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Characterization and Relatedness of Marine Vibrios Pathogenic to Fish: Deoxyribonucleic Acid Homology and Base Composition

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Deoxyribonucleic acid (DNA) isolated from several pathogenic marine vibrios was utilized in studies of base composition and nucleotide sequence relationships. Patterns of relatedness inferred from DNA criteria were found to correlate closely with those inferred from morphological, physiological, and serological analysis of the same organisms. The utility of the phenotypic and genotypic approaches to the determination of relatedness is discussed.

In recent years, a great deal of attention has been directed to the problem of microbial classification. The establishment of a taxonomy based on natural, phylogenetic relationships is impeded by the lack of evidence regarding evolutionary relatedness (2). Extensive structural differences and an abundance of developmental and paleontological evidence have enabled the classification of higher plants and animals to proceed with relative ease. The absence of such information concerning microorganisms has resulted in essentially artificial, arbitrary taxonomic systems for their classification.

The accepted criteria for classification of microorganisms are mostly morphological, physiological, biochemical, and serological. These phenotypic criteria represent the combined expression of many genetic loci, and it would be expected that the greater the number of characters considered, the closer the approximation to knowledge of genetic relatedness would be. This is the working hypothesis behind numerical taxonomy, but it involves possible weaknesses. Account is not taken of the origins and rate of development of phenotypic characters. In addition, weighting characters either equally or unequally is not justified by knowledge; yet, certainly characters do differ in their evolutionary significance (19, 20).

Another difficulty with phenotypic analysis is that, although two organisms may share the same character, suggesting relatedness, the enzyme pathways responsible may not be the same. Indeed, enzymes catalyzing the same reactions may be structurally quite different in different organisms. Thus, the occurrence of evolutionary convergence could mistakenly be interpreted as evidence of actual genetic homology.

Recently, however, new techniques have been developed which enable taxonomic studies to be made at the molecular level (10, 14). It is now possible to determine genetic homology directly via physicochemical studies of the deoxyribonucleic acid (DNA) molecule itself, in which the total genetic potential of an organism is stored. By use of DNA-agar (10, 16) and other methods (5, 8, 14), direct analysis of the degree of common polynucleotide sequences in nucleic acids from different sources is made by studying the formation of molecular hybrids.

The organisms used in this study were strains of marine bacteria considered to be vibrios which had been isolated during the course of epizootics. These pathogens were isolated from fish in the waters of the Pacific Northwest and of Europe and are described in detail in the preceding paper (17). Representative pseudomonads were also included, as there is some confusion in separation of vibrios from the several groups of pseudomonads owing to inadequate criteria of differentiation.

The vibrios and pseudomonads share many morphological, ecological, and physiological characteristics, and differentiation is usually based on vibrios having curved cells, having frequent fermentative potential, and lacking

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pigments. However, the criterion of cell curvature is extremely variable and may occur in both groups, often being dependent on culture age and growth medium. In addition, some organisms classified as vibrios are not fermentative, and many pseudomonads do not possess distinctive pigments. Definition of the groups (3) also involves other characteristics, including response to vibriostatic agents (18).

The experiments described below illustrate the usefulness of DNA duplex formation and base composition studies in the classification and determination of relatedness of bacteria. Correlations are made with physiological, cultural, and serological analyses of the same organisms (17). The results emphasize the strong compatibility of approaches based on genotypic and phenotypic characteristics.

MATERIALS AND METHODS

Strains and media. Vibrio strains were maintained in semisolid Brain Heart Infusion deeps (0.05% Difco Brain Heart Infusion, 2% yeast extract, 0.4% agar, 80% seawater, *p*H 7.7). Pseudomonad strains were carried on 1% Fleischmann's yeast extract-1% glucose slants.

Zymomonas mobilis was grown in a medium containing 0.5% Difco yeast extract, 0.5% tryptone, 1% glucose (*p*H 7.3) in large carboys without aeration at 30 C. All other organisms were grown in carboys of Difco Brain Heart Infusion (37 g/liter)-1% NaCl medium (*p*H 7.3), and were incubated with aeration at room temperature.

