Deoxyribonucleic Acid Reassociation Among Members of the Genus *Vibrio*

T. E. STALEY and R. R. COLWELL

Department of Microbiology, University of Maryland, College Park, Maryland 20742

Although application of deoxyribonucleic acid (DNA) reassociation techniques to the classification of vibrios has only recently been employed and, hence, complete data are unavailable, several generalizations regarding the results of such studies can be made. (i) *Vibrio cholerae*, *V. anguillarum*, and *V. parahaemolyticus* show intraspecies polynucleotide sequence variation from 80 to 100% and interspecies relatedness of approximately 20 to 30%. *V. parahaemolyticus* shows similar intraspecies variation and interspecies relatedness with *V. cholerae* and *V. anguillarum*, but considerably higher (60 to 70%) levels of relatedness to *V. alginolyticus*. It is not possible, at the present time, to place great reliance on other strain or species relatedness for the vibrios. (ii) Very little or no polynucleotide sequence relationship has been demonstrated between members of the genus *Vibrio* and the genera *Pseudomonas*, *Aeromonas*, *Zymomonas*, *Cytophaga*, and *Escherichia*. (iii) Based on the results of the majority of the reciprocal reactions presented, there appears to be no significant influence of genome size on DNA reassociation determinations within the genus *Vibrio*. Nonpathogenic marine vibrios constitute a particularly heterogeneous group with, as yet, no well-defined species.

The classification of gram-negative, rod-shaped bacteria is often accomplished by employing a determinative scheme such as that proposed by Shewan et al. (24). Accordingly, motile (by means of a polar flagellum), Kovac's oxidase-positive, fermentative (acid, without gas, produced from carbohydrates in Hugh and Leifson medium), pteridine (0/129)-sensitive organisms are placed in the genus *Vibrio*. This classical, or "alpha" taxonomic approach has, however, come under substantial reevaluation with the advent of numerical taxonomy and molecular genetic analysis. Numerical taxonomy procedures have, in general, provided results in good agreement with alpha taxonomy, and the correlation of molecular genetic analyses with alpha taxonomy often is also reasonably good. To date, speciation within the genus *Vibrio* has not been attempted on a completely quantitative basis and, as such, no widely accepted taxonomy for the genus has been proposed. It was not until the late 1960's that numerical taxonomy studies of members of this genus, in particular, *V. cholerae*, *V. parahaemolyticus*, and certain marine isolates, were begun. Furthermore, since that time only a few papers have appeared in the literature concerning the deoxyribonucleic acid (DNA) reassociation relationships among members of

the genus *Vibrio*. To date, Hanaoka et al. (15), Anderson and Ordal (1), Basden et al. (2), Kiehn and Pacha (18), Citarella and Colwell (9), and Staley and Colwell (27) have published molecular data for this genus. It is the intent of this paper to review the available literature concerning DNA relationships among strains of bacteria classified as members of the genus *Vibrio*. In addition, we will attempt an assessment of the validity of type and neotype strains as representative of a given species, the extent of inter- and intragenic DNA relationships, the usefulness of mol percent guanine plus cytosine determination as a measure of molecular genetic relatedness, and a comparison of the *Vibrio* genospecies with the taxospecies.

(This paper was presented at the 73rd Annual Meeting of the American Society of Microbiology, 7 May 1973, Miami, Florida.)

DNA reassociation technique. The current popularity of the DNA reassociation technique (also called DNA homology, hybridization, or molecular genetic analysis) is best documented by citing the large number of papers in the literature. The reports can be divided into two general categories: (i) those designed to differentiate selected reference strains from a relatively few isolates, and (ii) those designed to delineate the range of taxonomic groups. Both

types of studies are subject to potential intrinsic errors of the technique (incomplete pairing of base sequences, differences in genome size between reference and test strains, extrachromosomal DNA elements, variations in reassociation rates as a function of base composition, enriched sequences due to replication state, or low-level redundancy), all of which have been discussed elsewhere (7, 8, 23, 27). In spite of these limitations, both the differentiation and taxonomic range types of study have corroborated or complemented results of the alpha taxonomies. In some cases, unexpected, subtle differences not detectable by any other procedure have been revealed (7, 9, 27).

The technology applicable to the separation of single- from double-stranded DNA duplexes, the crucial step upon which all DNA reassociation calculations depend, has been amply discussed in the literature (5, 6, 13; T. E. Staley and R. R. Colwell, *Annu. Rev. Ecol. Syst.*, in press). The most important single consideration in using this technique as a taxonomic tool is the choice of reference strains against which all other strains are compared. Two lines of research are possible. One involves the use of type strains, designated by the individual describing a given taxon. The type strain may be the first isolate or may have special characteristics, as decided by the individual preparing the description of the taxon in question. The other line of research makes use of the hypothetical median organism (19), computed by numerical taxonomy and used to select the reference organism or type strain. Numerical taxonomy has not yet, unfortunately, been used extensively to select type strains. It would appear more logical to develop a classification by selection of strains representing those closely related to a median organism. Sets of strains can be subjected to numerical taxonomy analysis and the median organism calculated can be used as the reference strain for DNA reassociation determinations. In the few cases where other than a random selection of a reference strain was made (9, 10, 21), the correlation of percent DNA reassociation and percent similarity values were high. Unfortunately, Palleroni et al. (21) reported the selection of reference strains of *Pseudomonas stutzeri* and *Pseudomonas putida*, based on numerical analysis of phenetic characters, that were genetically atypical of both species. Nevertheless, the use of a hypothetical median organism for the selection of reference strains intuitively would seem the "best choice" for molecular taxonomy studies. No reports have yet appeared in the literature specifically addressing themselves to this problem, although

such studies would be a prerequisite for the development of a stable and reproducible microbial taxonomy.

As an additional precautionary note in interpreting DNA reassociation data, it should be kept in mind that, unless two reference strains have been shown to be >50% related to each other, any inference of nucleotide sequence relationship between these strains, derived from their relationship with a common test strain, may be purely conjectural.

Vibrio strains examined. In Table 1 are listed all the *Vibrio* strains examined by DNA reassociation techniques to date. It is evident that a major effort has been given to identification and classification of isolates pathogenic for humans (*V. cholerae* and *V. parahaemolyticus*) and fish (*V. anguillarum*). Unfortunately, very little interest has been shown in DNA sequence relationships of nonpathogenic vibrios. It is obvious that this situation must be remedied before a reliable and reproducible classification of vibrios can be accomplished.

All strains cited in Table 1 were assumed to be individual isolates. Any strain indicated by an investigator (or found by the authors) to be identical to any other strain is so noted in the last two columns of the table. Although we are not absolutely certain, it appears that *V. parahaemolyticus* strain 4 (strains SAK 4 and, probably, K4 also) is identical to the K4 pilot strains of Anderson and Ordal (1) and Hanaoka et al. (15), which are based on common O and K antigenic components. Similarly, *V. parahaemolyticus* strains 15 and A55, 23 and K-23 pilot, 5 and K-5 pilot, 1 and K-1 pilot, 17 and K-17 pilot appear to be identical strain pairs. Seven of the ten pilot strains used by Hanaoka et al. (15) were assumed to be identical to strains similarly designated by Anderson and Ordal (1).

