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The Genus *Aeromonas*: Biochemical Characteristics, Atypical Reactions, and Phenotypic Identification Schemes

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A total of 193 strains representing 14 different *Aeromonas* genomospecies were evaluated for 63 phenotypic properties to create useful tables for the reference identification of mesophilic aeromonads. Only 9 of 62 biochemical tests (14%) yielded uniform results, and the fermentation of certain carbohydrates was found to be linked to specific species. A number of unusual or aberrant properties for the genus *Aeromonas* were also detected in the collection of 428 strains (193 in the phenotypic study, 235 in a retrospective review). These tests included susceptibility to the vibriostatic agent, fermentation of *m*-inositol and D-xylose, hydrolysis of urea, and the lack of cytochrome oxidase activity. Fermentation of melibiose was linked to raffinose fermentation in all *Aeromonas* species except *A. jandaei*. Keys are provided for clinical laboratories choosing to identify aeromonads to species level based upon initial Møeller decarboxylase and dihydrolase reactions. In addition, several new tests were identified that help to separate members of the *A. caviae* complex (*A. caviae, A. media,* and *A. eucreonophila*).

The genus *Aeromonas* has undergone a number of taxonomic and nomenclature revisions over the past 15 years. Although originally placed in the family *Vibrionaceae* (34), which also included the genera *Vibrio, Photobacterium,* and *Plesiomonas*, subsequent phylogenetic investigations indicated that the genus *Aeromonas* is not closely related to vibrios but rather forms a monophyletic unit in the γ-3 subgroup of the class *Proteobacteria* (25, 31). These conclusions necessitated the removal of *Aeromonas* from the family *Vibrionaceae* and transfer to a new family, the *Aeromonadaceae* (8). Similarly, only five species of *Aeromonas* were recognized 15 years ago (21), three of which (*A. hydrophila, A. sobria,* and *A. caviae*) existed as phenospecies, that is, a named species containing multiple DNA groups, the members of which could not be distinguished from one another by simple biochemical characteristics. Subsequent systematic investigations have resulted in the number of valid published genomospecies rising to 14 (23), and it is anticipated that additional species will be described because rare strains have been identified that do not reside in any established *Aeromonas* species.

The genus *Aeromonas* comprises important human pathogens causing primary and secondary septicemia in immunocompromised persons, serious wound infections in healthy individuals and in patients undergoing medicinal leech therapy, and a number of less well described illnesses such as peritonitis, meningitis, and infections of the eye, joints, and bones (18). Gastroenteritis, the most common clinical manifestation, remains controversial (18). While there are a number of well-described cases of *Aeromonas*-associated gastroenteritis in the literature, it still remains unproven whether most fecal isolates recovered from symptomatic persons are the etiologic agent responsible for the diarrheal syndrome. One theory to explain this contradiction is that only specific subsets of *Aeromonas* are pathogenic for humans and that biotyping schemes need to be developed to differentiate environmental from clinical strains (23).

One of the major difficulties in the identification of *Aeromonas* strains to species level concerns the current number of recognized taxa ($n = 14$) and the lack of clear-cut phenotypic tables useful in distinguishing each of these groups from the others (17). This problem has arisen because taxonomic studies often report only selected biochemical characteristics on newly described species and then compare these results to phenotypic data from previously published studies on genetically related taxa. Although the tests used may be the same in each study, the growth conditions, medium composition, inoculation procedure, and incubation conditions may vary considerably, potentially affecting results (12, 15).

In some instances, commercial systems have been used to generate the data, and it is not always clear how closely microidentification test results parallel results obtained by conventional methodology (2). Furthermore, many of the biochemical schemes currently used in clinical laboratories to identify aeromonads predate the description of newer taxa (1, 5, 7, 22). This fact calls into question whether previously selected biochemical tests used to identify older *Aeromonas* species are applicable to the identification of those described more recently. Finally, the diversity in testing methodologies has hampered the development of consistent phenotypic properties and typing schemes with which to identify most *Aeromonas* strains, if necessary, to species level in the clinical laboratory.

In this study we present cumulative biochemical data on almost 200 *Aeromonas* strains representing each of the 14 recognized species and propose possible schemes for their identification in the clinical laboratory (Table 1).

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TABLE 1. Type and reference cultures of *Aeromonas* strains used in this investigation[a]

Species	No. of strains	Type strain	Reference culture(s)
A. hydrophila	25	ATCC 7966	
A. bestiarum	16	CDC 9533-76 (=ATCC 51108)	ATCC 14715, NCMB 1134
A. salmonicida	13		CDC 434-84 (=Popoff C316)
A. caviae	25	ATCC 15468	
A. media	11	ATCC 33907	ATCC 35950, CDC 862-83 (=Popoff C239), CDC 435-84 (=Popoff C233)
A. eucrenophila	9	NCMB 74 (=ATCC 23309)	NCMB 73, LMG 16179, LMG 17062
A. sobria	2	CIP 7433 (=CDC 9538-76, =Popoff 208)	CDC 9540-76 (=Popoff 215)
A. veronii bv sobria[b]	25		ATCC 9071, CDC 437-84 (=ATCC 51106)
A. jandaei	15	CDC 787-80 (=ATCC 49568)	ATCC 49569, ATCC 49570, ATCC 49571
A. veronii bv veronii[c]	10	ATCC 35624	ATCC 35622, ATCC 35623, ATCC 35625, ATCC 35626
A. schubertii	12	CDC 2446-81 (=ATCC 43700)	CDC 9180-81 (=ATCC 43701), CDC 2508-86 (=ATCC 43945), CDC 463-83 (=ATCC 43947)
A. trota	16	AH2 (=ATCC 49657)	MOB/A8 (=ATCC 49658), NMRI-208 (=ATCC 49661), AS-66 (=ATCC 49660), AS-370 (=ATCC 49659)
A. encheleia	4	CECT 4342 (=ATCC 51929)	CECT 4341 (=ATCC 51930), CECT 4340 (=ATCC 51931), CECT 4343
A. allosaccharophila	3	ATCC 51208 (=CECT 4199)	ATCC 35942 (=CDC 715-84), LMG 14058 (=CECT 4200)
A. popoffii	7	LMG 17541 (=CIP 105493)	LMG 17542, LMG 17543, LMG 17544, LMG 17545, LMG 17546, LMG 17547