For the isolation of radiolabeled DNA, strains were grown in 200-ml cultures of Brain Heart Infusion (9 g/liter)-1% NaCl (*p*H 7.3) with 20 μc each of ^{14}C-adenine and ^{14}C-uracil (5 mc/mmole; New England Nuclear Corp., Boston, Mass.).

The organisms used in this investigation are listed in Table 1. The vibrio strains include members of each of the three serotypes reported in our previous study (17). Two strains of serotype 1 were selected because these organisms were isolated during different outbreaks of disease. Strain 2.1, one of the first vibrios isolated, was obtained from chinook salmon in seawater, whereas strain 30, though a marine vibrio, was isolated from steelhead trout in freshwater. Two strains of serotype 3 which differed slightly in their antigenic composition also were studied.

DNA isolation. DNA was extracted by use of a modification of the technique of Marmur (12) described by Berns et al. (1). Cells were washed and suspended in water. An equal volume of 4 × SSC [SSC (standard saline-citrate) is 0.15 M NaCl, 0.015 M NaCl, 0.015 M trisodium citrate, *p*H 7.0] containing 54% sucrose and 0.4 sodium lauryl sulfate (SLS) was added to the mixture, and the mixture was heated to 60 to 72 C for 15 min with the addition, if necessary, of additional 25% SLS.

Cell lysates were then incubated at 37 C with Pronase for at least 1 hr and dialyzed overnight against 2 × SSC. The lysates were then phenol-extracted at 60 C and chilled, after which the aqueous phase precipitated with two volumes of ethyl alcohol. DNA was collected on stirring rods and dissolved in 0.1 × SSC.

TABLE 1. *Strains of bacteria*

Strain	Fish host	Source
Northwest U.S. vibrios		
2.1	Chinook salmon	E. J. Ordal
30	Steelhead trout	E. J. Ordal
53	Chinook salmon	E. J. Ordal
56	Pacific herring	E. J. Ordal
60	Pacific herring	E. J. Ordal
European vibrios (*V. anguillarum*)		
NCMB6 (type A)	Cod	J. M. Shewan
V-2911 (type C)	Finnock	I. W. Smith
V-2916 (type C)	Finnock	I. W. Smith
Pseudomonads		
Aeromonas hydrophila (A. H. X.)	Rainbow trout	E. J. Ordal
Zymomonas mobilis (ZMC-1)		E. J. Ordal
Pseudomonas aeruginosa		J. Pate
Other bacteria		
Cytophaga succinicans strain 8		E. J. Ordal

Nucleic acid contents were measured in terms of ultraviolet (UV) absorbance at 260 nm on a Beckman DU spectrophotometer. Boiled ribonuclease was added at a 1:100 (w/w) ratio to nucleic acid. The mixture was incubated at 37 C for at least 45 min, and phenol extraction was performed at room temperature; DNA was precipitated with ethyl alcohol and dissolved in 0.1 × SSC. Storage was at 4 C over $CHCl_3$.

DNA duplex formation. Duplex formation experiments were conducted by the method of Hoyer et al. (10). DNA fragments of approximately 0.5 × 10^6 molecular weight were prepared by shearing 1 mg/ml solutions of DNA at 10,000 psi in a French pressure cell (16).

DNA-agar was prepared according to the following method (16). DNA at a concentration of 200 μg/ml in 0.1 × SSC was denatured by boiling for 5 min, chilled, and concentrated. This solution was boiled for an additional 2 min and added to an equal volume of molten 8% Oxoid Ionagar in 2 × SSC. These components were mixed thoroughly and rapidly solidified by ice-chilling. The resulting DNA-agar was granulated by forcing it through a screen twice, and was then washed in three changes of 2 × SSC overnight at 60 C. DNA-agar preparations were stored over $CHCl_3$ in tightly sealed bottles at 4 C.

The DNA content in the agar preparations was determined by measuring the UV absorbance at 260 nm after dissolving samples in boiling 5 M $NaClO_4$. DNA content was expressed in micrograms of DNA per gram (wet weight).