Intragenetic DNA relationships. To assess quantitatively the molecular genetic relationships among members of the genus *Vibrio*, the available DNA reassociation data were analyzed using a single-linkage clustering method similar to that used in numerical taxonomy. The triangular matrix obtained is shown in Fig. 1. Although the paucity of data available precludes a precise interpretation of the matrix, some generalizations concerning group relationships are, nonetheless, possible. We have constructed Table 2 to illustrate certain of these relationships.

V. cholerae shows intraspecies variation of approximately 80 to 100%, as does *V. anguillarum*, *V. parahaemolyticus*, and *V. alginolyticus*. *V. cholerae* shows only 20 to 38% relatedness to the other groups. No data were

TABLE 1. *Vibrio* strains employed in DNA reassociation studies

Species	Strain	Antigenic composition or serotype	Mol % G + C	Source and/or isolation date	Place	Reassociation technique ^a	Comments	Reference ^b
<i>V. cholerae</i>	NIH 35A3 ^c	Inaba (10)	48 (9)	Early 1940s (10)	India (10)	FS-HA (9, 27), MI-C (1)		9 ^c , 27, 1
	C-401	Inaba (10)	48 (9)	1953 (10)	India (10)	FS-HA		9
	ATCC 14035	Ogawa	48 (9)	Neotype strain (10)	India (10)	FS-HA	Same as NCTC 8021	9
	P33/58	Ogawa	48 (9)	1958 (10)	India (10)	FS-HA		9
	NIH 41	Ogawa	48 (9)	Early 1940s (10)	India (10)	FS-HA		9
	VC-9	Ogawa	48 (9)	1960 (10)	E. Pakistan (10)	FS-HA		9
	El Tor HK-1	Ogawa	48 (9)	1961 (10)	Hong Kong (10)	FS-HA		9
	El Tor 2A-62	Ogawa	48 (9)	1962 (10)	New Guinea (10)	FS-HA		9
	El Tor SLH 29803	Ogawa	48 (9)	1961 (10)	Manila (10)	FS-HA		9
	El Tor NCTC 2890	Inaba (10)	48 (9)	1905 (10)	Egypt (10)	FS-HA	Same as ATCC 582	9
	El Tor NCTC 6563	Inaba (10)	48 (9)	1933 (10)	Egypt (10)	FS-HA	Same as NCTC 8457	9
	El Tor ATCC 14033	Inaba (10)	48 (9)	1930 (10)	Egypt (10)	FS-HA		9
	NCTC 4715 ^c	O Group V (10)	48 (9)	1934 (10)	Egypt (10)	FS-HA		9
	NCTC 8042 ^d	O Group II (10)	48 (9)	1932 (10)	Nanking (10)	FS-HA		9
	S-860		48 (9)	1962 (10)	E. Pakistan (10)	FS-HA		9
<i>V. cholerae</i> <i>Vibrio</i> sp.	El Tor ^c	O Group III (10)	48 (9)	1932 (10)	Nanking (10)	FS-HA, AI-D		15
	NCTC 4711 ^{c, d}	O Group IV (10)	48 (9)	1932 (10)	Kasauli (10)	FS-HA, AI-D		9, 15 ^c
	NCTC 4716 ^c	O Group III (10)	48 (9)	1916 (10)		FS-HA		9, 15 ^c
<i>V. anguillarum</i>	NCTC 30 ^c		44 (1)	Diseased finnock (1959) (26)	Scotland	AI-C, MI-C	Type C of I. W. Smith	18, 1 ^c
	V-2911 ^c		41 (15)	Diseased finnock (1959) (26)	Scotland	AI-C, MI-C	Type C of I. W. Smith	18, 1 ^c
	V-2916	1, 3, 4, 5 (20)			Japan	MI-C	From S. Egusa	1
	PB-1	0, 4			Washington	MI-C	From J. Baross	1
	AEOY1 (7) ^{c, f}			Chinook salmon	Washington	MI-C		1
	BB61-b ^f				Washington	MI-C	From J. Baross	1
	SOY-11	0, 2; K, 3		Chinook salmon	Washington	AI-C, MI-C		18, 1
	2.1	1, 2, 3, 4 (20)	44 (15)	Chinook salmon	Washington	MI-C		1
	4.3	1, 2, 3, 4 (20)		Chinook salmon	Washington	AI-C, MI-C		18 ^c , 1
	30 ^c	1, 2, 3, 4 (20)	44 (15)	Steelhead trout	Washington	MI-C		1
	73			Steelhead trout	Washington	MI-C		1
	83			Steelhead trout	Washington	MI-C		1
	SOY-2A ^d	0, 2; K, 3			Washington	MI-C	From J. Baross	1
	SOY-1 ^e	0, 2; K, 3			Washington	MI-C	From J. Baross	1
	PB-15		46 (15)		Japan	MI-C	From S. Egusa	1
<i>V. piscium</i> <i>V. metschnikovii</i> <i>V. coticolus</i> <i>V. piscium</i> <i>V. ichthyoderms</i> <i>V. anguillarum</i>	NCMB 571 ^c				Scotland	MI-C, AI-D	From J. M. Shewan (Hoshina strain)	1, 15 ^c
	IAM 1039 ^c					AI-D		15
	NCMB 701 (25) ^c				Washington	AI-D		15
	SWA50 ^d	0, 2; K, 3			Scotland	MI-C		1
	NCMB 407				Denmark	AI-C, FS-HA, MI-C	Strain PL-1 of Hodgkiss and Shewan Bagge and Bagge strain (ATCC 19264)	18 ^{c, e, n} , 1
	NCMB 6 ^c	1, 3, 4, 5 (18)	46 (18)	Ulcerous cod lesion	Japan	MI-C, AI-D	From S. Egusa	1
<i>V. ichthyoderms</i>	PB-39 ^c							
	NCMB 1291 ^c							15

[illegible]

