Numerical Taxonomy Study of *Vibrio* and *Spirillum* spp.

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One hundred and seven strains of *Vibrio* and *Spirillum* were examined for 175 morphological, physiological, biochemical, and nutritional characteristics. The results were analyzed by the method of numerical taxonomy. The deoxyribonucleic acid base composition of the *Spirillum* strains was also determined. Marked phenetic differences were found between *Vibrio* and *Spirillum*, with species of the genus *Vibrio* demonstrating more versatile biochemical capabilities. The numerical taxonomy and molecular genetic data are in agreement with the recently suggested assignment of selected *Spirillum* strains to the genera *Oceanospirillum* and *Aquaspirillum*.

Interest in the classification of the genus *Vibrio* has focused on the pathogenic species, with attention directed toward the role of *Vibrio cholerae* as an infectious agent. Other members of the genus have been less completely studied. More recently, *Vibrio parahaemolyticus*, a causative agent of gastroenteritis in the United States (5, 9) and other countries (1, 2), has been the subject of intensive research (3, 13, 22). Although *Vibrio alginolyticus* infections in man have been recorded (24), the pathogenic properties of this and other *Vibrio* species have not been examined fully.

Vibrio spp. are common inhabitants of seawater and marine sediments, and the relationship of members of this genus to other aquatic genera, such as *Pseudomonas* and *Spirillum*, has not been sufficiently explored. Morphological similarities between curved *Vibrio* and *Spirillum* spp. suggested to bacteriologists at the turn of the century that a strong relationship existed between these two groups. Thus, *Vibrio* was included as a genus in the family *Spirillaceae*. Recent but preliminary observations of *Vibrio* and *Spirillum* strains, included as reference strains in taxonomic studies, suggested a low level of similarity between the two genera (8). Identification of species within the genus *Spirillum* has been based, to a significant extent, on morphology (26-28). Extensive study of 39 *Spirillum* strains accomplished by Hy-lemmon and his associates (12) led to the suggestion of a reclassification based on ecological differences, salt tolerance, and deoxyribonucleic acid (DNA) base composition. Our study was undertaken to determine the taxonomic relationships of the genera *Vibrio* and

Spirillum, as well as of selected species within these genera.

MATERIALS AND METHODS

Details concerning source and place of isolation of 107 cultures included in this study are given in Table 1. The maintenance medium consisted of yeast extract (Difco) (1.0%), proteose peptone (Difco) (1.0%), and a salts solution as a diluent for the marine strains. The salts solution consisted of NaCl (2.34%), MgSO₄·7H₂O (0.69%), and KCl (0.075%). A multi-point inoculation system (15) was used where appropriate. Other media were inoculated by the spot drop technique (7) or with a drop from a Pasteur pipette.

Cultures were examined for the following: colonial and cultural characteristics, Gram reaction, presence of flagella, fluorescence under ultraviolet light, and production of a water-soluble or -insoluble pigment. Presence of cytochrome oxidase, catalase, arginine dihydrolase, and ornithine, lysine, and arginine decarboxylases was observed, as were hydrolysis of aesculin, casein, starch, Tweens 20, 40, 60, and 80 and urea, digestion of agar, and liquefaction of gelatin. Acid formation from adonitol, arabinose, cellobiose, dextrin, dulcitol, ethanol, fructose, galactose, glycerol, inositol, inulin, lactose, maltose, mannose, melibiose, melibiose, raffinose, rhamnose, salicin, sorbitol, sucrose, trehalose, and xylose were recorded, as were production of ammonia, carbonate, dihydroxyacetone, hydrogen sulfide, indole, lecithinase, levan, melanin, and phosphatase. Oxidative versus fermentative utilization of glucose was tested. Sensitivity to iodoacetate, the vibriostatic agent 0/129 (pteridine), and to antibiotics (penicillin [10 Ug], dihydrostreptomycin [10 mg], chloromycetin [30 mg], erythromycin [15 mg], kanamycin [30 mg], aureomycin [30 mg], novobiocin [30 mg], polymyxin B [30 mg], terramycin [30 mg], and tetracycline [30 mg]) were tested. Reduction of nitrates and nitrites, presence of trimethylamine oxidase, the methyl red, Voges-Proskauer, and litmus milk reactions were also determined. The abilities to utilize acetate, citrate, formate, and malonate as sole carbon sources, ammonium phosphate as a sole nitrogen source, and β -alanine, DL-alanine, arginine, Casamino Acids, lysine, phenylalanine, and