Competitive DNA-binding experiments were conducted by incubating radiolabeled DNA fragments

with homologous DNA-agar in the presence of "cold" fragments of competitor DNA. Reaction mixtures contained 4 μg of labeled DNA, various amounts of unlabeled DNA, 0.1 ml of 20 × SSC, and distilled water to 1 ml total volume. DNA fragments were denatured by boiling and then were rapidly chilled prior to the preparation of reaction mixtures. The reaction mixtures were again boiled and chilled just before use. Portions (0.5 ml) of reaction mixtures were incubated with 0.5-g amounts of DNA-agar in silicone coated glass vials at 60 C for at least 15 hr.

Unreacted DNA was washed out of the DNA-agar samples with 10 tubes each containing 15 ml of 2 × SSC at 60 C. In this procedure, the DNA-agar sample was held in a glass tube by a nylon screen (10) and moved from tube to tube at intervals of 20, 15, and thereafter 10 min in each tube. To elute the DNA which had formed stable duplexes at 60 C, the temperature was raised to 72 C and the ionic strength was lowered to 0.01 × SSC, conditions strongly favoring strand separation. This was accomplished in a manner similar to the washing, with seven tubes and intervals of 20, 20, 15, 15, 10, 10, and 10 min in each tube.

The radioactivity of each reaction mixture was measured before incubation, and samples of the wash buffer and eluant were measured, as a check on full recovery of radioactivity. Radioactivity was determined by cold precipitation in 5% trichloroacetic acid in the presence of carrier DNA to a total of 100 μg of DNA per sample. Precipitated DNA was collected on membrane filters, dried, and counted in an automatic scintillation spectrometer.

Determination of mole per cent guanine plus cytosine (GC) in DNA. Mole per cent GC in DNA samples was determined by measuring the characteristic denaturation temperatures (T_m) in 0.2 M Na^+ and applying the equation, $\%GC = (T_m - 69.3)(100)/0.41$ (13). UV absorbance at 260 nm was monitored on a Beckman DU spectrophotometer with automatic temperature control and absorbance recorder. The midpoint in the hyperchromic rise of the UV at 260 nw was taken as the T_m. Three determinations were made on each DNA sample, and the standard deviation was always less than ±0.4 C. Thus, the average values for per cent GC content are within ±1 mole % GC.

RESULTS

Table 2 describes the characteristics of the DNA-agar and labeled DNA preparations. It should be remarked that, although there was day-to-day variation in the absolute binding of homologous, labeled DNA, on a given day it was consistently reproducible. Thus, in these studies, duplicate controls were included in every experiment. All data from each experiment were calculated relative to the controls of the same day.

Levels of binding depression observed in competition experiments were independent of the particular batch of DNA-agar used, despite differences in the concentration of DNA in the agar. Binding depression was solely dependent on the sources of the interacting DNA molecules.

Table 3 gives the measured T_m and per cent GC values for the organisms studied. It is notable that all of the vibrios exhibit values in the 41 to 46% GC range except for strain 53, which has the very high value of 59% GC. On the other hand, *Zymomonas mobilis* (*Pseudomonas linderi*) with 49% GC is notably low for classification with the pseudomonads.

Equilibrium centrifugation and chemical base analyses of the *P. aeruginosa* DNA preparation (J. Pate, *unpublished data*) both indicated 66% GC, which agrees very well with the value as determined in this study by thermal denaturation and is the published value for the bacterium (9).

Values for per cent GC have also been published for *A. hydrophila* and *V. anguillarum* NCMB6, and the published values agree with the findings presented here (9). The value for *C.*

TABLE 2. *Characteristics of labeled DNA and DNA-agar preparations*

Source of DNA	Specific activity (counts per min per μg of DNA)	Wet wt (μg of DNA/g of DNA-agar)	Range of binding of homologous labeled DNA[a] (%)	Binding of labeled DNA to pure agar[a] (%)
Vibrio strain 30	567	136	35–45	1.7
		164	25–30	
V. anguillarum NCMB6	511	250	50–60	1.6
		352	45–60	

[a] Labeled DNA (2 μg) incubated with 0.5 g of DNA-agar or agar alone.

TABLE 3. *DNA thermal denaturation temperature and mole per cent GC*

Strain	T_m (C)	Mole % GC
Vibrios		
V-2916	86.1	41.0
60	86.2	41.2
V-2911	87.0	43.2
2.1	87.2	43.7
30	87.2	43.7
56	87.9	45.4
NCMB6	88.0	45.6
53	93.4	58.8
Pseudomonads		
Z. mobilis (ZMC-1)	89.2	48.6
A. hydrophila (A. H. X.)	93.1	58.1
P. aeruginosa	96.2	65.7
Other bacteria		
C. succinicans	85.0	38.0

succinicans is within the range observed for other *Cytophaga* species (9).