[a] Abbreviations: ATCC, American Type Culture Collection (Manassas, Va.); CDC, Centers for Disease Control and Prevention (Atlanta, Ga.); NCMB, National Collection of Marine Bacteria (Aberdeen, Scotland); LMG, Laboratorium voor Microbiologie (Ghent, Belgium); CIP, Collection of the Institut Pasteur (Paris, France), CECT, Colección Española de Cultivos Tipo (Valencia, Spain).
[b] Hybridization group 8, ornithine decarboxylase negative.
[c] Hybridization group 10, ornithine decarboxylase positive.

MATERIALS AND METHODS

Strains. A total of 193 strains were investigated in this study (Table 1); approximately 75% were from clinical specimens, with the remainder (≈25%) from animals, fish, or environmental sources. The taxa included in these investigations were *A. hydrophila, A bestiarum, A. salmonicida, A. caviae, A. media, A. eucrenophila, A sobria* (*sensu stricto*), *A. veronii* (two biotypes), *A. jandaei*, *A. schubertii*, *A. trota, A. encheleia, A. allosaccharophila,* and *A. popoffii*. Included within these 193 strains were the type strains for 13 *Aeromonas* species and 37 reference strains received from national and international culture collections. The *A. salmonicida* type strain, ATCC 33658^T (subspecies *salmonicida*), was not included in this study as it represents strains typically recovered from fish (salmonids) that grow at lower temperatures (20°C), produce a brownish pigment, and are nonmotile and indole negative. The *A. salmonicida* strains included in this study primarily originated from clinical material (feces) and from environmental samples (water). These strains were motile, grew well at 35°C, were indole positive, and did not produce melanin-like pigments; however, they did belong to hybridization group 3 (*A. salmonicida*) by DNA pairing studies.

Of these 193 strains, 152 (79%) had previously been identified to species level by DNA binding. The remaining 41 strains fit the classic phenotypic definition for their respective taxa. All strains were maintained as working cultures in motility agar deeps at room temperature during the course of these investigations and were periodically transferred to retain viability. In addition to this initial group of 193 strains, we retrospectively reviewed biochemical data on another 235 isolates in our collection.

Biochemical tests. *Aeromonas* strains (n = 193) were tested for 63 phenotypic traits. These tests were performed in a conventional format as previously described, and appropriate positive and negative controls were included for each test and with each lot of prepared medium (1, 13, 19). Liquid medium or agar slants were inoculated from overnight tryptone broth cultures grown at 35°C or, in the case of *A. popoffii* and *A. sobria* CIP 7433 (*sensu stricto*), 25°C. Plates (e.g., to assay elastase) were inoculated from overnight growth on heart infusion agar slants. Biochemical or enzymatic tests performed by plate assays included DNase and elastase activities, elaboration of a β-hemolysin, polypectate (pectinase) degradation, and expression of a staphylysin (1, 16).

Several new tests were added, including clearing of tyrosine-containing (0.5%) plates (tyrosinase), detection of alkylsulfatase activity (plate), and Jordan's tartrate (13, 20). Carbohydrate fermentation reactions were performed in extract broth (Acumedia Manufacturers, Inc., Baltimore, Md.) containing 1% (wt/vol) of the desired sugar and 1% (vol/vol) Andrade's indicator. All reagents (sugars and substrates) were obtained from Sigma (St. Louis, Mo.). Gas production from D-glucose fermentation was determined in carbohydrate fermentation broths containing Durham tubes. All tests were incubated at 35°C (polypectate and pigment, 25°C) with the exception of *A. popoffii* and *A. sobria* CIP 7433 (*sensu stricto*), which were incubated at 25°C because preliminary experiments had indicated that much better growth occurred at lower rather than higher temperatures.

Tests were read daily for 7 days with the following exceptions: tests for *o*-nitrophenyl-β-D-galactopyranoside (ONPG) and susceptibility to O/129 (2,4-diamino-6,7-diisopropylpteridine, 150 μg), ampicillin (10 μg), and cephalothin (30 μg) were read at 1 day; tests for KCN, malonate, gluconate oxidation, pyrazinamidase, and Jordan's tartrate were read at 2 days; tests for DL-lactate and urocanic acid utilization and Voges-Proskauer were read at 3 days; and tests for urea hydrolysis, citrate utilization, ornithine and lysine decarboxylase, and arginine dihydrolase activity were read at 4 days. A subset of these strains (n = 27) were additionally evaluated for the ability to ferment rare or unusual sugars. Carbohydrates tested included β-gentobiose, glucamine, glucose 1-phosphate, glucose 6-phosphate, inulin, lactulose, D-lyxose, maltotriose, palatinose, sedoheptulose, stachyose, D-tagatose, D-turanose, and xylitol. In addition, to determine the relative extent of phenotypic variation in the genus, the biochemical test results of 235 additional *Aeromonas* strains identified by the Microbial Diseases Laboratory were reviewed.

RESULTS

Biochemical properties. Most *Aeromonas* strains produced tan to buff-colored colonies on Trypticase soy agar (Becton-Dickinson, Cockeysville, Md.) when incubated at 25°C for 2 to 5 days. *A. encheleia* was extremely slow in pigment production, with all four strains yielding tan colonies only after 7 days of incubation. Rare strains, such as *A. bestiarum* ATCC 14715, *A. media* ATCC 33907 and ATCC 35950, and a strain of *A. eucrenophila,* produced brown to dark brown colonies on Trypticase soy agar. This pigmentation was similar to the melanin-like pigment produced by many fish isolates of *A. salmonicida* (10).