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TABLE 1. *Strains used*

Strain no.	Investigator	Source
FDA 3525, 3782, 4203, 10734, 10981, 11587, 11590, 11883, 12042, 11759, 3AC41 MD 8657-71 a + b, 8658-71 a + b, 8659-71, 8700-71 a + b, 2882 RMD 341-A, 341-B, 341-D	M. Fishbein, F.D.A. Washington, D. C. Maryland Department of Health P. Haefner, Virginia Institute of Marine Science L. Waddell, Fla.	Crab isolates from food poisoning outbreak in Bainbridge, Md. Crab isolates from food poisoning outbreak in Bainbridge, Md. Fish kill isolates, Va.
Mullet 2, CP Mullet, Blue Gill A1 → Eb		Fish kill isolates, Fla. Isolates from pompano and permit Phage host
V-6, V-12, V-14 Thompson K4, K5, S12T, S12O Landry 1, 2, 3, 4 N.H. 4, 12, 29, 31 EB101, WP1, A55, Hirakata, Toyonaka BCL, BOH 8RR7, 9RR212, 10RR10	H. Nakanishi, Japan J. Thompson, Canada P. Landry, Tex. C. Bartley, N.H. K. Tamura, Japan J. Baross, Ore. T. Kaneko, Canada	Rhode River area of Chesapeake Bay, Md. Control strains used routinely in numerical taxonomy studies in our laboratory S.E. Atlantic Ocean, <i>Spirillum</i> spp.
Ps207, Ox-Sawyer, <i>A. fischeri</i> , SAK 3, FC1011, <i>Micrococcus</i> CB234, <i>E. coli</i> , <i>Spirillum</i> FC281-a 3S2	J. D. Oliver, N. Carolina	Marine spirilli
ATCC 12753, <i>Spirillum atlanticum</i>	N. Krieg, Virginia Polytechnic Institute	
ATCC 12754, <i>Spirillum beijerinckii</i> ATCC 19192, <i>Spirillum halophilum</i> ATCC 19191, <i>Spirillum japonicum</i> ATCC 11336, <i>Spirillum linum</i> ATCC 11337, <i>Spirillum lunatum</i> ATCC 19193, <i>Spirillum minutulum</i> ATCC 19259, <i>Spirillum anulus</i> ATCC 7566, <i>Spirillum tenue</i> ATCC 14667, <i>Spirillum delicatum</i> ATCC 11334, <i>Spirillum giesbergeri</i> ATCC 12639, <i>Spirillum itersonii</i> subspecies <i>itersonii</i> ATCC 15280, <i>Spirillum metamorphum</i> ATCC 11332, <i>Spirillum polymorphum</i> ATCC 15279, <i>Spirillum putridiconchylum</i> ATCC 12638, <i>Spirillum serpens</i> ATCC 15278, subspecies <i>serpens</i> ATCC 11335, <i>Spirillum serpens</i> subspecies <i>azotum</i> ATCC 9786, <i>Spirillum sinuosum</i> ATCC 15387, <i>Spirillum peregrinum</i> ATCC 19624 a + b, <i>Spirillum gracile</i> ATCC 19625, <i>Spirillum gracile</i> ATCC 19626, <i>Spirillum gracile</i> ATCC 11331, <i>Spirillum itersonii</i> subspecies <i>vulgatum</i> V.P.I. 104, 8230, 9072, 101	N. Krieg	Freshwater spirilla

proline as sole sources of carbon and nitrogen were tested, as was the ability to grow at selected temperatures ranging from 4 to 37 C, in salt concentrations ranging from 0 to 10%, and at pH values from 4 to 10. Methods and details of the procedures followed have been published elsewhere (7, 8, 18).