Tables 4 and 5 demonstrate DNA interactions in which heterologous DNA fragments compete with labeled, homologous DNA fragments for binding sites in agar-fixed, homologous DNA. With vibrio strains NCMB6 and 30, DNA from most of the vibrios competed strongly. Strains 56 and 60 showed little competition, and strain 53, the pseudomonads, and *C. succinicans* did not compete at all with the homologous reactions.

The ability of heterologous DNA to compete with the formation of labeled homologous DNA duplexes relative to homologous competition should give an indication of the similarity of the two types of DNA. Thus, these data suggest that most of the vibrios are quite similar, that strains 56 and 60 are much less similar, and that strain 53, the pseudomonads, and *C. succinicans* are not at all related to the reference vibrios.

It is also possible to determine whether the similarities two or more organisms show to the reference organism represent a common genetic homology, by adding the DNA from the organisms in question to the same competition reaction (10). The extent of additive competition should reflect the degree of nonoverlapping of the genetic homologies. Attempts to discern whether additive competition existed in mixtures of the DNA competitors used in this study were not conclusive, but suggest that, in the cases examined, there were probably no additive effects. This implies that common genetic homologies exist among these organisms.

The average values given in Table 5 are based on a minimum of at least three individual experiments. Individual experiments were included in Table 4 to suggest the reproducibility of the method. The present resolution meets well the requirements of this study.

Table 6 represents the same data described in Tables 4 and 5 as a percentage depression of the binding of labeled DNA by unlabeled competitor DNA. In this manner, the relative abilities of heterologous DNA molecules to bind to the reference DNA-agar is demonstrated. For example, strain 2.1 DNA depressed strain 30 binding to about the same degree as strain 30 DNA itself. This implies a very high degree of homology among the polynucleotide sequences of these two organisms.

If it is assumed that two organisms have the same complement of DNA, then each should depress the binding reaction of the other to the same relative degree. Indeed, this is the case here. Strain NCMB6 depressed the reaction of strain 30 to a relative value of 78%, and the reciprocal interaction showed strain 30 depressing the

TABLE 4. *Competitive interactions with Vibrio strain 30 labeled DNA for homologous DNA-agar binding sites*

Source of competitor DNA	Relative percentage of labeled DNA bound[a]			
	250 μg of competitor DNA		500 μg of competitor DNA	
	Trials	Avg	Trials	Avg
Vibrio strains				
30	15, 19, 18, 18, 17, 16, 16, 14, 19	17	14, 12, 11, 11, 10, 14, 7, 9	11
NCMB6	38, 35, 33	35	31, 29, 26, 36	31
2.1	21, 17, 19	19	13, 13	13
V-2911	34, 39	37	35, 38	37
V-2916	43, 43	43	35, 42	39
56	81	81	81	81
60	89, 92, 92	91	99, 96	97
56 + 60[b]	74, 79, 84, 90	82	72, 72, 98, 90	83
NCMB6 + 60[b]	33, 31, 43	36	30, 29, 43	34
53	98	98	104	104
Cytophaga succinicans	107	107	119	119

[a] Percentages are relative to control reaction mixtures which contained no competitor DNA.

[b] The indicated amount of DNA is the amoun of *each* competitor DNA in the reaction mixture

TABLE 5. *Competitive interactions with V. anguillarum NCMB6 labeled DNA for homologous DNA-agar binding sites*

Source of competitor DNA	Avg relative percentage of labeled DNA bound[a]	
	250 μg of competitor DNA	500 μg of competitor DNA
Vibrio strains		
NCMB6	16	10
V-2911	23	21
V-2916	22	23
30	31	28
2.1	29	27
56	79	78
60	92	91
56 + 60[b]		80
53	111	108
Pseudomonads		
ZMC-1	97	104
A. H. X.	88	100

[a] Percentages are relative to control reaction mixtures which contained no competitor DNA.