TABLE 1—Continued

Species	Strain	Antigenic composition or serotype	Mol % G + C	Source and/or isolation date	Place	Reassociation technique ^a	Comments	Reference ^a
<i>V. alginolyticus</i>	15-2 ^c	Untypable		Food poisoning	Japan	AI-D	From H. Zen-Yoji	15
	CDC 8198		45	Saltwater swimmer sores	Georgia	FS-HA	From R. M. Twedt	27
	BCL 1-42-1		46	Bay clams	Washington	FS-HA	From J. Baross	27
	NH4		46	Estuarine water (1970)	New Hampshire	FS-HA	From C. Bartley	27
	21-1 ^c	Untypable		Food poisoning	Japan	AI-D		15
	11-1 ^c	Untypable		Food poisoning	Japan	AI-D		15
	ATCC 17749 ^c		47 45 (27)	Spotted horse mackerel	Japan	ML-C, FS-HA	<i>V. Miyamoto XII-53 (Oceanomonas alginolytica)</i>	15, 27
	V-390				Japan	ML-C	From H. Zen-Yoji	1
	V-373				Japan	ML-C	From H. Zen-Yoji	1
	V-385				Japan	ML-C	From H. Zen-Yoji	1
<i>Vibrio</i> sp.	V-375				Japan	ML-C	From H. Zen-Yoji	1
	V-374 ^c				Japan	ML-C	From H. Zen-Yoji	1
	V-393				Japan	ML-C	From H. Zen-Yoji	1
	V-389				Japan	ML-C	From H. Zen-Yoji	1
	V-388				Japan	ML-C	From H. Zen-Yoji	1
	V-386				Japan	ML-C	From H. Zen-Yoji	1
	166-70		46	Fish	Japan	FS-HA	Japanese strain no. 166-70	27
	(Hanaoka) ^c		45	Atlantic seawater	Georgia	AI-D	From G. Omori	15
	EW288		46	Pompano nose erosion	Florida	FS-HA	From G. Krantz	27
	B1		44			FS-HA		27
<i>V. tyrogenus</i> <i>Vibrio</i> sp.	Deneke ^c		45*	Herring	Washington	ML-C		15
	b60 ₁ ^c	1 (20)	41 (18)	Pacific herring	Washington	AI-C, ML-C	From E. J. Ordal	1
	60			Pacific herring	Washington	ML-C		1
	62			Silver salmon	Washington	ML-C		1
<i>V. cholerae</i> <i>Vibrio</i> sp.	BB-10			Estuarine sediment	Maryland	FS-HA	(Luminescent)	27
	OGAWA 5596		47	Herring	Washington	ML-C		1
	8RR7		45	Herring	Washington	ML-C		1
	GH-20 ^c			Silver salmon	Washington	ML-C		1
	90			Herring	Washington	ML-C		1
	BB-3			Herring	Washington	ML-C		1
	GH-10			Herring	Washington	ML-C		1
	56		45 (18)	Pacific herring ^d	Washington	AI-C, ML-C		18, 1
	63			Herring	Washington	ML-C		1
	GH-15			Herring	Washington	ML-C		1
	GH-41			Herring	Washington	ML-C		1
	BB-6			Silver salmon	Washington	ML-C		1
	b61			Herring	Washington	ML-C		1
	92			Herring	Washington	ML-C		1
	94			Herring	Washington	ML-C		1
	POYA12 ^c		47	Estuarine plankton (1971)	Maryland	FS-HA	From J. Baross	27
	9RR212		39	Seawater (1962)	Japan	FS-HA	(Luminescent)	27, 9, — ^{c, n}
	5144 ^c							

	IORR 10 73-2 ^a MB-22 PS 207 ATCC 25918	Untypable	47	Estuarine water (1971) Food poisoning Pacific cod skin Seawater	Maryland Japan Oregon Hawaii	FS-HA AI-D FS-HA FS-HA FS-HA	(Luminescent)	27 15 9 9 — ^a — ^a — ^a 9 9 18 2 2 2 2 2
<i>V. parahaemolyticus</i> <i>Vibrio</i> sp. <i>V. marinus</i> <i>V. fischeri</i>							"Marine" vibrio MB-22 Same as <i>V. fischeri</i> , ATCC 15382 P. Bauman 61 (<i>Photobacterium fischeri</i>) Same as ATCC 27364 <i>V. fischeri</i> ATCC 15381 Japanese strain no. 5146-62 Japanese strain no. 5162-62 From E. J. Ordal From F. W. Frank From E. O. King	
<i>V. psychroerythrus</i> <i>V. marinus</i> <i>Vibrio</i> sp.	NRC 1004c ^a MP-1 ^b 5146 5162 53 Bovine R9 ^c Ovine 751 Human 662 Avian 1802 GB ^c Avian 1803 C Bovine 655	2 (20)	40 (11) 41 — ^a 39 39 59 33 35 33 32 32 64	Flounder eggs Seawater Food Food Chinook salmon Bovine fetus Lamb Human blood Chicken gall bladder Chicken cecal contents Bovine semen	Norway Oregon Japan Japan Washington Connecticut Connecticut Connecticut Connecticut	FS-HA FS-HA FS-HA FS-HA AI-C DT		

^a Abbreviations: FS-HA, free solution-hydroxyapatite; AI-D, agar immobilized-direct; AI-C, agar immobilized-competitive; MI-D, membrane immobilized-direct; MI-C, membrane immobilized-competitive; DT, DNA template.

^b References listed in order according to technique used for reassociation.

^c Strains used as reference organisms (i.e., source of radioactive DNA fragments).

^d Non-*cholera* vibrios.

^e This strain is presumed to be the same as AEO 1 (7) listed in Table 1, reference 1.

^f Received by Anderson and Ordal (1) as *V. parahaemolyticus*.

^g This strain is presumed to be that received by Anderson and Ordal (1) from J. Baross as *V. parahaemolyticus* and, as such, is incorrectly footnoted in Table 3, reference 1.

^h M. Sochard, personal communication.

ⁱ These pilot strains were used by Hanaoka et al. (15) and by Anderson and Ordal (1) and are assumed to be identical.

^j Serotype given in reference 1 presumably should have been 0, 1; K-25.

^k Mol % G + C given as 41 in Table 2 and 45 in Table 3, reference 1 (the latter was assumed to be the correct value).

^l Source incorrectly given in reference 1 as silver salmon (*O. kisutch*).

^m Originally received from R. Sakazaki as *V. parahaemolyticus* biotype 2, subsequently reclassified as *V. alginolyticus* (18) and recently as a *Vibrio* sp. (1, 27).

ⁿ Previously unpublished data of this laboratory (from Table 2).

^o Obligate psychrophiles.

^p Presumed to be either PB-30 or PB-34 as listed in Table 1, reference 1.

^q Abstracts of the Annual Meeting of the American Society for Microbiology, p. 58, 1972.

FIGURE 1

— V PARAHAEVOLYTICUS

FIG. 1. Intragenetic polynucleotide sequence relationships expressed as a total triangular DNA reassociation matrix. Asterisk indicates the strain used as the reference. In the case of reciprocal reactions, only one of the two values was used (consult Table 3 for reciprocal reaction values).