Results from each of the above tests were scored for analysis by computer. In the coding system employed,

1 indicated positive result, 0 indicated negative result, and 3 indicated that the test was not applicable or not performed. The Jaccard coefficient, S_j , with negative matches excluded, was used (21). The strains were clustered by a single linkage technique, using the GTP-2 program. Feature frequencies were computed for the strain clusters using the GTP-3 program (8).

The guanine plus cytosine content (moles percent

G+C) of purified DNA extracted from selected strains in this study was determined by previously described methods (6, 17).

RESULTS

One hundred and seven strains were examined for 175 coded characteristics. Characters uniformly negative included the following: growth in KCN, utilization of adonitol, dulcitol, inositol, inulin, melezitose, raffinose, and rhamnose, digestion of agar, and growth at 4 C. None of the strains produced gas from the carbohydrates tested. With the exception of a reference strain, *Pseudomonas fluorescens* strain Oxford-Sawyer, the tests for pigmentation, such as Sabouraud agar and King, Ward, and Raney agar, and the tests for fluorescence (i.e., Paton medium and Haynes medium) were negative. All strains grew at 25 C and in the pH range 6 to 9 and were sensitive to 10 µg of chloromycetin.

The similarity matrix obtained from the computer analysis is shown in Fig. 1. Eight clusters and nine strains which did not cluster with any of the eight groups were noted. Four clusters were identified as *V. cholerae* (group 1), *V. parahaemolyticus* (group 2), marine *Spirillum* strains (group 6), and freshwater *Spirillum* strains (group 7). The remaining clusters consisted of two or three strains each. Of the latter, groups 3, 4, and 5 showed some similarity to *V. parahaemolyticus*, whereas group 8 did not demonstrate significant relationships with any of the other strains studied. Characters found to be useful in differentiating the major groupings are listed in Table 2.

Intra- and intergroup similarity (S) values are given in Table 3. A set of *V. cholerae* strain data, analyzed in an earlier published study (8), was included here solely for purposes of comparison. The *V. cholerae* cluster was homogeneous (intragroup % S = 85) and demonstrated an intergroup similarity of approximately 75% with *V. parahaemolyticus*. A low degree of similarity (38 to 39%) was observed between *Vibrio* spp. and *Spirillum* spp. and the other clusters obtained in this study.

Despite varied geographical sources, the *V. parahaemolyticus* strains (cluster 2) displayed a high intragroup similarity value, S = 83%. A very high degree of relatedness, S ≥ 92%, was noted among eight *V. parahaemolyticus* isolates (labeled FDA strains) and *V. parahaemolyticus* FC1011. Several of these FDA strains of *V. parahaemolyticus* and strains of this species obtained from the Maryland Department of Health (isolated from the same *V. parahaemolyticus* outbreak in Bainbridge, Md.

in 1971 [9]) were found to be related at S ≥ 90%. Eleven strains isolated from Pompano fish in mariculture were highly related to each other and, interestingly, were sucrose utilizers. The *V. parahaemolyticus* phage hosts V-6 and V-14 were included in the cluster at S = 77 and 78%. These strains differed from the clustered strains and from each other mainly in antibiotic sensitivities, cultural and colonial morphology, and salt and pH tolerances.

The three luminescent organisms (cluster 3) demonstrated an intragroup similarity of 85% and were included in the *V. parahaemolyticus* cluster at S = 80%. Although similar to *V. parahaemolyticus* in cultural and colonial characteristics, these strains could be differentiated on the basis of bioluminescence, inability to utilize arginine, citrate, or formate, and requirement for added sodium chloride.

Clusters 4 and 5, consisting of two strains each, did demonstrate some degree of similarity with *V. parahaemolyticus*. The Pompano isolates (cluster 4), which can be described as polarly flagellated, gram-negative, fermentative, non-gas-producing rods, were unable to utilize citrate, dextrin, fructose, formate, glycerol, maltose, malonate, or trehalose or to hydrolyze starch or gelatin, and lacked both the lysine and ornithine decarboxylases. The difference in biochemical abilities, in conjunction with the low (S = 71%) similarity to *V. parahaemolyticus*, precludes their identification as members of this species. The data obtained on cultural and colonial characteristics, biochemical abilities, and mol% G+C, permit a similar conclusion concerning the Thompson cultures (cluster 5), which joined the *V. parahaemolyticus* cluster at S = 69%.

