic, and parasitic infections. 6th ed. American Public Health Association. Washington, DC.

Felter, R. A., R. R. Colwell, G. B. Chapman. 1969. Morphology and round body formation in *Vibrio marinus*. J. Bacteriol. 99:326–335.

Finkelstein, R. A. 1973. Cholera. Crit. Rev. Microbiol. 2:553–623.

Fischer, B. 1984. Die Bakterien des Meeres nach den Untersuchungen der Plankton-Expedition unter gleichzeitiger Berucksichtigung einiger alterer und neuerer Untersuchungen. Ergebnisse der Plankton-Expedition der Humbolt-Stiftung. 4:1–83.

Fischer, B. 1887. Bacteriologische untersuchungen auf einer reise nach westindien. II Ueber einen lichtentwickelnden im meerswasser gefundenen spaltpitz. Zeitschr. F. Hyg. 2:54–95.

Fitzgerald, J. M. 1977. Classification of luminous bacteria from the light organ of the Australian Pinecone fish, *Cleidopus gloriamaris*. Archiv. Microbiol. 112:153–156.

Ford, W. W. 1927. Textbook of bacteriology. W. B. Saunders Co. Philadelphia, Pa.

Forsberg, C. W., J. W. Costerton, R. A. MacLeod. 1970. Separation and localization of cell wall layers of a Gram-negative bacterium. J. Bacteriol. 104:1338–1353.

Fox, G. E., E. Stackebrandt, R. B. Hespell, J. Gibson, J. Maniloff, T. A. Dyer, R. S. Wolfe, W. E. Balch, R. S. Tanner, L. J. Magrum, L. B. Zablen, R. Blakemore, R. Gupta, L. Bonen, B. J. Lewis, D. A. Stahl, K. R. Leuhrsen, K. N. Chen, C. R. Woese. 1980. The phylogeny of procaryotes. Science 209:457–463.

Fujino, T., G. Sakaguchi, R. Sakazaki, Y. Takeda (ed.). 1974. International symposium on *Vibrio parahaemolyticus*. Saikon Publishing Company, Ltd. Tokyo.

Furniss, A. L., J. V. Lee, T. J. Donovan. 1978. The *Vibrios*. Public Health Laboratory Service Monograph Series no. 11. Maidstone Public Health Laboratory. Her Majesty's Stationery Office. London.

Garcia, M. T., A. Ventosa, F. Ruiz-Berraquero, M. Kocur. 1987. Taxonomic study and amended description of *Vibrio costicola*. Int. J. Syst. Bacteriol. 37:251–256.

Gauthier, M. J. 1976. Morphological, physiological, and biochemical characteristics of some violet pigmented bacteria isolated from seawater. Canad. J. Microbiol 22:138–149.

Gee, D. L., P. Baumann, L. Baumann. 1975. Enzymes of D-fructose catabolism in species of *Beneckea* and *Photobacterium*. Archiv. Microbiol. 103:205–207.

Gibbons, N. E., K. B. Pattee, J. G. Holt (ed.). 1981. Supplement to *Index Bergeyana*. Williams and Wilkins. Baltimore, Maryland.

Giovannoni, S. J., L. Margulis. 1981. A red *Beneckea* from Laguna Figueroa, Baja California. Microbios 30:47–63.

Golten, C., W. A. Scheffers. 1975. Marine vibrios isolated from water along the Dutch coast. Netherlands J. Sea Res. 9:351–364.

Grimes, D. J., P. R. Brayton, P. A. West, F. L. Singleton, R. R. Colwell. 1986. The probabilistic identification of *Vibrio* spp. isolated from surface seawater with special reference to *Vibrio campbellii*. Let. Appl. Microbiol. 2:93–95.

Grimes, D. J., J. Stemmler, H. Hada, E. B. May, D. Maneval, F. M. Hetrick, R. T. Jones, M. Stoskopf, R. R. Colwell. 1984. *Vibrio* species associated with mortality of sharks held in captivity. Microb. Ecol. 10:271–282.

Guerinot, M. L., D. G. Patriquin. 1981. N_2-fixing vibrios isolated from the gastrointestinal tract of sea urchins. Can. J. Microbiol. 27:311–317.

Guerinot, M. L., P. A. West, J. V. Lee, R. R. Colwell. 1982. *Vibrio diazotrophicus* sp. nov., a marine nitrogen-fixing bacterium. Int. J. Syst. Bacteriol. 32:350–357.