Of the 62 biochemical characteristics evaluated for all 193 strains of *Aeromonas*, only 9 tests (15%) yielded uniform re-

TABLE 2. Biochemical properties of *Aeromonas* species

Trait[a]	% of strains showing trait								
	A. hydrophila (*n* = 25)	*A. bestiarum* (*n* = 16)	*A. salmonicida* (*n* = 13)	*A. caviae* (*n* = 25)	*A. media* (*n* = 11)	*A. eucrenophila* (*n* = 9)	*A. sobria* (*n* = 2)	*A. veronii*[b] (*n* = 35)	
								HG 8	HG 10
Motility	100	100	69	88	82	100	0	96	100
Catalase	100	100	100	100	100	100	50	100	90
Indole	96	100	100	84	100	89	100	100	100
ONPG	100	100	100	100	100	100	100	100	100
Urea	0	0	0	8	0	0	0	0	0
KCN	100	81	77	92	73	100	0	68	0
Citrate	88	12	85	88	55	0	100	52	90
Acetate	100	94	92	92	73	45	100	100	90
Malonate	0	0	0	0	0	0	0	0	0
VP	92	63	62	0	0	0	0	88	70
LDC	100	50	46	0	0	0	100	96	100
ODC	0	0	0	0	0	0	0	0	100
ADH	100	88	69	92	64	56	0	100	0
Gelatin	96	75	100	84	55	89	0	100	100
PPA	60	25	46	72	73	22	100	100	100
Pectate[c] (25°C)	0	0	38	0	0	0	0	0	0
DNase	100	63	69	92	82	78	0	84	60
Lipase	100	88	92	76	82	89	100	92	90
Glucose (gas)	92	69	77	0	0	78	50	92	90
Acid from:									
Adonitol	0	0	0	0	0	0	0	0	0
Amygdalin	4	0	8	4	0	0	0	0	0
L-Arabinose	84	100	100	100	100	78	0	12	10
Cellobiose	0	19	69	100	100	56	100	20	80
Glycerol	96	100	100	68	55	11	100	96	100
m-Inositol	0	0	0	0	0	0	0	0	0
Lactose	16	13	92	60	64	11	0	12	40
Maltose	100	100	92	100	100	100	100	92	100
Mannose	100	100	100	32	100	100	100	100	100
D-Mannitol	96	100	100	100	100	100	100	100	100
Melibiose	0	0	0	4	0	0	0	4	0
α-Methyl-D-glucoside	56	38	46	0	0	0	50	16	90
Raffinose	0	0	0	4	0	0	0	4	0
L-Rhamnose	24	69	0	0	0	22	0	0	0
Salicin	76	56	31	76	28	67	0	0	100
D-Sorbitol	0	0	85	4	0	0	0	0	0
Sucrose	100	94	100	100	100	33	100	100	100
Esculin	92	81	85	76	55	78	0	0	100
GCF	92	75	69	0	0	33	100	68	10
Gluconate	64	13	0	0	0	0	0	60	70
DL-Lactate	80	0	0	96	45	0	0	0	0
Urocanic	12	94	100	100	100	0	0	0	0
Tartrate	0	0	0	0	0	0	0	0	100
NaCl (0%)	100	100	100	96	100	100	100	100	100
NaCl (3%)	100	94	100	96	100	89	0	100	100
PZA	24	50	31	88	18	100	0	56	0
β-Hemolysis	100	94	69	52	45	89	0	100	100
Staphylysin[c]	84	38	46	0	0	0	0	0	0
Alkylsulfatase[c]	12	0	0	4	0	33	50	40	90
Elastase[c]	72	13	46	0	0	0	0	0	0
Tyrosine[c]	0	0	0	8	0	0	0	32	10
Ampicilin R[d]	100	94	85	100	73	100	50	100	100
Cephalothin R[d]	72	81	77	100	73	78	0	0	20
O/129 R[d]	100	100	100	100	100	67	100	100	90

[a] Test results were read at 48 h unless otherwise noted. Abbreviations: ONPG, *o*-nitrophenyl-β-D-galactopyranoside; VP, Voges-Proskauer; LDC, lysine decarboxylase; ODC, ornithine decarboxylase; ADH, arginine dihydrolase; PPA, phenylpyruvic acid (phenylalanine deaminase); GCF, H_2S production from cysteine; PZA, pyrazinamidase; O/129, 2,4-diamino-6,7-diisopropylpteridine; R, resistance to indicated compound or antimicrobial agent.

[b] HG 8, *A veronii* biogroup sobria (ornithine decarboxylase negative; *n* = 25); HG 10, *A veronii* biogroup veronii (ornithine decarboxylase positive; *n* = 10).

[c] Tests were read at 96 h.

[d] Tests were read at 24 h.

sults. These reactions were the presence of cytochrome oxidase and nitrate reductase, fermentation of D-glucose and trehalose, failure to utilize mucate, and the inability to produce acid from D-arabitol, dulcitol, erythritol, and xylose. The remaining 53 tests that yielded variable results are listed in Table 2. The positive reactions listed in Table 2 were for 48 h (2 days). This endpoint was chosen because clinical laboratories rarely read biochemical test results on rapid growers for more than 48 h.