The *Spirillum* strains formed two groups, clusters 6 (marine) and 7 (freshwater), which were clearly distinct from each other and from the other groups. Although a low intergroup similarity (S = 46%) was observed for these two groups, neither group, by itself, was markedly homogeneous (cluster 6, S = 66%; cluster 7, S = 64%). Differentiation of the groups was possible on the basis of morphology and sodium chloride requirement (Table 2). Individual freshwater *Spirillum* strains could be distinguished by their ability to hydrolyze gelatin, utilize Casamino Acids, arginine, DL-alanine, β-alanine, lysine, and proline. There was, however, no definitive set of characteristics which served to identify this group of strains. *Spirillum lunatum*, originally classified as a marine strain and listed by the American Type Culture Collection as such, did not demonstrate a require-

ment for sodium chloride and was not found to be closely related to any of the other *Spirillum* strains.

Of the nine unclustered strains, five were reference strains, viz., *Escherichia coli*, *Micrococcus* sp., *Pseudomonas* sp., *Vibrio marinus* strain PS 207, and *Achromobacter fischeri*. The marine isolate, strain 3S2, demonstrated low similarity (61%) to the other spirilla.

TABLE 2. Summary of differential characteristics

Character	Cluster							
	V. cholerae	V. parahaemolyticus				Marine spirilla	Fresh-water spirilla	
	1	2	3	4	5	6	7	8
Total no.	29	56	3	2	2	7	21	3
Oxidative (glucose)	29	56	3	2	2	0	0	3
Fermentative (glucose)	29	56	3	2	2	0	0	3
Arabinose	0	21	0	2	0	0	0	0
Cellobiose	18	27	3	2	0	0	2	2
Dextrin	29	49	3	0	0	0	0	0
Galactose	29	56	3	0	2	0	0	0
Glycerol	28	41	2	2	0	0	0	3
Maltose	29	54	3	2	0	0	0	0
Mannitol	29	56	1	2	2	0	0	0
Mannose	26	55	3	2	2	0	0	0
Melibiose	0	5	2	0	0	0	0	0
Sucrose	28	3	0	0	2	0	0	0
Trehalose	29	56	3	0	0	0	0	3
Xylose	0	0	0	2	0	0	0	0
Amylose	28	56	3	0	0	0	0	0
Gelatinase	29	53	3	0	0	0	6	0
Lipase	29	56	3	2	2	3	6	0
Oxidase	29	56	3	2	2	6	21	0
Catalase	29	56	3	2	2	7	20	3
Formate	28	41	0	0	0	0	14	0
Acetate	29	56	3	2	2	0	17	0
Casamino Acids	29	56	3	2	2	0	18	0
L-Arginine	29	46	0	0	2	0	3	0
L-Proline	29	56	1	2	2	0	11	0
Indole	29	56	3	0	2	0	0	0
NH ₄ H ₂ PO ₄	29	48	2	2	2	0	21	3
NaCl required	0	56	3	2	2	7	0	3

V6, V14, and *S. lunatum*, also unclustered strains, have already been discussed. None of the *Vibrio* or *Spirillum* strains demonstrated S values greater than 40% with the Pompano isolates which comprised cluster 8. These three organisms were found to be gram-positive, fermentative, motile rods.