Gundersen, K. 1976. Cultivation of microorganisms. 3.1 Bacteria. 301–356. O. Kinne (ed.) Marine ecology, vol. 3, part 1. Wiley. London.

Hada, H. S., P. A. West, J. V. Lee, J. Stemmler, R. R. Colwell. 1984. *Vibrio tubiashii* sp. nov., a pathogen of bivalve mollusks. Int. J. Syst. Bacteriol. 34:1–4.

Harrell, L. W., A. J. Noyotny, M. H. Schiewe, H. O. Hodgins. 1976. Isolation and description of two vibrios pathogenic to Pacific salmon in Puget Sound, Washington. Fishery Bull. 74:447–449.

Harvey, E. N. 1915. The effect of certain organic and inorganic substances upon light production by luminous bacteria. Biol. Bull. Marine Biol. Lab. 29:308–311.

Harvey, E. N. 1940. Living light. Princeton University Press.

Harwood, C. S. 1978. *Beneckea gazogenes* sp. nov., a red, facultatively anaerobic, marine bacterium. Curr. Microbiol. 1:233–238.

Hastings, J. W., G. W. Mitchell. 1971. Endosymbiotic bioluminescent bacteria from the light organ of pony fish. Biological Bull. 141:261–268.

Hastings, K. W., K. H. Nealson. 1977. Bacterial bioluminescence. Ann. Rev. Microbiol. 31:549–595.

Hastings, J. W., K. H. Nealson. 1981. The symbiotic luminous bacteria. 1332–1345. M. P. Starr, H. Stolp, H. G. Trüper, A. Balows, and H. G. Schlegel (ed.) The prokaryotes. Springer-Verlag. Berlin.

Hendrie, M. S., W. Hodgkiss, J. M. Shewan. 1970. The identification, taxonomy and classification of luminous bacteria. J. Gen. Microbiol. 64:151–169.

Hickman, F. W., J. J. Farmer III, D. G. Hollis, G. R. Fanning, A. G. Steigerwalt, R. E. Weaver, D. J. Brenner. 1982. Identification of *Vibrio hollisae* sp. nov. from patients with diarrhea. J. Clin. Microbiol. 15:395–401.

Hidaka, T., M. Sakai. 1968. Comparative observation of the inorganic salt requirements of the marine and terrestrial bacteria. Bull. Misaki Marine Biol. Institute. Kyoto University. 12:125–149.

Hollis, D. G., R. E. Weaver, C. N. Baker, C. Thornsberry. 1976. Halophilic *Vibrio* species isolated from blood cultures. J. Clin. Microbiol. 3:425–431.

Hugh, R., R. Sakazaki. 1972. Minimal number of characters for the identification of *Vibrio* species, *Vibrio cholerae*, and *Vibrio parahaemolyticus*. J. Conf. Public Health Lab. Dir. 30:133–137.

Hughes, J. M., D. G. Hollis, E. J. Gangarosa, R. E. Weaver. 1978. Non-cholera vibrio infections in the United States. Ann. Intern. Med. 88:602–606.

Humm, H. J. 1946. Marine agar-digesting bacteria of the south Atlantic coast. Bulletin No. 3 of the Duke University Marine Laboratory. 45–75.

Huq, M. I., A. K. M. J. Alam, D. J. Brenner, G. K. Morris. 1980. Isolation of *Vibrio*-like group, EF-6, from patients with diarrhea. J. Clin. Microbiol. 11:621–624.

Ingraham, J. L. 1962. Temperature relationships. 285–288. I. C. Gunsalus and R. Y. Stanier (ed.) The bacteria, vol. IV. Academic Press. New York.

Jannasch, H. W. 1968. Competitive elimination of Enterobacteriaceae from sea water. Appl. Microbiol. 16:1616–1618.

Jannasch, H. W., C. O. Wirsen. 1977. Retrieval of concentrated and undecompressed microbial populations from the deep sea. Appl. Environ. Microbiol. 33:360–367.

Jannasch, H. W., C. O. Wirsen, C. D. Taylor. 1976. Undecompressed microbial populations from the deep sea. Appl. Environ. Microbiol. 32:360–367.

Jean-Jacques, W., K. R. Rajashekaraiah, J. J. Farmer, III, F. W. Hickman, J. G. Morris, C. A. Kallick. 1981. *Vibrio metschnikovii* bacteremia in a patient with cholecystitis. J. Clin. Microbiol. 14:711–712.