TABLE 2—*Continued*

% of strains showing trait					
A. jandaei (*n* = 15)	*A. schubertii* (*n* = 12)	*A. trota* (*n* = 16)	*A. encheleia* (*n* = 4)	*A. allosaccharophila* (*n* = 3)	*A. popoffii* (*n* = 7)
100	100	100	100	100	100
100	100	100	100	100	100
100	8	100	100	100	43
100	83	100	100	100	57
0	0	0	0	0	0
60	8	13	100	33	57
87	58	94	0	33	100
100	75	94	100	100	43
0	0	6	0	0	14
87	17	0	0	0	86
100	83	100	0	100	0
0	0	0	0	33	0
100	92	94	25	67	100
93	83	100	0	100	100
93	67	94	50	100	86
0	0	0	0	0	0
93	58	100	100	0	57
93	92	0	100	100	100
100	0	69	75	100	100
0	0	0	0	33	0
0	0	0	0	0	0
0	0	0	0	67	57
13	0	100	0	100	0
100	0	81	100	100	100
0	0	0	0	33	0
0	0	0	0	0	0
100	100	100	100	100	100
100	92	100	100	100	100
100	0	69	100	100	100
47	0	0	0	33	0
33	0	0	0	33	100
0	0	0	0	33	0
0	0	0	75	67	0
0	0	0	0	0	0
0	0	0	0	0	0
0	0	19	75	100	0
0	0	0	50	67	0
47	0	6	75	33	100
60	0	0	0	0	0
7	58	88	0	0	71
7	0	75	75	0	57
0	0	0	0	33	0
100	92	100	100	100	100
100	100	100	100	100	100
0	0	13	0	33	0
100	75	50	75	33	0
0	0	0	0	0	0
33	83	94	0	0	29
0	0	0	0	0	0
0	8	13	0	0	0
93	92	6	100	100	100
67	25	100	50	100	100
100	100	100	100	100	100

Although all 193 *Aeromonas* strains were oxidase positive, several other tests useful in the differentiation of aeromonads from vibrios and plesiomonads gave variable results (Table 2).

Two strains of *A. eucrenophila* and one strain of *A. veronii* biogroup veronii produced small zones of growth inhibition in the presence of the vibriostatic agent O/129. One *A. caviae* strain required 72 h to grow in nutrient broth containing 0% and 3% NaCl. Both *A. sobria* strains (*sensu stricto*, fish isolates), one *A. bestiarum,* and one *A. eucrenophila* strain failed to grow in nutrient broth supplemented with 3% salt. A strain of *A. allosaccharophila* was found to ferment *m*-inositol, a characteristic typically associated with *Plesiomonas shigelloides*. Three enzymatic activities, production of elastase, polypectate (pectinase) degradation, and hydrolysis of *Staphylococcus au-*

TABLE 3. Atypical phenotypic properties of the genus *Aeromonas*

Test	Atypical reaction	No. of strains	Species (no. of strains)	Source	Phenotype frequency (%)[a]
Oxidase	−	1	*Aeromonas* sp. (1)	Feces (1)	0.2
Urea	+	12	*A. caviae* (12)	Feces (11), wound (1)	2.5
Mucate	+	1	*Aeromonas* sp. (1)	Blood (1)	0.2
Pectate	+	5	*A. salmonicida* (4), *Aeromonas* sp. (1)	Water (3), environment (1), unknown (1)	1.2
Acid from:					
Amygdalin	+	9	*A. hydrophila* (4), *Aeromonas* spp. (2), *A. caviae* (2), *A. salmonicida* (1)	Feces (6), blood (1), environmental (1), unknown (1)	2.1
D-Arabitol	+	1	*Aeromonas* sp. (1)	Environmental (1)	0.2
m-Inositol	+	3	*A. allosaccharophila* (1), *Aeromonas* spp. (2)	Feces (2), unknown (1)	0.7
Melibiose	+	20	*A. jandaei* (7), *A. caviae* (5), *Aeromonas* spp. (5), *A. hydrophila* (1), *A. allosaccharophila* (1)	Feces (11), blood (2), wound (2), gall bladder (1), environmental (3)	4.6
			A. veronii bv sobria (1)	Unknown (1)	
raffinose	+	13	*A. caviae* (5), *Aeromonas* spp. (5), *A. hydrophila* (1), *A. allosaccharophila* (1), *A. veronii* bv sobria (1)	Feces (8), wound (1), gall bladder (1), environmental (2), unknown (1)	3.0
L-rhamnose	+	33	*A. hydrophila* (13), *A. bestiarum* (11), *A. encheleia* (3), *A. allosaccharophila* (2), *A. eucrenophila* (2), *Aeromonas* spp. (2)	Fish (8), environmental (7), water (4), birds (2), animal (1), feces (4), blood (2), wound (1), eye (1), unknown (3)	7.7
D-sorbitol	+	22	*A. salmonicida* (11), *A. caviae* (5), *A. hydrophila* (4), *Aeromonas* spp. (2)	Feces (9), urine (1), wound (1), water (3), environmental (5), birds (1), unknown (2)	5.1
trehalose	−	2	*A. caviae* (1), *A. veronii* bv sobria (1)	Feces (1), sputum (1)	0.5
D-xylose	+	2	*Aeromonas* spp. (2)	Feces (1), environmental (1)	0.5

[a] Cumulative phenotypic frequency based upon 428 strains (193 strains listed in Table 2 and an additional 235 retrospectively reviewed).

reus cell wall components (stapholysin), were associated only with the *A. hydrophila* complex (*A. hydrophila sensu stricto, A. bestiarum,* and *A. salmonicida*). The fermentation (or lack thereof) of some methyl pentose, disaccharides, and alcohol carbohydrates was also linked to specific genomospecies.

Most strains that fermented L-rhamnose belonged to *A. bestiarum, A. encheleia,* or *A. allosaccharophila*. Almost half of all *A. jandaei* strains fermented the disaccharide melibiose. Fermentation of D-sorbitol, previously linked to *A. salmonicida* (19), was almost exclusively associated with this species, as 85% of strains tested fermented this alcoholic sugar; only one other strain with similar abilities (*A. caviae*) was identified in this survey. The inability to ferment D-mannitol was primarily restricted to *A. schubertii* (14) and some strains of *A. trota* (6).