Code no.	Nomenspecies	Strain	Code no.	Nomenspecies	Strain
1.	<i>V. cholerae</i>	NIH 35A3	57.	<i>V. parahaemolyticus</i>	15
2.	<i>V. cholerae</i>	C-401	58.	<i>V. parahaemolyticus</i>	23
3.	<i>V. cholerae</i>	ATCC 14035	59.	<i>V. parahaemolyticus</i>	1
4.	<i>V. cholerae</i>	P33/58	60.	<i>V. parahaemolyticus</i>	13
5.	<i>V. cholerae</i>	NIH 41	61.	<i>V. parahaemolyticus</i>	17
6.	<i>V. cholerae</i>	VC-9	62.	<i>V. parahaemolyticus</i>	*A55
7.	<i>V. cholerae</i>	El Tor HK-1	63.	<i>V. parahaemolyticus</i>	*8-3
8.	<i>V. cholerae</i>	El Tor 2A-62	64.	<i>V. parahaemolyticus</i>	*K-1 pilot
9.	<i>V. cholerae</i>	El Tor SLH 29803	65.	<i>V. parahaemolyticus</i>	*K-2 pilot
10.	<i>V. cholerae</i>	El Tor NCTC 2890	66.	<i>V. parahaemolyticus</i>	*K-4 pilot
11.	<i>V. cholerae</i>	El Tor NCTC 6563	67.	<i>V. parahaemolyticus</i>	*K-8 pilot
12.	<i>V. cholerae</i>	El Tor ATCC 14033	68.	<i>V. parahaemolyticus</i>	*K-14 pilot
13.	<i>Vibrio</i> sp.	NCTC 4715	69.	<i>V. parahaemolyticus</i>	*K-18 pilot
14.	<i>Vibrio</i> sp.	NCTC 8042	70.	<i>V. parahaemolyticus</i>	*K-19 pilot
15.	<i>Vibrio</i> sp.	S-860	71.	<i>V. parahaemolyticus</i>	*K-20 pilot
16.	<i>V. cholerae</i>	*El Tor	72.	<i>V. parahaemolyticus</i>	*K-23 pilot
17.	<i>Vibrio</i> sp.	*NCTC 4711	73.	<i>V. parahaemolyticus</i>	*K-24 pilot
18.	<i>Vibrio</i> sp.	*NCTC 4716	74.	<i>V. parahaemolyticus</i>	*ATCC 17802
19.	<i>Vibrio</i> sp.	NCTC 30	75.	<i>V. parahaemolyticus</i>	T3937-1
20.	<i>V. anguillarum</i>	*V-2911	76.	<i>V. parahaemolyticus</i>	K-22 pilot
21.	<i>V. anguillarum</i>	V-2916	77.	<i>V. parahaemolyticus</i>	K-10 pilot
22.	<i>V. anguillarum</i>	PB-1	78.	<i>V. parahaemolyticus</i>	K-21 pilot
23.	<i>V. anguillarum</i>	AEOY1 (7)	79.	<i>V. parahaemolyticus</i>	K-25 pilot
24.	<i>V. anguillarum</i>	BB61-b	80.	<i>V. parahaemolyticus</i>	T3880-1
25.	<i>V. anguillarum</i>	SOY-11	81.	<i>V. parahaemolyticus</i>	T3960-1
26.	<i>V. anguillarum</i>	2.1	82.	<i>V. parahaemolyticus</i>	T3979-1
27.	<i>V. anguillarum</i>	4.3	83.	<i>V. parahaemolyticus</i>	1POY3A
28.	<i>V. anguillarum</i>	*30	84.	<i>V. parahaemolyticus</i>	K-15 pilot
29.	<i>V. anguillarum</i>	73	85.	<i>V. parahaemolyticus</i>	K-17 pilot
30.	<i>V. anguillarum</i>	83	86.	<i>V. parahaemolyticus</i>	K-28 pilot
31.	<i>V. anguillarum</i>	SOY-2A	87.	<i>V. parahaemolyticus</i>	K-3 pilot
32.	<i>V. anguillarum</i>	SOY-1	88.	<i>V. parahaemolyticus</i>	K-5 pilot
33.	<i>V. anguillarum</i>	PB-15	89.	<i>V. parahaemolyticus</i>	T3991-1
34.	<i>V. piscium</i>	*NCMB 571	90.	<i>V. parahaemolyticus</i>	*15-1
35.	<i>V. metschnikovii</i>	*IAM 1039	91.	<i>V. parahaemolyticus</i>	CDC 8198
36.	<i>V. costiculus</i>	*NCMB 701	92.	<i>V. parahaemolyticus</i>	BCL 1-42-1
37.	<i>V. piscium</i>	SWA50	93.	<i>V. parahaemolyticus</i>	NH4
38.	<i>V. ichthyoderms</i>	NCMB 407	94.	<i>V. parahaemolyticus</i>	*21-1
39.	<i>V. anguillarum</i>	*NCMB 6	95.	<i>V. parahaemolyticus</i>	*11-1
40.	<i>V. anguillarum</i>	PB-39	96.	<i>V. alginolyticus</i>	*ATCC 17749
41.	<i>V. ichthyoderms</i>	*NCMB 1291	97.	<i>V. alginolyticus</i>	V-390
42.	<i>V. anguillarum</i>	*NCMB 829	98.	<i>V. alginolyticus</i>	V-373
43.	<i>V. parahaemolyticus</i>	*4	99.	<i>V. alginolyticus</i>	V-385
44.	<i>V. parahaemolyticus</i>	K4	100.	<i>V. alginolyticus</i>	V-375
45.	<i>V. parahaemolyticus</i>	*MD 8657-71	101.	<i>V. alginolyticus</i>	V-374
46.	<i>V. parahaemolyticus</i>	Nic shrimp	102.	<i>V. alginolyticus</i>	V-393
47.	<i>V. parahaemolyticus</i>	21	103.	<i>V. alginolyticus</i>	V-389
48.	<i>V. parahaemolyticus</i>	T249	104.	<i>V. alginolyticus</i>	V-388
49.	<i>V. parahaemolyticus</i>	12RR82	105.	<i>V. alginolyticus</i>	V-386
50.	<i>V. parahaemolyticus</i>	V6	106.	<i>V. alginolyticus</i>	166-70
51.	<i>V. parahaemolyticus</i>	*FC1011	107.	<i>V. alginolyticus</i>	*(Hanaoka)
52.	<i>V. parahaemolyticus</i>	2RR236	108.	<i>Vibrio</i> sp.	EW288
53.	<i>V. parahaemolyticus</i>	FDA crab	109.	<i>Vibrio</i> sp.	B1
54.	<i>V. parahaemolyticus</i>	1EB11	110.	<i>V. tyrogenes</i>	*Deneke
55.	<i>V. parahaemolyticus</i>	4A-A	111.	<i>Vibrio</i> sp.	*b60 ₂
56.	<i>V. parahaemolyticus</i>	5	112.	<i>Vibrio</i> sp.	60

FIGURE 1 legend—Continued
List of strains used in the total triangular DNA reassociation matrix in Fig. 1