Jesudason, M. V., C. P. Thangavelu, M. K. Lalitha. 1984. Rapid screening of fecal samples for *Vibrio cholerae* by a coagglutination technique. J. Clin. Microbiol. 19:712–713.

Johnson, F. H., I. V. Shunk. 1936. An interesting new species of luminous bacteria. J. Bacteriol. 31:585–592.

Joseph, S. W., J. B. Kaper, R. R. Colwell. 1982. *Vibrio parahaemolyticus* and related halophilic vibrios. CRC Crit. Rev. Microbiol. 10:77–124.

Kelly, M. T., J. W. Peterson, W. E. Sarles, M. Romanko, D. Martin, B. Hafkin. 1982. Cholera on the Texas gulf coast. J. Am. Med. Assoc. 247:1598–1599.

Kennedy, S. F., R. R. Colwell, G. B. Chapman. 1970. Ultrastructure of a marine psychrophilic *Vibrio*. Canad. J. Microbiol. 16:1027–1031.

Kinne, O. 1976. Cultivation of marine organisms: water-quality management and technology. 19–36. O. Kinne (ed.) Marine ecology, vol. III, part 1. Wiley. London.

Kodama, T., S. Tanaguchi. 1976. Sodium-dependent growth and respiration of a nonhalophilic bacterium, *Pseudomonas stutzeri*. J. Gen. Microbiol. 96:17–24.

Kusuda, R., J. Yokoyama, K. Kawai. 1986. Bacteriological study on cause of mass mortalities in cultured black sea bream fry. Bull. Jpn. Soc. Sci. Fisheries 52:1745–1752.

Lapage, S. P. 1974. Practical aspects of probabilistic identification of bacteria. Int. J. Syst. Bacteriol. 24:500–507.

Larsen, H. 1962. Halophilism. 297–342. I. C. Gunslaus and R. Y. Stanier (eds.) The bacteria, vol. IV. Academic Press. New York.

Lee, J. V., T. J. Donovan, A. L. Furniss. 1978a. Characterization, taxonomy, and emended description of *Vibrio metschnikovii*. Int. J. Syst. Bacteriol. 28:99–111.

Lee, J. V., P. Shread, A. L. Furniss. 1978b. The taxonomy of group F organisms: relationships to *Vibrio* and *Aeromonas*. J. Appl. Bacteriol. 45:ix.

Lee, J. V., P. Shread, L. Furniss, T. N. Bryant. 1981. Taxonomy and description of *Vibrio fluvialis* sp. nov. (synonym group F vibrios, group EF6). J. Appl. Bacteriol. 50:73–94.

Lehmann, K. B., R. Neuman. 1896. Atlas and Grundriss der Bakteriologie und Lehrbuch der speciellen bakteriologischen Diagnostik, 1st ed. J. F. Lehmann. München.

Leifson, E. 1960. Atlas of bacterial flagellation. Academic Press. New York.

Levin, R. E., R. H. Vaughn. 1968. Spontaneous spheroplast formation by *Desulfovibrio aestuarii*. Canad. J. Microbiol. 14:1271–1276.

Ljungh, A., T. Wadstrom. 1983. Toxins of *Vibrio parahaemolyticus* and *Aeromonas hydrophila*. J. Toxicol. Toxin Rev. 1:257–307.

Lockwood, D. E., A. S. Kreger, S. H. Richardson. 1982. Detection of toxins produced by *Vibrio fluvialis*. Infect. Immun. 35:702–708.

Lodeiros, C., J. Bolinches, C. P. Dopazo, A. E. Toranzo. 1987. Bacillary necrosis in hatcheries of *Ostrea edulis* in Spain. Aquaculture 65:15–30.

Love, M., D. T. Teebken-Fisher, J. E. Hose, J. J. Farmer III, F. W. Hickman, G. R. Fanning. 1981. *Vibrio damsela*, a marine bacterium, causes skin lesions on the damselfish *Chromis punctipinnis*. Science 214:1139–1140.

MacLeod, R. A. 1965. The question of the existence of specific marine bacteria. Bacteriol. Rev. 29:9–23.

MacLeod, R. A. 1968. On the role of inorganic ions in the physiology of marine bacteria. Adv. Microbiol. of the Sea 1:95–126.