Differences among strains grouped within the genera *Vibrio* and *Spirillum* and between the marine and freshwater spirilla were corroborated by mol% G+C data (Table 4). For example, a G+C of 41.6 mol%, obtained for the Thompson S-12-O culture, substantiates the consideration of the two organisms comprising cluster 5 as a species separate from *V. parahaemolyticus* which has a mol% G+C of 45 ± 1 . The mol% G+C determined for the

TABLE 4. DNA base composition of strains included in this study

ATCC	Designation	T _m (C)	G+C (mol %)
19192	<i>Spirillum halophilum</i>	86.8	42.7
19193	<i>S. minutum</i>	97.0	44.1
11336	<i>S. linum</i>	89.3	49.0
12753	<i>S. atlanticum</i>	89.7	49.8
19191	<i>S. japonicum</i>	88.2	46.1
12754	<i>S. beijerinckii</i>	88.4	46.1
	<i>Spirillum</i> sp. FC281-a	94.9	62.4
14667	<i>S. delicatum</i>	95.0	62.7
7566	<i>S. tenue</i>	95.7	64.4
11332	<i>S. polymorphum</i>	94.8	62.2
11331	<i>S. itersonii</i>	94.5	61.4
12639	<i>S. itersonii</i>	94.0	60.2
11334	<i>S. giesbergeri</i>	92.6	56.8
15387	<i>S. peregrinum</i>	94.0	60.2
9786	<i>S. sinuosum</i>	92.9	57.6
19259	<i>S. anulus</i>	93.0	57.8
15279	<i>S. putridiconchylum</i>	90.6	52.0
15278	<i>S. serpens</i>	89.9	50.0
11335	<i>S. serpens</i>	95.3	63.4
12638	<i>S. serpens</i>	89.8	50.0
19624a	<i>S. gracile</i>	95.7	64.4
19624b	<i>S. gracile</i>	95.9	64.9
19625	<i>S. gracile</i>	95.4	63.7
19626	<i>S. gracile</i>	95.6	64.1
15280	<i>S. metamorphum</i>	89.8	50.0
11337	<i>S. lunatum</i>	94.3	61.0

TABLE 3. Inter- and intragroup similarity values

1. <i>Vibrio cholerae</i>	85							
2. <i>Vibrio parahaemolyticus</i>	75	83						
3. Luminescent strains	70	70	85					
4. Thompson isolates	65	68	38	91				
5. Pompano isolates	68	68	40	65	90			
6. Marine spirillum	39	39	41	39	46	66		
7. Freshwater spirillum	38	38	41	40	40	40	64	
8. Pompano isolates	38	38	41	38	38	40	38	93
Group no.	1	2	3	4	5	6	7	8

marine spirilla strains ranged from 42.7 to 49.8. Interestingly, there was a close correspondence between the mol% G+C and percent S within this group. *Spirillum halophilum* and *Spirillum minutulum* were 86% related phenetically and demonstrated a G+C composition of 42.7 and 44.1 mol%, respectively. *Spirillum linum* and *Spirillum atlanticum* (S = 82%) revealed a DNA base composition of 49.0 and 49.8 mol%, respectively. *Spirillum beijerinckii* and *Spirillum japonicum* were found to be 72% similar, phenetically, and demonstrated a G+C of 46.1 mol% (Table 4). The high correlation observed between similarity values and DNA base composition was not found for all the freshwater spirilla. Within the freshwater group, the G+C ranged from 50.0 to 64.4 mol%. Indeed, several of the more closely related species, namely, *Spirillum serpens* strains 11335 and 12638, demonstrated markedly different DNA base compositions, 63.4 and 50 mol% G+C. Data for other strains in these groups are given in Table 4.

In summary, the *V. cholerae* and *V. parahaemolyticus* strain clusters were found to be related (S = 75%) to each other but to differ significantly from both of the *Spirillum* groups (S = 40%). Furthermore, the freshwater and marine isolates did not share high similarity values (S = 46%). Although *V. parahaemolyticus* and the marine *Spirillum* spp. shared similar overall DNA base composition, the similarity values obtained indicated a rather low relationship between these groups. The freshwater *Spirillum* spp. yielded G+C values ranging from 50 to 64.4 mol%, markedly different from those of the other major clusters.

DISCUSSION

Analysis of results obtained in this study of *Vibrio* and *Spirillum* spp. illustrates some of the problems encountered in bacterial taxonomy. Morphology, as a primary factor in bacterial classification, should be critically evaluated. The importance of the source of isolation in classification has not been clearly established, nor has the level of relatedness indicating membership in a given genus or species been agreed upon by taxonomists.