Code no.	Nomenspecies	Strain	Code no.	Nomenspecies	Strain
113.	<i>Vibrio</i> sp.	62	131.	<i>Vibrio</i> sp.	*5144
114.	<i>Vibrio</i> sp.	BB-10	132.	<i>Vibrio</i> sp.	10RR10
115.	<i>V. cholerae</i>	OGAWA 5596	133.	<i>V. parahaemolyticus</i>	*73-2
116.	<i>Vibrio</i> sp.	8RR7	134.	<i>Vibrio</i> sp.	MB-22
117.	<i>Vibrio</i> sp.	*GH-20	135.	<i>V. marinus</i>	PS 207
118.	<i>Vibrio</i> sp.	90	136.	<i>V. fisherii</i>	*ATCC 25918
119.	<i>Vibrio</i> sp.	BB-3	137.	<i>V. psychroerythrus</i>	NRC 1004
120.	<i>Vibrio</i> sp.	GH-10	138.	<i>V. marinus</i>	MP-1
121.	<i>Vibrio</i> sp.	56	139.	<i>Vibrio</i> sp.	5146
122.	<i>Vibrio</i> sp.	63	140.	<i>Vibrio</i> sp.	5162
123.	<i>Vibrio</i> sp.	GH-15	141.	<i>Vibrio</i> sp.	53
124.	<i>Vibrio</i> sp.	GH-41	142.	<i>Vibrio</i> sp.	*bovine R9
125.	<i>Vibrio</i> sp.	BB-6	143.	<i>Vibrio</i> sp.	ovine 751
126.	<i>Vibrio</i> sp.	b61	144.	<i>Vibrio</i> sp.	human 662
127.	<i>Vibrio</i> sp.	92	145.	<i>Vibrio</i> sp.	*avian 1802 GB
128.	<i>Vibrio</i> sp.	94	146.	<i>Vibrio</i> sp.	avian 1803 C
129.	<i>Vibrio</i> sp.	POYAJ 2	147.	<i>Vibrio</i> sp.	bovine 655
130.	<i>Vibrio</i> sp.	9RR212			

available on relationships of *V. cholerae* with the marine fish pathogens.

V. anguillarum was found to be 20 to 38% related to *V. cholerae*, *V. parahaemolyticus*, and *V. alginolyticus*. Based on a rather limited number of observations, *V. anguillarum* appears to be only 25 to 30% related to the marine fish pathogens studied by Anderson and Ordal (the strain designations GH-20 and b60₂ are synonymous with the group designations II and I, respectively, in Fig. 1). The proposal by Hendrie et al. (16) that *V. anguillarum* includes *V. piscium* and *V. ichthyodermis* strains is supported by the results shown in Fig. 1.

V. parahaemolyticus appears to be related to *V. cholerae* and *V. anguillarum* at about 20 to 30% and *V. alginolyticus* at 58 to 70%. Based on only two determinations, the fish pathogens (I and II) were 27 to 28% related to *V. parahaemolyticus*.

V. alginolyticus (most likely including strains 166-70 and "Hanaoka" based on their relationships with the other species) was, in addition to the relationships already cited, only 29 to 32% related to the two marine fish pathogens.

The extreme molecular genetic heterogeneity noted among the miscellaneous marine vibrios can be seen in Fig. 1 and Table 3. We suggest that these strains (including *Vibrio* sp. EW288 and B1, *V. tyrogenus* strain Deneke, and *Vibrio* sp. 8RR7) be examined further before being designated or accepted as species within the genus *Vibrio*.

The single-linkage analysis procedure separated *V. cholerae* strain Ogawa 5596 from *V. cholerae*. However, based on the relationship of this strain with *V. alginolyticus* (29 and 32%) and *V. parahaemolyticus* (31%), it should be included in *V. cholerae*.

The animal pathogens appeared to be heterogeneous; however, relationships of these strains with strains of the other groups were not examined.

Extrapolating from the work of Brenner et al. (7), who studied well-characterized strains of the genera *Escherichia* and *Salmonella* and found them to be 40 to 50% related to each other on the basis of nucleotide homologies, it seems likely that the genus *Vibrio* may, as presently defined, encompass more than one genus if uniform levels of molecular genetic relatedness are to be used as the index of relatedness for the genus *Vibrio*. Based on the data given in Table 2, two and possibly three new genera showing high species relatedness (>80%) and no more than 25 to 35% DNA relatedness for the genus *Vibrio*. Thus, based on the data given in Table 2, two and possibly three new genera showing high species relatedness (>80%) and no more than 25 to 35% DNA molecular genetic terms. Significant correlation of the numerical taxonomy and DNA reassociation data would support such a proposal. However, it must be kept in mind that interpretation of reassociation values as species or genus levels of taxonomic relatedness re-

TABLE 2. *Intragenetic polynucleotide sequence relationships (expressed as percentage of relative binding) within the genus Vibrio*

Organism	<i>V. cholerae</i>	<i>V. anguillarum</i>	<i>V. parahaemolyticus</i>	<i>V. alginolyticus</i>
<i>V. cholerae</i>	82-100(18) ^a			
<i>V. anguillarum</i>	22-38(8)	80-100(26) ^b		
<i>V. parahaemolyticus</i>	20-33(19)	21-31(9)	81-100(70)	
<i>V. alginolyticus</i>	29-33(4)	31(1)	58-70(34) ^c	80-96(16)
Marine fish pathogens	ND ^d	3 ^e -30(6)	27-28(2)	29-32(2)
Miscellaneous marine vibrios	4-21(7)	0-19(7)	4-26(10)	ND

^a Parentheses contain number of strains examined within designated range.

^b The values of 69% for the *V. anguillarum* strain 30* /*V. anguillarum* V-2916 and 71% for the *V. anguillarum* strain 30* /*V. anguillarum* V-2911 reactions indicated by Kiehn and Pacha (18) seem to be rather low (24 to 26%), since Anderson and Ordal (1) have reported the reciprocal of the latter reaction to be 95%. (Strains followed by asterisks indicate reference strains.)

^c Assuming *V. alginolyticus* 166-70 and "Hanaoka" are >80% homologous to any one of the strains in the *V. alginolyticus* group.

^d ND, no data available.

^e Probably too low for the same reason given in footnote *b* above.

TABLE 3. *Polynucleotide sequence relationships among various marine vibrios (previously unpublished data of this laboratory)*

Organism	<i>V. psychroerythrus</i> NRC 1004 ^a		<i>Vibrio</i> sp. 5144 ^a		<i>V. anguillarum</i> NCMB6 ^a		Mol % G + C ^{c,d}
	% Relative binding, 60 C ^{b,d}	Δ T _m (e) ^d	% Relative binding, 60 C ^{b,d}	Δ T _m (e) ^d	% Relative binding, 60 C ^{b,d}	Δ T _m (e) ^d	
<i>V. psychroerythrus</i> NRC1004	100		1	8.5	6	8.0	40
<i>Vibrio</i> sp. 5144	8	5.0	100		6*	9.0*	39
<i>V. anguillarum</i> NCMB 6	5	3.5	2	6.5	100		45
<i>V. marinus</i> MP-1	6	5.5*	1	7.5	9*	8.0*	41
<i>V. fischeri</i> ATCC 25918	3	4.5	2	7.0	9	10.0	40

^a Source of ³²P-labeled DNA fragments.

^b Percentage of actual binding at 60 C was 90, 89, and 85%, respectively, for *V. psychroerythrus* NRC1004, *Vibrio* sp. 5144, and *V. anguillarum* NCMB6 homologous reactions. Self-reassociation amounted to between 5 and 10% of the homologous reaction with zero-time binding amounting to <0.5% (calculations and procedure for determining % relative binding described in reference 27).

^c Calculated from thermal mid-point determined spectrophotometrically.