Merkel, J. R., E. D. Traganza, B. B. Mukherjee, T. B. Griffin, J. M. Prescott. 1964. Proteolytic activity and general characteristics of a marine bacterium, *Aeromonas proteolytica* sp. n. J. Bacteriol. 87:1227–1233.

Miwatani, T., Y. Takeda. 1976. *Vibrio parahaemolyticus*: a causative bacterium of food poisoning. Saikon Publishing Co. Tokyo (Available from Maruzen Company, Ltd., Import and Export Dept., P.O. Box 5050, Tokyo International 100-31 Japan.)

Mitchell, R., J. C. Morris. 1969. The fate of intestinal bacteria in the sea. 811–817. S. H. Jenkins (ed.) Advances in water pollution research. Pergamon Press. Oxford.

Moebus, K. 1972. Bactericidal properties of natural and synthetic sea water as influenced by addition of low amounts of organic matter. Marine Biol. 15:81–88.

Morishita, H., H. Takada. 1976. Sparing effects of lithium ion on the specific requirement for sodium ion for growth of *Vibrio parahaemolyticus*. Canad. J. Microbiol. 22:1263–1268.

Morris, G. K., M. H. Merson, I. Huq, A. K. M. G. Kibrya, R. Black. 1979. Comparison of four plating media for isolating *Vibrio cholerae*. J. Clin. Microbiol. 9:79–83.

Morris, J. G., H. G. Miller, R. Wilson, C. O. Tacket, D. G. Hollis, F. W. Hickman, R. E. Weaver, P. A. Blake. 1982. Illness caused by *Vibrio damsela* and *Vibrio hollisae*. Lancet i:1294–1297.

Morris, J. G., R. Wilson, B. R. Davis, I. K. Wachsmuth, C. F. Riddle, H. G. Wathen, R. A. Pollard, P. A. Blake. 1981. Non-0 group 1 *Vibrio cholerae* gastroenteritis in the United States: clinical, epidemiologic, and laboratory characteristics of sporadic cases. Ann. Intern. Med. 94:656–658.

Muroga, K., Y. Jo, K. Masumura. 1986. *Vibrio ordalii* isolated from diseased ayu *Plecoglossus altivelis* and rockfish *Sebastes schlegeli.* (In Japanese). Jpn. Fish Pathol. 21:239–244.

Nishibuchi, M., N. C. Roberts, H. B. Bradford Jr., R. J. Seidler. 1983. Broth medium for enrichment of *Vibrio fluvialis* from the environment. Appl. Environ. Microbiol. 46:425–429.

Nishibuchi, M., R. J. Seidler. 1983. Medium-dependent production of extracellular enterotoxins by non-O1 *Vibrio cholerae*, *Vibrio mimicus*, and *Vibrio fluvialis*. Appl. Environ. Microbiol. 45:228–231.

Pacini, F. 1854. Osservazione microscopiche e deduzioni patologiche sul Cholera Asiatico. Gaz Med. Ital. Toscana Firenze 6:405–412.

Pavia, A. T., J. A. Bryan, K. L. Maher, T. R. Hester, Jr., J. J. Farmer III. 1989. *Vibrio carchariae* infection after a shark bite. Ann. Intern. Med. 111:85–86.

Payne, W. J., R. G. Eagon, A. K. Williams. 1961. Some observations on the physiology of *Pseudomonas natriegems*, nov. spec. Antonie van Leeuwenhoek. J. Microbiol. Serol. 27:121–128.

Pfister, R. M., P. R. Burkholder. 1965. Numerical taxonomy of some bacteria isolated from antarctic and tropical sea waters. J. Bacteriol. 90:863–872.

Pflüger, E. 1875. Beiträge zur lehre von der respiration. I. Uber die physiologische verbrennung in dem lebendigen organismen.Arch. F. D. Ges. Physiol. 10:251–367.

Pien, F., K. Lee, H. Higa. 1977. *Vibio alginolyticus* infections in Hawaii. J. Clin. Microbiol. 5:670–672.

Pollitzer, R. 1959. Cholera. World Health Organization Monograph Series no. 43. World Health Organization. Geneva.

Poole, M. D., J. D. Oliver. 1978. Experimental pathogenicity and mortality in ligated ileal loop studies of the newly reported halophilic lactose-positive *Vibrio* sp. Infect. Immun. 20:126–129.