Fermentation of unusual carbohydrates. Because many *Aeromonas* species are difficult to identify with a limited number of biochemical characteristics, we explored whether the fermentation of unusual carbohydrates might be useful as an aid to species identification. Thirteen type strains and 37 reference strains of *Aeromonas* (Table 1) representing each of the 14 nomenspecies were used to screen for potentially useful characters. Eight carbohydrates yielded uniformly negative test results: β-gentobiose, glucamine, inulin, D-lyxose, sedoheptulose, stachyose, D-tagatose, and xylitol. All 27 strains fermented maltotriose, although most isolates (>80%) required 96 h to produce acid from this carbohydrate. Palatinose was fermented by five of six strains of the *A. hydrophila* complex, by both *A. popoffii* strains tested, and by one *A. veronii* biogroup veronii isolate. Subsequently all *A. popoffii* strains were tested and found to be palatinose positive. Turanose fermentation was detected in only 5 of the 27 strains tested (19%) and then only after prolonged incubation (4 to 7 days). Three other sugars (lactulose, glucose 1-phosphate, and glucose 6-phosphate) yielded potentially discriminatory results (see *A. caviae* complex).

Atypical phenotypic properties. In addition to the data presented in Table 2, we retrospectively reviewed laboratory data on an additional 235 *Aeromonas* strains. These strains were identified to genomospecies level as *A. hydrophila, A. caviae, A. veronii* biogroup sobria, or *Aeromonas* sp. (could not be placed in a defined taxon based upon biochemical characteristics). Of the 12 characteristics listed in Table 3, five of these phenotypes were not detected in the original survey of 193 strains (Table 2). These tests include fermentation of D-arabitol and D-xylose, mucate utilization, and failure to produce cytochrome oxidase and acid from trehalose. The most common atypical biochemical characteristics observed included fermentation of L-rhamnose, D-sorbitol, and melibiose and urea hydrolysis. An oxidase-negative strain was recovered from the feces of a 16-day-old male infant with gastroenteritis in 1992. Because of the negative oxidase reaction, it was originally thought to be a possible *Chromobacterium violaceum* isolate. Subsequent biochemical testing in our laboratory identified this strain as an oxidase-negative *A. caviae* complex member (confirmed by the Centers for Disease Control).

TABLE 4. Propeties of raffinose- and melibiose-positive *Aeromonas* strains

Species	No. of strains	Dates of isolation	Rapid[a] fermentation of:	
			Melibiose	Raffinose
A. jandaei	7	1980–1996	+	−
A. caviae	5	1986–1997	+	+
Aeromonas spp.	5	1993–1999	+	+
A. hydrophila	1	1991	+	+
A. veronii bv sobria	1	1982	+	+
A. allosaccharophila	1	1984	+	+

[a] Within 24 to 48 h.

Coexpression of raffinose and melibiose fermentation. Twenty strains were identified that fermented either melibiose (Mel^+) or raffinose (Raf^+) or both among the 428 strains analyzed (Table 2, Table 3). With the exception of *A. jandaei* ($n = 7$), for which all strains were Mel^+ Raf^-, the remaining 13 strains (63%) were uniformly Mel^+ Raf^+ (Table 4). These Mel^+ Raf^+ strains were isolated over a 17-year period and were recovered from diverse clinical and environmental specimens. Expression of the Mel^+ Raf^+ phenotype was rapid (24 to 48 h) and was observed in four different genomospecies plus a number of isolates ($n = 5$) that could not be assigned to a taxon.

Identification schemes for aeromonads. Because of the increasing number of recognized *Aeromonas* species and the number of strains with unusual or atypical biochemical reactions, it is becoming increasingly difficult to identify older and newer members of this genus. One approach to identifying aeromonads to species level would be to rely on the results of Møeller decarboxylase and dihydrolase reactions to narrow the list of potential groups to a minimum (Fig. 1). For instance, at present ornithine decarboxylase-positive strains (group 1) could only be either *A. veronii* biogroup veronii (most common) or *A. allosaccharophila* (one strain to date), while lysine decarboxylase-negative, ornithine decarboxylase-negative, and arginine dihydrolase-negative strains (group 3) would be restricted to the *A. caviae* complex (*A. caviae, A. media,* and *A. eucrenophila*) or to *A. encheleia*. However, by far the majority of clinical isolates would fall into either group 2 or 4, requiring additional tests to identify isolates to either the complex or genomospecies level.

Strains could be placed into one of these three complexes based upon a limited number of tests, most of which are either included in commercial identification systems or routinely used in laboratories to identify other groups of bacteria (Table 5). *A. popoffii* ($n = 7$), *A. encheleia* ($n = 4$), *A. allosaccharophila* ($n = 3$), and *A. sobria* ($n = 2$) were not included because of the limited number of available strains and unusual growth requirements (25°C) for some species (*A. popoffii*). For instance, strains that were both esculin negative (Esc^-) and L-arabinose negative (Ara^-) would be restricted to the *A. sobria* (phenospecies) complex, as only one strain outside of this group (an *A. eucrenophila* isolate) was Esc^- Ara^-. Similarly, negative Voges-Proskauer and/or gas from glucose reactions would help separate the *A. caviae* complex from *A. trota* strains, which are lysine decarboxylase positive, and *A. schubertii* strains, which are Ara^-. However, three strains of *A. bestiarum* which were lysine decarboxylase negative, Voges-Proskauer negative, and gas-from-glucose negative would be misidentified to complex level with this system.