^d Arithmetic average of triplicate determinations (asterisk indicates average of duplicate determinations).

mains equivocal and will remain so until some agreement is reached among taxonomists and molecular geneticists.

Intergeneric DNA relationships. The use of one or, in some cases, several characteristics to differentiate genera (namely the scheme of Shewan (24) for differentiating *Vibrio* from other gram-negative genera, such as *Aeromonas*, based on the sensitivity to compound 0/129) is obviously open to serious question. Nevertheless, the few data available (Table 4) indicate that no, or very few, polynucleotide sequences are held in common between any of the *Vibrio*

strains examined and selected strains of *Aeromonas*, *Pseudomonas*, *Zymomonas*, *Cytophaga*, or *Escherichia*. The corroboration of results using such schemes with molecular genetic criteria might be explained by the fact that the single characteristics selected to separate the genera represent "critical" metabolic pathways. Hence, the presence or absence of the given character sums up chains of metabolic events, thus serving to demark generic separations. The data provided in Table 4 could be interpreted as indicating low levels of relatedness (6 to 13%) between vibrios and selected

TABLE 4. Intergeneric polynucleotide sequence relationships (expressed as percentage of relative binding) of the genus *Vibrio*^a

Strain	<i>V. cholerae</i>		<i>V. anguillarum</i>		<i>V. costicollus</i>		<i>V. parahaemolyticus</i>				<i>V. alginolyticus</i>		<i>Vibrio</i> sp.			Mol % G + C
	El Tor	V-2911	30 ^b	NCMB ^c	NCMB 701	4	MD. 8657-71	A55	ATCC 17802	ATCC 17449	GH-20	Bovine R9	1802GB			
<i>Aeromonas hydrophila</i> AB 364	9				13			7								58
<i>Aeromonas hydrophila</i> (A.H.X.)				0												
<i>Pseudomonas aeruginosa</i> lishima	8				8			6								49
<i>Zymomonas mobilis</i> (ZMC-1)				0	0											38
<i>Cytophaga succinicans</i> strain 8			0													
<i>Escherichia coli</i> ^d <i>Escherichia coli</i> K-12(W1485)	1	0			1	4	6	4	5	8	4	0	0			50

^a Johnson et al. (17) found *Vibrio* sp. "Vibrio 01" (ATCC 11711) to be 64% related to *Bacterium anitratum* type B5W, 25% to *Alcaligenes haemolysans* ATCC 17988, 26% to *Achromobacter metalcaligenes* ATCC 17909, and 27% to *Moraxella lwoffii* ATCC 17986, but it is very doubtful that this strain should be placed in the genus *Vibrio*.

^b Referred to as *V. anguillarum* 30 in reference 18 and *Vibrio* sp. strain 30 in reference 1.

^c Kiehn and Pacha, 1969 (7).

^d Strain not specified.

Pseudomonas and *Aeromonas* strains. However, until a more complete set of data is available, with many more "typical" strains included, such relationships are merely conjectural. Molecular genetic relatedness values of $<5\%$ (as seen in Table 4 for the *Zymomonas*, *Cytophaga*, and *Escherichia/Vibrio* reactions) approach the limits of accuracy of the technique and, as such, may represent background "noise." In addition, the problem of assessing mispaired base sequences in heteroduplexes of DNA obtained from distantly related organisms becomes particularly acute and, in fact, has been ignored by most workers until recently.

At the moment the extent of our knowledge allows us only to say that, in the few DNA reassociation studies reported, there appears to be very little polynucleotide sequence relatedness among members of the genus *Vibrio* and some members of *Aeromonas*, *Pseudomonas*, *Zymomonas*, *Cytophaga*, or *Escherichia*.

Necessity of reciprocal DNA reactions. The molecular genetic relationship (percentage of DNA reassociation) of organism A with organism B can be determined by annealing radioactive, single-stranded DNA fragments of strain A with nonradioactive, single-stranded DNA fragments of strain B. Similarly, the reciprocal relationship of strain B to A can be assessed by using radioactivity labeled B in place of A. Theoretically, within the experimental error of the particular technique in question (usually ± 1 to 5%), the relative values of such reciprocal reactions should be identical. As shown in Table 5, 15 of 19 values for such reciprocal reactions lie within an acceptable experimental error range. Four of the values for reciprocal reactions showed differences of 7% (*V. psychroerythrus* NRC 1004/*Vibrio* sp. 5144), 8% (*V. parahaemolyticus* A55/*V. costicola* NCMB 701), 24% (*V. anguillarum* 30/*V. anguillarum* V-2911), and 37% (*V. parahaemolyticus* ATCC 17802 and *V. alginolyticus* V-374). The first two reciprocal value differences, although slightly outside of the anticipated error, might well fall within the accuracy of the given experiment. Alternatively, assuming these differences are real, an additional explanation for such results might be that the genome sizes (i.e., the total polynucleotide sequence complement of the cell, including possible episomal, temperate phage, and/or doubly replicated regions due to multiple growing points, as well as redundant sequences) of the strains used for the reciprocal reactions are substantially different.

The magnitude of the difference in genome size, generating a constant, significant reciproc-

cal value difference, increases as the number of polynucleotide sequences which are held in common between the two strains being compared decreases. This concept might best be illustrated by the following example. Two strains, A and B, are reciprocally reacted and are found to be related as $A/B = 90\%$ and $B/A = 80\%$ (a reciprocal difference of 10%). This situation can only arise if $B > A$, since B must contain all the homologous sequences present in A, plus 20% nonhomologous sequences. The genome size of B relative to A can then be calculated. Since 80% of the sequences in B, held in common with A, represent 90% of the sequences in A, some percentage (χ) of the sequences in B must equal 100% of the sequences in A. By simple proportions, χ , in this case, is 1.12, i.e., the genome of strain B is 12% larger than A. Using the same rationale, the genome size difference between two strains, C and D (reciprocally related, $C/D = 70\%$ and $D/C = 60\%$), with a 10% reciprocal value difference as above, is 1.17 (i.e., the genome of D is 17% larger than C). Similarly, a relative genome size difference ($F > E$ by 33%) can be calculated from data, indicating that $E/F = 40\%$ and $F/E = 30\%$.

Furthermore, as the difference in reciprocal reaction values increases, so does the magnitude of the difference in genome size.

In short, what we have said is that reciprocal value differences among highly related strains are indicative of smaller genome size differences than the same reciprocal value differences among less highly related strains. Also, as the reciprocal value differences increase, so do the relative genome size differences, and also conversely.