Pratt, D. 1974. Salt requirements for growth and function of marine bacteria. 3–15. R. R. Colwell and R. Y. Morita (ed.) Effect of the ocean environment on microbial activities. University Park Press. Baltimore.

Pujalte, M. J., A. Arnau, C. Amaro, E. Alcaide, E. Garay. 1983. Numerical taxonomy of vibrio strains isolated from the coastal area of Valencia (Spain). INSERM. 114:185–197.

Pujalte, M.-J., E. Garay. 1986. Proposal of *Vibrio mediterranei* sp. nov.: A new marine member of the genus *Vibrio*. Int. J. Syst. Bacteriol. 36:278–281.

Reichelt, J. L., P. Baumann. 1973. Taxonomy of the marine, luminous bacteria. Archiv Mikrobiol. 94:283–330.

Reichelt, J. L., P. Baumann. 1974. Effect of sodium chloride on growth of heterotrophic marine bacteria. Archiv. for Microbiol. 97:329–345.

Reichelt, J. L., P. Baumann. 1975. *Photobacterium mandapamensis* Hendrie et al., a later subjective synonym of *Photobacterium leiognathi* Boisvert et al. Int. J. Syst. Bacteriol. 25:208–209.

Reichelt, J. L., P. Baumann, 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.

Reichelt, J. L., K. Nealson, J. W. Hastings. 1977. The specificity of symbiosis: pony fish and luminescent bacteria. Arch. Microbiol. 112:157–161.

Richter, O. 1928. Natrium: Ein notwendiges nahrelement fur eine marine mikroarophile Leuchtbakterie. Anzeiger der Akademie der Wissenschaften, Wien, Mathematische-Naturwissenschaftliche Klasse. 101:261–292.

Rubin, S. J., R. C. Tilton. 1975. Isolation of *Vibrio alginolyticus* from wound infections. J. Clin. Microbiol. 2:556–558.

Ruby, E. G., E. P. Greenberg, J. W. Hastings. 1980. Planktonic marine luminous bacteria: species distribution in the water column. Appl. Environ. Microbiol. 39:302–306.

Ruby, E. G., J. G. Morin. 1978. Specificity of symbiosis between deep-sea fishes and psychrotrophic luminous bacteria. Deep-Sea Research 25:161–167.

Ruby, E. G., K. H. Nealson. 1976. Symbiotic association of *Photobacterium fischeri* with the marine luminous fish *Monocentris japonica*: a model of symbiosis based on bacterial studies. Biological Bulletin of the Marine Biology Laboratory, Woods Hole. 151:574–586.

Ruby, E. G., K. H. Nealson. 1978. Seasonal changes in the species composition of luminous bacteria in nearshore seawater. Limnology and Oceanography. 23: 530–533.

Russell, H. L. 1891. Untersuchungen über im golf von Neapel lebende bakterien. Zeit. Hyg. 11:165–206.

Ryan, W. J. 1976. Marine vibrios associated with superficial septic lesions. J. Clin. Pathol. 29:1014–1015.

Sakazaki, R. 1968. Proposal of *Vibrio alginolyticus* for the biotype 2 of *Vibrio parahaemolyticus*. Japan. J. Med. Sci. and Biol. 21:359–362.

Sakazaki, R., A. Balows. 1981. The genera *Vibrio*, *Plesiomonas*, and *Aeromonas*. 1272–1301. M. P. Starr, H. Stolp, H. G. Trüper, A. Balows, and H. G. Schlegel (ed.) The prokaryotes. Springer-Verlag. Berlin.

Sakazaki, R., S. Iwanami, H. Fukumi. 1963. Studies on the enteropathogenic, facultatively halophilic bacteria, *Vibrio parahaemolyticus*. I. Morphological, cultural and biochemical properties and is taxonomic position. Jpn. J. Med. Sci. Biol. 16:161–188.

Sakazaki, R., T. Shimada. 1977. Serovars of *Vibrio cholerae* identified during 1970–1975. Jpn. J. Med. Sci. Biol. 30:279–282.

Schiewe, M. H., J. H. Crosa, E. J. Ordal. 1977. Deoxyribonucleic acid relationships among marine vibrios pathogenic to fish. Canad. J. Microbiol. 23:954–958.

Schiewe, M. H., T. J. Trust, J. H. Crosa. 1981. *Vibrio ordalii* sp. nov.: A causative agent of vibriosis in fish. Cur. Microbiol. 6:343–348.