[b] The amount of DNA indicated is the amount of *each* competitor DNA in the reaction mixture.

TABLE 6. *Binding depression of labeled DNA to homologous DNA-agar by unlabeled competitor DNA*[a]

Source of labeled DNA and DNA-agar	Source of competitor DNA	Binding depression	Binding depression relative to homologous depression
		%	%
Vibrio strain 30	*Vibrio* strains		
	30	89	100
	2.1	87	98
	NCMB6	70	78
	V-2911	63	71
	V-2916	61	69
	56	19	22
	60	3	3
	53	−4	0
	C. succinicans	−19	0
V. anguillarum NCMB6	*Vibrio* strains		
	NCMB6	90	100
	30	72	80
	2.1	73	81
	V-2911	80	88
	V-2916	77	85
	56	22	25
	60	9	10
	53	−8	0
	Pseudomonads		
	ZMC-1	−4	0
	A. H. X.	0	0

[a] Data are based on the average values of per cent labeled DNA bound in competition experiments (Tables 4 and 5) at the 500-μg level of competitor DNA. These values were subtracted from 100% to reflect the extent of binding depression in these experiments.

reaction of strain NCMB6 to a relative value of 80%. In a direct experiment, without competitor DNA, the binding of labeled DNA from strains 30 and NCMB6 to strain NCMB6 DNA-agar was compared. It was found that the degree to which strain 30 DNA bound to strain NCMB6 DNA-agar was 87% as great as that of homologous strain NCMB6 DNA. Thus, the relative extent of binding to a heterologous DNA suggests the same magnitude of relatedness as can be inferred from competition experiments.

Figure 1 illustrates graphically the data from Table 4. Represented are typical curves of competition in DNA-binding reactions. Depression of the binding of labeled DNA to homologous DNA-agar is seen to be greatest when unlabeled competitor DNA from the homologous strain was used, as would be predicted. In each case, 500 μg of homologous competitor DNA depressed the binding reaction about 90%.

DISCUSSION

To draw conclusions from DNA-binding experiments, it is essential to understand the nature of the binding reaction. Recent studies have demonstrated that the temperature of duplex formation, relative to the natural T_m of the interacting DNA molecules, is a critical parameter in the formation of specific complementary double strands (11, 15). It was determined that the reaction products formed at progressively lower temperatures contain a rapidly increasing proportion of unstable, nonspecific products. Thus, at T_m 25 C duplexes were formed which had essentially the same thermal stability as native DNA, but at T_m 40 C the predominant products were unstable and did not represent duplexes of homologous nucleotide sequences (11). By varying the reaction temperature, there was formed a series of intermediate products representing various degrees of partially redundant nucleotide sequences (15).

In the determination of sequence homology in

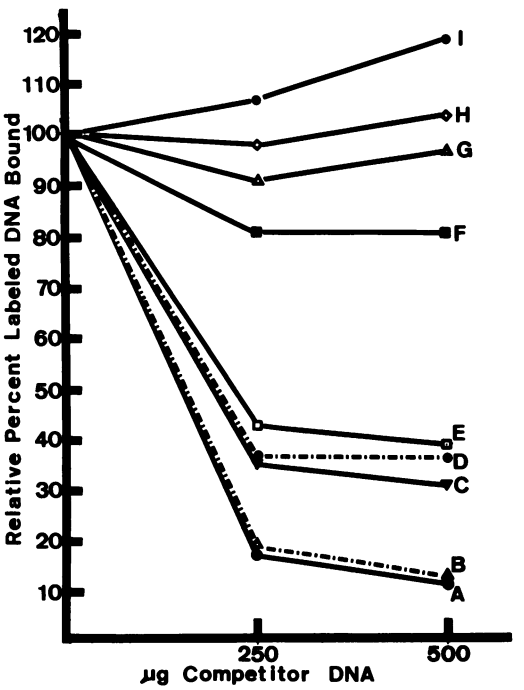

FIG. 1. *Competitive effect of nonradiolabeled DNA in the binding of radiolabeled strain 30 DNA to strain 30 DNA-agar. The experimental values are the average values listed in Table 4. The sources of the nonradiolabeled DNA are as follows: strain 30 (A), strain 2.1 (B), strain NCMB6 (C), strain V-2911 (D), strain V-2916 (E), strain 56 (F), strain 60 (G), strain 53 (H), and C. succinicans (I).*