Using this argument, we can calculate (again, assuming the reciprocal difference values to be significant) that the genome size of *V. psychroerythrus* NCR 1004 is eight times larger than that of *Vibrio* sp. 5144; *V. parahaemolyticus* A55 is 37% larger than that of *V. costicola* NCMB 701; *V. anguillarum* 30 is 36% larger than that of *V. anguillarum* V-2911; and *V. alginolyticus* V-374 is 113% larger than that of *V. parahaemolyticus* ATCC 17802. Almost certainly, the first estimate is incorrect due to severe errors involved in determinations at that level, whereas the second and third estimates seem reasonable. The last estimate, although seemingly reasonable, is most probably incorrect because of the unusually low reciprocal value of 33% obtained for the *V. alginolyticus* V-374/*V. parahaemolyticus* ATCC 17802 reaction. Since Anderson and Ordal (1) have shown nearly identical reciprocal values for their *V.*

TABLE 5. Reciprocal polynucleotide sequence relationships among vibrios used as reference strains

Organism	<i>V. cholerae</i>		<i>V. anguillarum</i>		<i>V. costicola</i>	<i>V. anguillarum</i>	<i>V. parahaemolyticus</i>				<i>V. alginolyticus</i>		<i>Vibrio</i> sp.		<i>V. psychroerythrus</i>	
	NIH 35A3	EI Tor	V-2911	30	NCMB 701	NCMB 6	4	MD 8657-71	FC 1011	A55 17802	ATCC 17749	V-374	b60 ₂	GH-20		5144
<i>V. cholerae</i> NIH 35A3	100															
<i>V. cholerae</i> EI Tor		100														
<i>V. anguillarum</i> V-2911			100													
<i>V. anguillarum</i> 30			95/71 ^a	100												
<i>V. costicola</i> NCMB 701					100											
<i>V. anguillarum</i> NCMB 6			90/88 ^a	78/80		100										
<i>V. parahaemolyticus</i> 4	20/18 ^a						100									
<i>V. parahaemolyticus</i>	21/-						92/91	100								
MD 8657-71																
<i>V. parahaemolyticus</i>							88/87	91/90	100							
FC1011										100						
<i>V. parahaemolyticus</i>		25/25			22/30											
A55																
<i>V. parahaemolyticus</i>	33/-		31/-				88/-	89/-		100						
ATCC 17802																
<i>V. alginolyticus</i> ATCC	30/-		31/-				55/-	59/-		67/66	100					
17749																
<i>V. alginolyticus</i> V-374	32/-											100				
<i>Vibrio</i> sp. b60 ₂			30/29							70/33	80/82					
<i>Vibrio</i> sp. GH-20			30/26							27/-	32/-		100			
<i>Vibrio</i> sp. 5144	4/-					6/2	26/ ^b	24/-		28/-	29/-		22/22	100		
<i>V. psychroerythrus</i> NRC 1004						6/5									100	100

^a Reciprocal cross not done by the same investigator.^b Citarella and Colwell (9) reported 4% for *V. parahaemolyticus* 4*/*Vibrio* sp. 5144 reaction.

parahaemolyticus ATCC 17802/*V. alginolyticus* ATCC 17749 and *V. alginolyticus* ATCC 17749/*V. alginolyticus* V-374 reactions, it necessarily follows that the *V. alginolyticus* V-374/*V. parahaemolyticus* ATCC 17802 reaction cannot be 33%, but rather near 70%.

In general, the majority of the reciprocal reactions performed to date have indicated relatively small (usually less than 10 to 15%) differences in genome content among members of the genus *Vibrio* examined, especially among those strains exhibiting relatedness values of 20 to 25% or more.

Needless to say, many of the reciprocal reactions that are missing in Table 5 must be performed before we can conscientiously say that differences in genome content, regardless of the nature of the source, cannot severely influence interpretation of DNA reassociation data derived from studies with vibrios.

Furthermore, although one would prefer to use every strain as a reference strain, the relatively few data in Table 5 as yet do not indicate significant changes in the taxonomic groupings derived from Fig. 1 and compiled in Table 2. Obviously, a complete matrix of the data in Fig. 1 would be beneficial in more clearly defining taxonomic groupings and sorting out strains that now appear to cluster with a group merely on their common relationship to members of the groups. However, it may well be that the judicious selection of several reference strains (4 to 6, perhaps) from each species cluster, compared with as many of the *Vibrio* strains as possible, might accomplish the same purpose. In addition, much more work has to be done with nonpathogenic vibrios before any valid molecular genetic limits of relatedness, with themselves or with other species, can be assigned.

Correlation of DNA reassociation data and mol percent guanine plus cytosine. The nucleotide base composition (mol percent guanine plus cytosine [mol % G+C]) of bacteria has been used in the past as a criterion for classifying organisms. The advent of DNA reassociation has, by and large, superseded this technique because the G + C content of a particular DNA preparation tells nothing of the crucial arrangement of bases in sequence. Hence, the existence of organisms with identical G + C content but very little phenetic or molecular genetic relationship with each other can be shown. As DeLey (12) has pointed out, the chance of two organisms being closely related decreases as the difference between their mean nucleotide composition increases, and, in fact, the chance nearly reaches zero

when the mean nucleotide compositions differ by at least 18 to 30%. Since the G + C content of most of the species presently in the genus *Vibrio* ranges from 39 to 49% (9), the likelihood that some DNA relatedness exists among some of these species is, thus, reasonably high, although the DNA relatedness between organisms at each end of the G + C range would predictably be very low.

Since the organisms listed in Table 1 are arranged according to polynucleotide sequence relationship, any correlation with nucleotide composition should be apparent. *V. cholerae* (including "classical" and El Tor vibrios) possess an overall G + C content of 48%; *V. anguillarum*, 44 to 46% (one exception was *V. anguillarum* V-2911, which was 41%); *V. parahaemolyticus*, 44 to 47%; *V. alginolyticus*, 45 to 47%; marine fish pathogens, 41 to 45%; and the miscellaneous marine vibrios, 39 to 59%.

These results, in general, agree well with those published by Bianchi (4) using organisms classified by numerical taxonomy procedures. The vast range of values for the miscellaneous marine vibrios, spanning 20% G + C, reaffirms the heterogeneity of this group as determined by DNA reassociation and numerical taxonomy procedures. However, a range of approximately 10% G + C has been shown for the genera *Pseudomonas*, *Mycobacterium*, and *Flexibacter*.

DISCUSSION

Deoxyribonucleic acid hybridization studies among members of the genus *Vibrio* have been useful in classifying and identifying isolates thought to be identical by other criteria. However, the application of this technique in the development of a taxonomy for the genus has not been an outstanding success for several reasons. Firstly, only species pathogenic for man or animals have received significant attention, and, most assuredly, these represent only a few of the species of the genus as it is now defined. Secondly, the usefulness of the results of these studies (aside from corroborative evidence obtained from other classification methods) have been severely limited by the fact that arbitrarily selected strains have been employed as the reference strains. Thirdly, reciprocal reactions have not always been carried out. Furthermore, until recently, the consideration of mispaired base sequences in yielding unduly high % DNA reassociation values, particularly in reactions involving more distantly related strains, has been neglected. In addition, with the exception of the noteworthy

study of Anderson and Ordal (1), no generalized attempt has been made to compare several strains from a number of species within the genus. It is hoped that the obvious gaps, as evident in the data provided in Fig. 1, will be filled in the future. The delineation of species within the very heterogeneous group of organisms presently lumped together as miscellaneous marine vibrios may then be accomplished.