Shaffer, N., E. S. do Santos, P. Andreason, J. J. Farmer, III. 1989. Rapid laboratory diagnosis of cholera in the field. Trans. Royal Soc. Trop. Med. Hyg. 83:119–120.

Shandera, W. X., J. M. Johnston, B. R. Davis, P. A. Blake. 1983. Disease from infection with *Vibrio mimicus*, a newly recognized *Vibrio* species. Ann. Intern. Med. 99:169–171.

Shewan, J. M., M. Vernon. 1974. Genus I. *Vibrio* Pacini 1854, 411. 340–345. R. E. Buchanan and N. E. Gibbons (ed.) Bergey's manual of determinative bacteriology, 8th ed. Williams and Wilkins Co. Baltimore.

Shilo, M., T. Yetinson. 1979. Physiological characteristics underlying the distribution patterns in the Mediterranean Sea and the Gulf of Elat. Appl. Environ. Microbiol. 38:577–584.

Shinoda, S., T. Honda, Y. Takeda, T. Miwatani. 1974a. Antigenic difference between polar monotrichous and peritrichous flagella of *Vibrio parahaemolyticus*. J. Bacteriol. 120:923–928.

Shinoda, S., R. Kariyama, M. Ogawa, Y. Takeda, T. Miwatani. 1976. Flagellar antigens of various species of the genus *Vibrio* and related genera. Int. J. Syst. Bacteriol. 26:97–101.

Shinoda, S., T. Miwatani, T. Honda, Y. Takeda. 1974b. Antigenicity of flagella of *Vibrio parahaemolyticus*. 193–197. T. Fujino, G. Sakaguchi, R. Sakazaki and T. Takeda (ed.) International symposium on *Vibrio parahaemolyticus*. Saikon Publishing. Tokyo.

Shinoda, S., K. Okamoto. 1977. Formation and function of *Vibrio parahaemolyticus* lateral flagella. J. Bacteriol. 129:1266–1271.

Sieburth, J. M. 1979. Sea microbes. Oxford University Press. New York.

Simonson, J. G., R. J. Siebeling. 1988. Coagglutination of *Vibrio cholerae*, *Vibrio mimicus*, and *Vibrio vulnificus* with anti-flagellar monoclonal antibody. J. Clin. Microbiol. 26:1962–1966.

Sinderman, C. J. 1966. Diseases of marine fish. Academic Press. London.

Sinderman, C. J. 1970. Principal diseases of marine fish and shellfish. Academic Press. New York.

Singleton, R. J., T. M. Skerman. 1973. A taxonomic study by computer analysis of marine bacteria from New Zealand waters. J. Royal Soc. New Zealand, 3:129–140.

Smith, F. B. 1938. An investigation of a taint in rib bones of bacon. The determination of halophilic vibrios (n.spp.). Proc. Royal Soc. Queensland 49:29–52.

Smith, H. L., Jr. 1979. Serotyping of non-cholera vibrios. J. Clin. Microbiol. 10:85–90.

Smith, I. W. 1961. A disease of finnock due to *Vibrio anguillarum*. J. Gen. Microbiol. 24:247–252.

Stanier, R. Y. 1941. Studies on marine agar digesting bacteria. J Bacteriol. 42:527–559.

Stanier, R. Y. 1971. Toward an evolutionary taxonomy of the bacteria. 595–604. A. Perez-Miravete and D. Palaez (ed.) Recent advances in microbiology. Associacion Mexicana de Microbiologia. Mexico.

Stanier, R. Y., E. A. Adelberg, J. Ingraham. 1976. The microbial World. 4th ed. 612–629.Prentice Hall. Englewood Cliffs, NJ.

Stanier, R. Y., N. J. Palleroni, M. Doudoroff. 1966. The aerobic pseudomonads: a taxonomic study. J. Gen Microbiol. 43:159–271.

Stephen, S., A. L. Vaz, I. Chandreshekara, K. N. A. Rao. 1978. Characterization of *Vibrio alginolyticus (Beneckea alginolytica)* isolated from the fauna of Arabian sea. Indian J. Med. Res. 68:7–11.

Tacket, C. O., F. Hickman, G. V. Pierce, L. F. Mendoza. 1982. Diarrhea associated with *Vibrio fluvialis* in the United States. J. Clin. Microbiol. 16:991–992.