DNA from different organisms of similar per cent GC, the results of low temperature in binding reactions can suggest considerable homology where little or none exists (11). Theoretically, with increasingly stringent reaction conditions, one selects against even slight imperfections of complementarity and can approach determinations of absolute values of homology. The conditions employed in the present study meet the requirements necessary to essentially quantitate the degree of relatedness of the experimental organisms (11). This is verified by the fact that two DNA preparations from totally unrelated organisms with GC values similar to those of vibrios, the myxobacterium *C: succinicans* and *Z. mobilis*, did not compete at all in the binding reactions of vibrio strains 2.1 and NCMB6, respectively. The absence of nonspecific reaction products is also suggested by the very low levels of competition from the DNA of vibrio strains 56 and 60 in the binding reactions of vibrio strains 2.1 and NCMB6, even though the organisms are probably all in the same genus, have many common phenotypical characteristics, and exhibit the same GC composition.

Two other sources of nonspecific interaction in the DNA-binding reaction should be discussed. There exists the possibility of retention of labeled DNA by the agar-matrix alone. In the present studies it was determined that less than 2% of input labeled DNA is agar-bound, a negligible amount for the purposes of these experiments.

In addition, Fig. 1 demonstrates a nonspecific enhancement of binding with increasing amounts of nonradioactive, heterologous DNA. This is probably a result of protein or polysaccharide, or both, in the DNA preparations (8). Whether the presence of contaminating macromolecules stimulated DNA-DNA interactions, or somehow increased the retention of unbound DNA in the DNA-agar matrix, if the effect were relatively small in comparison with actual specific DNA binding, the rising slopes seen in the competition curves would only occur with relatively heterologous DNA preparations. Figure 1 would seem to bear out this prediction. Thus, the actual potentials of DNA molecules as competitors could be slightly greater than indicated.

The significance of GC content in DNA to classification and determination of relatedness is the following: if organisms are related to a high degree, their GC contents should be very similar. However, evidence of similar GC content does *not* imply that organisms are closely related, but only allows that the possibility exists. It should also be noted that, although large differences in GC content indicate great overall differences in DNA molecules, small regions may be homologous, and may represent genes that evolved at much slower rates than the bulk of the genome (10, 15). These conserved genes could have great significance as to the possible common ancestry of highly divergent organisms or groups. For example, it was found (6, 7) that in the genus *Bacillus*, whose various members differ in GC by as much as 20%, there was very little overall genetic homology between any individual species, but that certain cistrons, such as ribosomal ribonucleic acid (RNA), soluble RNA, and antibiotic resistance loci, were highly conserved in all species tested. It may be noted that these cistrons were *not* represented in organisms outside of the genus, strongly supporting the validity of the present taxonomic definition of this group. Thus, phenotypic uniqueness was ramified by evidence of a small core of common genes, even though individual species have diverged widely from each other.

The current problem of classification of the pseudomonads (3, 4) and vibrios (T. E. Lovelace and R. R. Colwell, Bacteriol. Proc., p. 18, 1968) exists because of strong similarities, weak and variable differences, and intermediate organisms that are usually arbitrarily placed in one or the other "genus." It would indeed be interesting to elucidate the relationships of the intermediate organisms to the two ends of the spectrum, and to search for possible conserved genes between these groups. The possibilities exist that these groups may have evolved from a common ancestor, and that certain genetic continuities may have been retained between them.

On the sole basis of the serological data provided in the preceding paper (17), the following relationships seem to exist among the vibrios studied. Members of the Northwest salmonid vibrios (strains 2.1, 4.3, 8, 14, 16, 17, 30, 78, 83, and 61-1) are indistinguishable, are fairly closely related to the European vibrios (strains NCMB6, V-2911, and V-2916), and are more distantly related to the Pacific herring vibrios (strains 56 and 60). The European vibrios also represent a homogeneous group, are related to the Northwest salmonid vibrios, and are more distantly related to the Pacific herring vibrios. The Pacific herring vibrios, while distantly related, have an antigen(s) in common with both the Northwest and European vibrios. Strain 53 has an antigen in common with strain 2.1 but appears very unrelated. It exhibits no relationship with the European vibrios.