In conclusion, the following generalizations can be made. (i) *V. cholerae*, *V. anguillarum*, and *V. parahaemolyticus* show intraspecies polynucleotide sequence variation from 80 to 100% and interspecies relatedness of approximately 20 to 30%. *V. parahaemolyticus* shows relatively high (60 to 70%) levels of relatedness to *V. alginolyticus*. It is not possible, at the present time, to place great reliance on other strain or species relatedness for the vibrios. (ii) Very little or no polynucleotide sequence relationship has been demonstrated between members of the genus *Vibrio* and members of the genera *Pseudomonas*, *Aeromonas*, *Zymomonas*, *Cytophaga*, or *Escherichia*. (iii) Based on the results of the majority of the reciprocal reactions presented, there appears to be no significant influence of genome size on DNA reassociation determinations within the genus *Vibrio*.

We reserve the right to make a dignified retreat from any one or all of the above mentioned conclusions as more DNA reassociation data accumulate for members of the genus *Vibrio*.

REPRINT REQUESTS

Address requests for reprints to: Dr. T. E. Staley, Department of Microbiology, University of Maryland, College Park, Md. 20742.

LITERATURE CITED

1. Anderson, R. S., and E. J. Ordal. 1972. Deoxyribonucleic acid relationships among marine vibrios. *J. Bacteriol.* **109**:696-706.
2. Basden II, E. H., M. E. Tourtellotte, W. N. Plastringe, and J. S. Tucker. 1968. Genetic relationship among bacteria classified as vibrios. *J. Bacteriol.* **95**:439-443.
3. Bauman, P., L. Bauman, and M. Mandel. 1971. Taxonomy of marine bacteria: the genus *Beneckea*. *J. Bacteriol.* **107**:268-294.
4. Bianchi, M. A. G. 1973. Composition en bases de L'ADN et position taxonomique de bactéries marines fermentant le glucose, du genre *Vibrio* et des genres voisins. *Arch. Mikrobiol.* **90**:131-140.
5. Boling, M. E. 1972. Homology between the deoxyribonucleic acids of *Haemophilus influenzae* and *Haemophilus parainfluenzae*. *J. Bacteriol.* **112**:745-750.
6. Brenner, D. J., G. R. Fanning, A. Rake, and K. E. Johnson. 1969. A batch procedure for thermal elution of DNA from hydroxyapatite. *Anal. Biochem.* **28**:447-458.
7. Brenner, D. J., G. F. Fanning, F. J. Skerman, and S. Falkow. 1972. Polynucleotide sequence divergence among strains of *Escherichia coli* and closely related organisms. *J. Bacteriol.* **109**:953-965.
8. Brenner, D. J., G. R. Fanning, K. E. Johnson, R. V. Citarella, and S. Falkow. 1969. Polynucleotide sequence relationships among members of the Enterobacteriaceae. *J. Bacteriol.* **98**:637-650.
9. Citarella, R. V., and R. R. Colwell. 1970. Polyphasic taxonomy of the genus *Vibrio*: polynucleotide sequence relationships among selected *Vibrio* species. *J. Bacteriol.* **104**:434-442.
10. Colwell, R. R. 1970. Polyphasic taxonomy of the genus *Vibrio*: numerical taxonomy of *Vibrio cholerae*, *Vibrio parahaemolyticus* and related *Vibrio* species. *J. Bacteriol.* **104**:410-433.
11. D'Aoust, J. Y., and D. J. Kushner. 1972. *Vibrio psychroerythrus* sp. n.: classification of the psychrophilic marine bacterium, NRC 1004.
12. DeLey, J. 1969. Compositional nucleotide distribution and theoretical prediction of homology in bacterial DNA. *J. Theoret. Biol.* **22**:89-116.
13. DeLey, J., and R. Tijtgaat. 1970. Evaluation of membrane filter methods for DNA/DNA hybridization. *Antonie van Leeuwenhoek J. Microbiol. Serol.* **36**:461-474.
14. Fukino, T., Y. Okuno, D. Nakada, A. Aoyama, F. Fukai, K. Murai, and T. Ueho. 1953. On the bacteriological examination of "shirasu" food poisoning. *Med. J. Osaka Univ.* **4**:299-304.
15. Hanaoka, M., Y. Kato, and T. Amano. 1969. Complementary examination of DNA's among vibrio species. *Biken J.* **12**:181-185.
16. Hendrie, M. S., W. Hodgkiss, and J. M. Shewan. 1971. Proposal that the species *Vibrio anguillarum* Bergman 1909, *Vibrio piscium* David 1972, and *Vibrio ichthyoderms* (Wells and ZoBell) Shewan, Hobbs, and Hodgkiss 1966 be combined as a single species, *Vibrio anguillarum*. *Int. J. Syst. Bacteriol.* **21**:64-68.
17. Johnson, J. L., R. S. Anderson, and E. J. Ordal. 1970. Nucleic acid homologies among oxidase-negative *Moraxella* species. *J. Bacteriol.* **101**:568-573.
18. Kiehn, E. D., and R. E. Pacha. 1969. Characterization and relatedness of marine vibrios pathogenic to fish: deoxyribonucleic acid homology and base composition. *J. Bacteriol.* **100**:1248-1255.
19. Liston, J., W. Wiebe, and R. R. Colwell. 1963. Quantitative approach to the study of bacterial species. *J. Bacteriol.* **85**:1061-1070.
20. Pacha, R. E., and E. D. Kiehn. 1969. Characterization and relatedness of marine vibrios pathogenic to fish: physiology, serology and epidemiology. *J. Bacteriol.* **100**:1242-1247.

21. Palleroni, N. J., R. W. Ballard, E. Ralston, and M. Douderoff. 1972. Deoxyribonucleic acid homologies among some *Pseudomonas* species. *J. Bacteriol.* **110**:1-11.
22. Sakazaki, R. 1968. Proposal of *Vibrio alginolyticus* for the biotype of *V. parahaemolyticus*. *Jap. J. Med. Sci. Biol.* **21**:359-362.
23. Seidler, R. J., and M. Mandel. 1971. Quantitative aspects of DNA reassociation: base composition, state of chromosome replication and polynucleotide homologies. *J. Bacteriol.* **106**:608-614.
24. Shewan, J. M., G. Hobbs, and W. Hodgkiss. 1960. A determinative scheme for the identification of certain Gram-negative bacteria, with special reference to the Pseudomonadaceae. *J. Appl. Bacteriol.* **23**:379-390.
25. Smith, F. B. 1938. An investigation of a taint in rib bones of bacon. The determination of halophilic vibrio (N. spp.) *Proc. Roy. Soc. Queensland* **49**:29-52.
26. Smith, I. W. 1961. A disease of finnock due to *Vibrio anguillarum*. *J. Gen. Microbiol.* **24**:247-252.
27. Staley, T. E., and R. R. Colwell. 1973. Polynucleotide sequence relationships among Japanese and American strains of *Vibrio parahaemolyticus*. *J. Bacteriol.* **104**:916-927.