Thompson, J., R. A. MacLeod. 1973. Na^+ and K^+ gradients and α-aminoisobutyric acid transport in a marine pseudomonad. J. Biol. Chem. 248:7106–7111.

Tison, D. L., M. T. Kelly. 1984. *Vibrio* species of medical importance. Diag. Microbiol. Infect. Dis. 2:263–276.

Tison, D. L., R. J. Seidler. 1983. *Vibrio aestuarianus* new species from estuarine waters and shellfish. Int. J. Syst. Bacteriol. 33:699–702.

Tubiash, H. S., P. E. Chanley, E. Leifson. 1965. Bacillary necrosis, a disease of larval and juvenile bivalve mollusks. I. Etiology and epizootiology. J. Bacteriol. 90:1036–1044.

Tubiash, H. S., R. R. Colwell, R. Sakazaki. 1970. Marine vibrios associated with bacillary necrosis, a disease of larval and juvenile bivalve mollusks. J. Bacteriol. 103:272–273.

Twedt, R. M., L. Spaulding, H. E. Hall. 1969. Morphological, cultural, biochemical, and serological comparisions of Japanese strains of *Vibrio parahaemolyticus* with related cultures isolated in the United States. J. Bacteriol. 98:511–518.

Ulitzur, S. 1974. Induction of swarming in *Vibrio parahaemolyticus*. Archiv. Microbiol. 101:357–363.

Ulitzur, S. 1975. The mechanism of swarming of *Vibrio alginolyticus*. Archiv. Microbiol. 104:67–71.

Wachsmuth, I. K. 1984. Laboratory detection of enterotoxin. 93–155. P. Ellner (ed.) Infectious diarrheal diseases: current concepts and laboratory procedures. Marcel Dekker, Inc., New York.

Wachsmuth, I. K., G. K. Morris, J. C. Feeley. 1980. *Vibrio*. 226–234. E. H. Lennette, A. Balows, W. J. Hausler, Jr., and J. P. Truant (ed.) Manual of clinical microbiology, 3rd ed. American Society for Microbiology. Washington, DC.

Watson, S. W., J. B. Waterbury. 1971. Charcteristics of two marine nitrite oxidizing bacteria, *Nitrospina gracilis* nov. gen. nov. sp. and *Nitrococcus mobilis* nov. gen. nov. sp. Archiv. Mikrobiol. 77:203–230.

Wiik, R., E. Egidius. 1986. Genetic relationships of *Vibrio salmonicida* sp. nov. to other fish pathogenic vibrios. Int. J. Syst. Bacteriol. 36:521–523.

Williamson, D. H., J. F. Wilkison. 1958. The isolation and estimation of poly-β-hyroxybutyrate inclusions in *Bacillus* species. J. Gen. Microbiol. 19:198–209.

World Health Organization. 1983. Manual for laboratory investigation of acute enteric infections. Diarrheal Diseases Control Program, World Health Organization. Geneva, Switzerland.

World Health Organization. 1990. Cholera in 1989. Weekly. Epidemiological Rec. 65:141–142.

World Health Organization Scientific Working Group. 1980. Cholera and other vibrio-associated diarrheas. Bull. W.H.O. 58:353–374.

Yabuuchi, E., T. Mitwatani, Y. Takeda, M. Arita. 1974. Flagellar morphology of *Vibrio parahaemolyticus* (Fujino et al.) Sakazaki, Iwanami and Fukumi 1963. Jpn. J. Microbiol. 18:295–305.

Yang, Y., L. P. Yeh, Y. Cao, L. Baumann, P. Baumann, J. S. Tang, 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.

Yetinson, T., M. Shilo. 1979. Seasonal and geographic distribution of luminous bacteria in the Eastern Mediterranean Sea and the Gulf of Elat. Appl. Environ. Microbiol. 37:1230–1238.

Zen-Yoji, H., S. Sakai, T. Terayama, Y. Kudo, T. Ito, M. Benoki, M. Nagasaki. 1965. Epidemiology, enteropathogenicity, and classification of *Vibrio parahaemolyticus*. J. Infect. Dis. 115:436–444.

ZoBell, C. E. 1946. Marine microbiology. Chronica Botanica Co. Waltham, Mass.

ZoBell, C. E. 1963. Domain of the marine microbiologist. 3–24. C. H. Oppenheimer (ed.) Marine microbiology. Thomas. Springfield, IL.