***A. hydrophila* complex.** As previously mentioned, any *Aeromonas* strain producing the enzyme elastase, pectinase, or staholysin belongs to the *A. hydrophila* complex. No strain of *A. encheleia* or *A. popoffii* was found to produce these activities. Tests useful in differentiating members of the *A. hydrophila* complex are listed in Table 6. Of the tests listed, the single most useful test was fermentation of D-sorbitol, which detected 85% of *A. salmonicida* strains. Other tests useful in separating

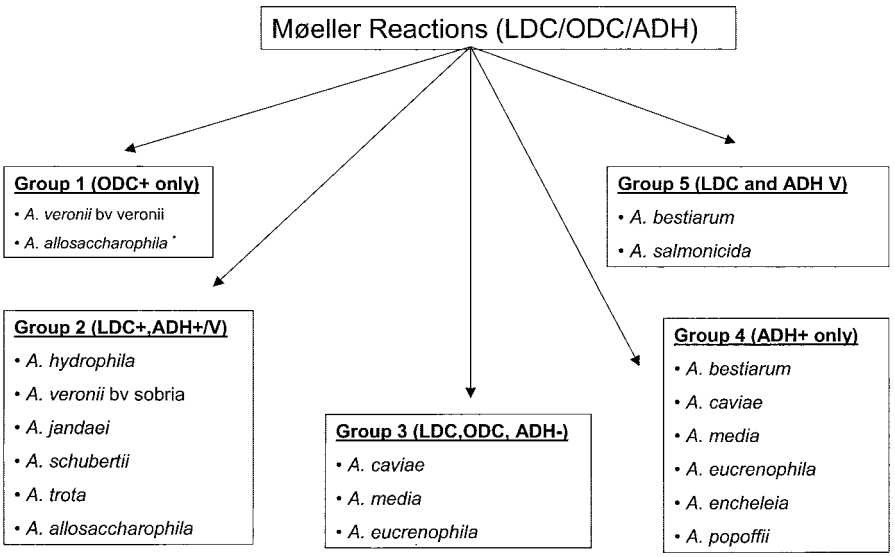

FIG. 1. Use of Møeller decarboxylase and dihydrolase reactions as a screening tool for recognition of potential species giving the indicated reaction. *, only rare strains of these species display this pattern. Abbreviations: LDC, lysine decarboxylase; ODC, ornithine decarboxylase; ADH, arginine dihydrolase; V, variable.

TABLE 5. Biochemical identification of *Aeromonas* to complex level

Test	No. of strains identified as belonging to[a]:		
	A. hydrophila complex (*A. hydrophila, A. bestiarum, A. salmonicida*)	*A. caviae* complex (*A. caviae, A. media, A. eucrenophila*)	*A. sobria* complex (*A. veronii* HG8, *A. jandaei, A. schubertii, A. trota*)
Esculin	87 (92, 81, 85)	71 (76, 55, 78)	0
Voges-Proskauer	74 (88, 63, 62)	0	54 (88, 87, 17, 0)
Glucose (gas)	81 (92, 69, 77)	16 (0, 0, 78)	87 (92, 100, 0, 69)
L-Arabinose	93 (84, 100, 100)	96 (100, 100, 78)	4 (12, 0, 0, 0)

[a] The first number is the overall percent positive for each complex for a given trait; the numbers in parentheses are percent positive for each species listed within that complex. Data are derived from Table 2.

members of this complex include utilization of DL-lactate and urocanic acid. Fermentation of L-rhamnose was a marker associated with a majority of *A. bestiarum* strains, but almost one-quarter of all *A. hydrophila* strains also produced acid from this sugar. Simmon's citrate was a useful test at 48 h, as was lactose fermentation. However, upon prolonged incubation (4 and 7 days, respectively), many *A. hydrophila* strains became positive, reducing the usefulness of these assays.

***A. caviae* complex.** Tests useful in differentiating members of the *A. caviae* complex (*A. caviae, A. media,* and *A. eucrenophila*) are listed in Table 7. In addition to previously described tests to separate members of this complex (1), several new tests useful in identifying members to genomospecies were found, including DL-lactate and urocanic acid utilization and fermentation of glucose 1-phosphate, glucose 6-phosphate, and lactulose. The latter three tests were identified in a limited survey of all *Aeromonas* species for the ability to ferment rare or unusual carbohydrates. When these tests were recognized, all 45 isolates of this complex were tested for acid production from glucose 1-phosphate, glucose 6-phosphate, and lactulose. A number of other tests were also useful in separating members of the *A. caviae* complex but were not as differential as those listed in Table 7. These tests include fermentation of sucrose, glycerol, and phenylpyruvic acid (1). Some tests formerly found to be useful (1), including β-hemolysis and H_2S production from cysteine, were less discriminatory in the resolution of species.

Several phenotypic changes within *A. caviae* strains (*sensu stricto*) were also noted in this study. The Microbial Diseases Laboratory began seeing urea-hydrolyzing (Ure^{+}) *A. caviae* strains as early as 1984, and this phenotype peaked between 1988 and 1991. Serotyping of random Ure^{+} *A. caviae* strains (courtesy of T. Shimada, National Institutes of Health, Tokyo, Japan) indicated that these isolates fell into several serogroups, including O:61, O:62, and OUK (unknown serogroup). A second noted difference was the frequency of β-hemolytic *A. caviae* strains. Early investigations found β-hemolysis to be a useful test in separating the *A. caviae* complex (usually negative) from the hemolytic phenospecies *A. hydrophila* and *A. sobria* (2, 16, 26, 30). However, in this study, over half (52%) of all *A. caviae* strains tested were beta-hemolytic on sheep blood agar within 48 h (Table 2). Furthermore, a retrospective review of *A. caviae* strains submitted to our laboratory for identification since 1996 indicated that 89% of these *A. caviae* isolates are beta-hemolytic.