After several nomenclatural reassignments, *V. parahaemolyticus*, which may or may not demonstrate cell curvature, has been placed in the genus *Vibrio*. Originally described as a *Pasteurella* sp., *V. parahaemolyticus* was subsequently placed in *Oceanomonas*, *Pseudomonas*, or *Aeromonas* by various investigators. In 1966, it was suggested that the genus *Vibrio*

should be redefined to indicate a type of motion rather than shape (10), with the net effect of reducing the weight placed on morphology. Recently, the suggestion was made that marine vibrios should be included in the genus *Beneckeia* (3). In this study, strains of *V. cholerae* and *V. parahaemolyticus* were found to have similarity values of 75% S, which can be interpreted as a marginal level of similarity for inclusion of these species in the same genus. Nevertheless, a closer relationship of *V. parahaemolyticus* with genera other than *Vibrio* is not precluded.

The high level of similarity shown by the luminescent bacteria with *V. parahaemolyticus* suggests that the former are representatives of a species related to *V. parahaemolyticus*. The biochemical data and G+C of the luminescent bacteria (47 mol%) correlate with data published by Shewan for a set of bioluminescent strains (20). Hendrie et al. suggested that *V. fischeri* and strains PS207 and MP-1 of *V. marinus* be put into the same species (11). The mol% G+C values published for these organisms are in the range 40 to 43, with PS207 = 43 mol% and MP-1 = 41 mol% (6), whereas the DNA base composition of the luminescent bacteria in group 3 of this study was found to be in the 47.1 to 47.3 mol% G+C range. Several differences in carbohydrate utilization were also observed among these strains. It has been proposed that strains with these characteristics be placed in a separate genus, the name of which is in dispute (*Lucibacterium* or *Beneckeia*). Further work involving DNA/DNA hybridization studies of several luminescent bacteria is planned to resolve this question.

Groups 4 and 5 showed high relationship with *V. parahaemolyticus* and *V. cholerae* but did not demonstrate all of the biochemical capabilities of these species. Furthermore, group 5 strains (Thompson strains) were found to be nonmotile under the conditions tested and in at least one case (Thompson strain S-12-O, G+C = 41.6 mol%) demonstrated significantly different overall DNA base composition.

Examination of the *V. parahaemolyticus* strain data revealed two strain subclusters of high intragroup similarity. In one case, namely that of the FDA Bainbridge strains, subcultures of the same organisms may be involved since the strains, although received from different laboratories, were obtained from the same outbreak.

The isolates from Pompano fish, all of which were sucrose positive, were acetoin negative and did not grow in 10% salt, indicating that they

may be sucrose-positive *V. parahaemolyticus*. Sakazaki noted only one sucrose-fermenting strain of *V. parahaemolyticus* among thousands of isolates from human diarrheal stools (19). However, sucrose-positive strains may be more common in marine or estuarine environments (G. Sakaguchi, R. Sakazaki, and Y. Taheda, Int. Symp. *Vibrio parahaemolyticus*, in press).

The low similarity values observed between strains of the genera *Vibrio* and *Spirillum* reflect not only morphological differences but also the fact that *Vibrio* spp. demonstrate more versatile biochemical capabilities. The S values, based on morphological, physiological, nutritional and biochemical tests, were low, supported by marked differences in mol% G+C of

the DNA for the *Vibrio* and freshwater *Spirillum* strains. On careful examination of the phenetic data, the DNA base composition determinations for the *Vibrio* spp. (46 to 47 mol%) and the marine *Spirillum* spp. (42 to 49.8 mol%) did not provide sufficient evidence to conclude that the genera are closely related.

Although several species of *Spirillum* have been identified and classified on the basis of morphology, biochemical properties were not used to define species of this genus until 1936 (G. Giesberger, Ph.D. thesis, Delft University, Netherlands, 1936). All of the original Giesberger strains were lost as a result of World War II. *Spirillum* spp. were reisolated by Williams and Rittenberg in 1957 (26), who relied on morphology as the primary factor in classification. Despite a similarity in length or width, the variability in amplitude, wave length, and cell width makes many *Spirillum* species visibly distinct.