Consideration of the cultural and physiological data given in the preceding paper (17) yields an interpretation similar to that derived from analysis of antigenic characteristics. With regard to 51 cultural and physiological properties, the marine

vibrios in this study are relatively homogeneous, and all are facultative fermenters of carbohydrates, the sole exception being strain 53. Because of this homogeneity it is relatively straightforward to assign a cultural-physiological archtype, i.e., in each of the 51 tests a clear majority of the organisms responded in the same way and this response will be regarded as typical. By this criterion, Northwest salmonid vibrio strains 30 and 14 and European vibrio (*V. anguillarum*) NCMB6 are archetypical.

Seven of the eight remaining Northwest salmonid vibrios are different in only one or two characters, and the remaining strain varies in four characters. The other two European vibrios are each different in three properties with two in common. The Pacific herring vibrios differ considerably; strain 56 varies in 6 characters and strain 60 differs in 11, with 4 in common. Of all the vibrios, strain 53, with 21 differing properties, is the most disparate. Although it has slightly curved cells, considered typical of vibrios, strain 53 uniquely produces a water-soluble pigment and is strictly oxidative in carbohydrate utilization, suggesting relationship with the pseudomonads. In summary, cultural and physiological analysis did not allow discrimination between the Northwest salmonid vibrios and the European vibrios but did suggest the more distant relationship of the Pacific herring vibrios, and the probable total unrelatedness of strain 53.

DNA competition experiments confirm and emphasize the interpretations from serology that the vibrios studied can be divided into three groups, that the Northwest salmonid groups and the European groups are quite closely related, and that the Pacific herring group is much more distantly related. Although competition experiments enable much more strain differentiation than was attained in the reported serological experiments (17), other serological techniques clearly discriminated between members of the various groups (*unpublished data*), including the European vibrios.

DNA competition experiments showed that, among the European vibrios, strains V-2911 and V-2916 are relatively more distantly related than strain NCMB6 to the Northwest salmonid vibrios. This conclusion is strongly supported by cultural, physiological, and serological data (17). Thus, *V. anguillarum* appears to be the pivotal organism. Of the members of the Northwest salmonid and the European vibrios that were tested, the *least* related to strain NCMB6 is strain 30, yet it is still 80% homologous. However, exhibiting 69% DNA homology, even strain V-2916 is quite closely related to Northwest vibrio strain 30.

Another notable strain differentiation achieved by DNA competition experiments is the finding that, among the Pacific herring vibrios, strain 60 is much more distantly related to the European vibrios (10% DNA homology) and to the Northwest salmonid vibrios (3% DNA homology) than is strain 56 (respectively, 25 and 22% DNA homology). This conclusion is strongly supported by the cultural and physiological data, and implied by the serological experiments. The regions of genetic homology in strains 56 and 60 relative to strains NCMB6 and 30 appear to be overlapping; i.e., they are related in the same way to strain NCMB6 and 30. The same holds true with homologous polynucleotide sequences in strains NCMB6 and 60 relative to strain 30.

Although strain 53 has a common antigen with strain 2.1 and has slightly curved cells, DNA competition experiments demonstrate no detectable homology with the vibrios. Its cultural-physiological characteristics and its high GC content suggest that it is a pseudomonad.

Values of GC composition paralleled the DNA competition experiments in that no two vibrio strains appeared identical except Northwest salmonid vibrio strains 2.1 and 30. These strains had identical GC content, were indistinguishable in DNA competition experiments, had the same serotype, and deviated in only 1 of 51 cultural and physiological characteristics. It is noteworthy that strains 2.1 and 30 were isolated at widely separated times and locations, strain 2.1 from a salmon in seawater and strain 30 from a trout in freshwater.

It has been demonstrated that strong correlations exist between phenotypic and genotypic analysis of a group of bacteria. Detailed serological, cultural, physiological, and DNA nucleotide sequence comparisons all suggested the same overall patterns of relatedness, but each approach ramified and expanded upon the data obtained from the others. In addition, it has been shown that recently developed techniques in molecular biology represent powerful contributions to the array of classical methods available to the taxonomist.

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