***A. sobria* complex.** In 1976, Popoff and Véron (28) identified what later turned out to be the *A. sobria* complex or phenospecies. The *A. sobria* complex was defined on the basis of a number of phenotypic traits, which included failure to hydrolyze esculin, failure to ferment (or utilize) L-arabinose and salicin, and failure to grow in KCN broth. With some minor exceptions (e.g., variable growth in KCN broth for some species), this complex is composed of the following nomenspecies: *A veronii* biogroup sobria (DNA hybridization group 8), *A. jandaei, A. schubertii,* and *A. trota*. The major phenotypic features useful in the separation of species within the *A. sobria* complex are listed in Table 8. Although *A. veronii* biogroup sobria and *A. jandaei* can only be distinguished from one an-

TABLE 6. Tests useful in the separation of members of the *A. hydrophila* complex

Test	Result[a]		
	A. hydrophila	*A. bestiarum*	*A. salmonicida*
Utilization of:			
Citrate	+ (92)	V (38)	+ (85)
DL-Lactate	V (84)	− (0)	− (0)
Urocanic acid	V (16)	+ (94)	+ (100)
Gluconate oxidation	V (64)	− (13)	− (0)
Acid from:			
Cellobiose	− (4)	V (38)	V (69)
Lactose	V (64)	− (13)	+ (92)
L-Rhamnose	V (24)	V (69)	− (0)
D-Sorbitol	− (0)	− (0)	+ (85)

[a] +, ≥85% of strains positive; −, <15% positive; V, 15 to 85% positive: results at 48 h (see Table 5 for abbreviations); numbers in parentheses indicate percent positive for test at the final day of reading: gluconate, 2 days; DL-lactate and urocanic acid, 3 days; citrate, 4 days; and carbohydrates, 7 days.

TABLE 7. Tests useful in the separation of members of the *A. caviae* complex

Test	Result[a]		
	A. caviae	*A. media*	*A. eucrenophila*
Utilization of:			
Citrate	+ (88)	V (82)	− (0)
DL-Lactate	+ (96)	V (56)	− (0)
Urocanic acid	+ (100)	+ (100)	− (0)
Gas from D-glucose	− (0)	− (0)	V (78)
PZA	+ (88)	V (18)	+ (100)
Acid from:			
Glucose 1-phosphate	− (4)	+ (100)	+ (100)
Glucose 6-phosphate	− (4)	+ (100)	+ (100)
Lactulose	V (68)	V (55)	− (0)
D-Mannose	V (32)	+ (100)	+ (100)

[a] See Table 6, footnote *a*. PZA, pyrazinamidase, 2 days; DL-lactate and urocanic acid, 3 days; citrate, 4 days; and carbohydrates, 7 days.

TABLE 8. Tests useful in the separation of members of the *A. sobria* complex

Test	Result[a]			
	A. veronii[b]	*A. jandaei*	*A. schubertii*	*A. trota*
Indole	+ (100)	+ (100)	V (17)	+ (100)
Voges-Proskauer	+ (92)	+ (87)	V (17)	− (0)
Lipase (corn oil)	+ (92)	+ (100)	+ (100)	− (0)
Gas from D-glucose	+ (92)	+ (100)	− (0)	V (69)
Acid from:				
Cellobiose	V (20)	V (20)	− (0)	+ (100)
Glycerol	+ (100)	+ (100)	− (0)	+ (94)
D-Mannitol	+ (100)	+ (100)	− (0)	V (69)
Sucrose	+ (100)	− (0)	− (0)	V (19)
Amp^r	+ (100)	+ (93)	+ (92)	− (6)

[a] See Table 6, footnote *a*. Amp^r, resistance to 10 μg of ampicillin, 1 day; Voges-Proskauer, 3 days; all other tests, 7 days.
[b] Biogroup sobria (DNA hybridization group 8).

other in Table 8 based upon sucrose fermentation, there are several other tests useful in separating these taxa. Two-thirds of *A. jandaei* strains are resistant to cephalothin, while all *A. veronii* biogroup sobria strains were susceptible or partially susceptible to this first-generation cephalosporin. Half of the *A. veronii* biogroup sobria strains were pyrazinamidase positive, and a third degraded L-tyrosine crystals; neither activity was associated with *A. jandaei* isolates. Also, most *A. jandaei* were citrate positive, while only half of *A. veronii* biogroup sobria strains utilized citrate. As previously mentioned, almost half (47%) of *A. jandaei* fermented melibiose, while only 1 of 25 *A. veronii* biovar sobria strains fermented this carbohydrate.

Both *A. schubertii* and *A. trota* were easily recognizable by exhibiting different reactions from both *A. veronii* biovar sobria and *A. jandaei* in the indole, Voges-Proskauer, and lipase tests and susceptibility to ampicillin. Several sugar reactions were additionally useful, although fermentation of glycerol by *A. schubertii* strains lost diagnostic significance upon prolonged incubation (>48 h) because many strains showed a delayed fermentation of this carbohydrate.

DISCUSSION

The results of the present investigation further document the extensive phenotypic diversity within the genus *Aeromonas* and of the 14 currently recognized *Aeromonas* species (Table 2). Of the more than 60 tests evaluated in the present investigation, only four characteristics exhibited uniform reactions for the more than 400 *Aeromonas* strains evaluated, 193 in the prospective study and 235 in the retrospective review. These four tests were fermentation of D-glucose, production of nitrate reductase, and failure to produce acid from either dulcitol or erythritol. The reasons for the increasing phenetic diversity observed in the genus are numerous. As new taxa are reported, unusual phenotypic properties for the genus are often described, such as in the case of D-mannitol-negative *A. schubertii* (14) and ampicillin-susceptible *A. trota* (6). Furthermore, as surveys analyze strains from environmental sources, an increase in phenotypic diversity from the normalized data (profiles) established for clinical strains should be expected (4). Examples of such variation include the isolation of D-xylose-positive *Aeromonas* strains from the Chesapeake Bay (27) and O/129-sensitive isolates recovered from Japanese tadpoles (33).