In 1972, Terasaki (23) published an extensive study of 49 *Spirillum* strains and included many characteristics previously examined by others for *Spirillum*. More recently, Hylemon et al. (12) suggested, on the basis of a very careful study, that organisms comprising the genus *Spirillum* should be reclassified into three genera—*Spirillum*, *Oceanospirillum*, or *Aquaspirillum*.

The genetic and phenetic data of the study reported here indicate differences among the *Spirillum* strain clusters. However, analyses of feature frequencies (Table 2) showed that there was no set of definitive characteristics, other than salt requirement demonstrated by the marine strains, which could be used to separate the *Spirillum* groups phenetically. Although individual strains could be distinguished, few characters were held in common by all members of the strain clusters. The clustering of strains which was noted is substantiated by DNA base composition data, i.e., the DNA base compositions of the freshwater spirilla and the marine strains were 50 to 64 mol% G+C and 42 to 46 mol% G+C, respectively. High G+C content of the DNA, as well as production of a water-soluble, fluorescent pigment and presence of poly- β -hydroxybutyrate granules, observed by other investigators, suggests that some relationships between the freshwater spirilla and members of the genus *Pseudomonas* may exist. It would be interesting and useful to determine the degree of DNA/DNA homology between several of these organisms and selected *Pseudomonas* spp. The marine strains included in this study were found to be easily differentiated from *Spirillum volutans*, the proposed

FIG. 2. Dendrogram showing the relationships among different strains.

type species. Hylemon et al. (12) examined the characteristics of *S. volutans* and discussed those which led them to propose that the marine strains be renamed *Oceanospirillum*.

Several subgroupings are apparent in an examination of the dendrogram shown in Fig. 2. In most cases, the subgroupings were characterized not only by a relatively high percent of similarity but also by similar DNA base composition and antigenic properties (16). Within the cluster of marine strains, members of the subgroups demonstrated a relatively high degree of similarity, suggesting a single species, namely *S. minutum* and *S. halophilum*, and *S. linum* and *S. atlanticum*. Although two other marine species, *S. japonicum* and *S. beijerinckii*, shared similar DNA base composition, they were easily differentiated on phenetic and morphological bases. VPI 101, related to the other marine strains at 67%, has been named *Oceanospirillum maris*, ATCC 27509 (12).

Several subgroups were detected within the freshwater *Spirillum* cluster. In general, these were subspecies groups, such as *S. serpens* and *Spirillum itersonii*. One strain, received as *S. serpens* 11335, demonstrated a DNA base composition of 63.4 mol% G+C. Most probably it is a substrain of, or related to, *S. serpens* 11330, the base composition of which is about 65 mol% G+C. VPI 8230 and 9072 have been identified by Hylemon et al. (12) as *Spirillum graniferum* and *Spirillum polymorphum*, respectively. VPI 104 (ATCC 27510), named *Aquaspirillum dispar* (12), demonstrated a 67% S to the cluster of freshwater strains. Although FC281-a and 3S2 demonstrated a spiral shape under certain conditions, they were not included in the calculation of *Spirillum* feature frequencies (Table 2) since they are capable of fermentative utilization of glucose. *S. lunatum*, originally classified as a marine *Spirillum*, is not a salt-requiring strain and differs in several properties (i.e., DNA composition and selected carbohydrate utilization tests) from the other marine spirilla. Indeed, a lower relationship with the *Spirillum* cluster was observed for *S. lunatum* than for any of the other spirilla examined.

In conclusion, marked phenetic differences were observed for the genera *Vibrio* and *Spirillum*, with species of the genus *Vibrio* demonstrating more diverse biochemical capabilities than the *Spirillum* spp. The numerical taxonomy and molecular genetic data support reassignment of selected *Spirillum* spp. to the genera *Oceanospirillum* and *Aquaspirillum*, as proposed by Hylemon et al. (12).

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REPRINT REQUESTS

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