For less well-characterized species, the true extent of phenotypic variation remains unknown, although, as more strains of these uncommon genomospecies are identified, greater phenetic diversity is likely to be found. Such diversity can be seen in the recent case report describing the isolation of an *A. media* strain that is lysine decarboxylase positive from the sputum of a patient with chronic bronchitis (11). The bottom line to this phenotypic diversity is that it will become increasingly difficult to identify *Aeromonas* isolates to species level without extensive arrays of biochemical tests. Fortunately, approximately 85% of clinical isolates fall into one of three recognizable genomospecies, that is, *A. hydrophila* (*sensu stricto*, HG1), *A. caviae* (*sensu stricto*, HG4), and *A. veronii* biotype sobria (*sensu stricto,* HG8, often incorrectly referred to as *A. sobria*).

Although increasing phenotypic diversity within the genus is now being recorded, *Aeromonas* isolates can in most cases be identified to phenospecies or genomospecies with fairly straightforward biochemical schemes and selected biochemical characteristics (Tables 5 to 8). Some tests, previously used in a number of identification schemes, now seem to be less useful than previously thought due to the expanding number of species and the need to generate an identification within a reasonable period of time (48 h). These tests include growth in KCN broth, fermentation of salicin, and production of a β-hemolysin. Growth in KCN, a test not commonly used by most clinical laboratories, appears less helpful now, since 60% of *A. veronii* biogroup sobria and *A. jandaei* strains grew in this broth. Likewise, fermentation of salicin has lost some of its discriminatory value, as 25% to 70% of members of the *A. hydrophila* complex fail to produce acid from this aglycone. Finally, the widespread emergence of beta-hemolytic *A. caviae* strains, one of the three most common species identified in the clinical laboratory, renders this trait of limited value in distinguishing *A. caviae* from *A. hydrophila* (*sensu stricto*) and *A. veronii* biovar sobria.

A 1996 Canadian study of 35 *A. caviae* strains of clinical origin found 6 (17%) to be beta-hemolytic on sheep blood agar plates (35). The hemolysin detected in that investigation appears to be unique to *A. caviae*. It may be that the increased incidence of hemolytic *A. caviae* strains is due to dissemination of clones bearing this hemolytic determinant or to horizontal transfer of hemolysin genes from other hemolytic *Aeromonas* species (e.g., *A. hydrophila* and *A. veronii* biotype sobria) to *A. caviae*. Whatever the reason, the incidence of beta-hemolytic *A. caviae* strains is on the rise and warrants attention, as it limits the use of this marker as an aid to species identification.

An interesting finding was the coexpression of rapid melibiose and raffinose fermentation by some strains of several *Aeromonas* species, excluding *A. jandaei*. This was an unexpected observation and suggests that these markers may be closely linked on the bacterial chromosome. Another possibility is that Mel$^+$ Raf$^+$ strains may be harbored on extrachromosomal elements that encode these metabolic activities. This hypothesis seems unlikely since most aeromonads do not routinely carry plasmids (only ≈25%), although it cannot be ruled out at present and will require further investigation. Similar to this observation was the initial presence of Ure$^+$ *A. caviae* strains

TABLE 9. Suggested and recommended tests useful in *Aeromonas* identification schemes

Laboratory type	Carbohydrate tests	Tube/slant tests	Plate tests
Clinical laboratory	L-Arabinose, D-glucose (gas), glycerol, D-mannitol, sucrose	Citrate, gelatin, indole Voges-Proskauer	Ampicillin and cephalothin susceptibility
Reference laboratory	Cellobiose, glucose 1-phosphate, glucose 6-phosphate	Gluconate, KCN, DL-lactate, urocanic acid	Elastase, stapholysin

in California in the late 1980s and early 1990s. These strains seem to have mostly disappeared by the mid-1990s and were most often associated with diarrheal disease. Because the serotype could not be determined for several strains, it is possible that one or more clones carrying the Ure^+ marker emerged during this period and subsequently declined in numbers. The reasons for this possible decline are unknown. However, in *Yersinia enterocolitica,* urease activity contributes to acid tolerance and may promote bacterial survival prior to infection (24). Thus, urease activity in select *Aeromonas* strains might provide a similar advantage.

The most important decision facing clinical laboratories is how far to proceed with the identification of *Aeromonas* species isolated from clinical material. For most moderate- to larger-sized hospitals or medical centers, it seems reasonable that isolates should be identified at least to phenospecies (Table 5). However, under a number of additional circumstances, medical facilities may want to proceed with definitive identifications. Some cases of gastroenteritis may fall into this category. Patients with hematologic malignancies may be more prone to gastrointestinal tract colonization with aeromonads than persons with other underlying conditions (32). Since the gastrointestinal tract is often the anatomic site from which bacteria disseminate to produce septicemia, and the pathogenic (invasive) potential of *Aeromonas* species varies, identifying isolates to species may be warranted in order to monitor such persons.

A second situation where species identification of strains may be justified is in reputed cases of chronic disease, such as gastroenteritis (29) or hepatobiliary disease (unpublished observations). In these instances, identifying *Aeromonas* isolates to species may resolve issues concerning whether a patient has chronic disease or has been reinfected by a different strain. Although no definitive outbreak involving *Aeromonas* has ever been described, there have been clusters of cases reported at long-term care (3) and day care (9) facilities where identification of strains to species was appropriate. Finally, isolates recovered from systemic infections such as blood should be good candidates for species identification, since it will help define the role of each genomospecies in serious clinical disease, thereby impacting prognosis and treatment. Potential useful tests for the identification of aeromonads by both clinical and reference microbiology laboratories under these circumstances are listed in Table 9.

REFERENCES

1. **Abbott, S. L., W. K. W. Cheung, S. Kroske-Bystrom, T. Malekzadeh, and J. M. Janda.** 1992. Identification of *Aeromonas* strains to the genospecies level in the clinical laboratory. J. Clin. Microbiol. **30:**1262–1266.
2. **Altwegg, M., A. G. Steigerwalt, R. Altwegg-Bissig, J. Lüthy-Hottenstein, and D. J. Brenner.** 1990. Biochemical identification of *Aeromonas* genospecies isolated from humans. J. Clin. Microbiol. **28:**258–264.
3. **Bloom, H. G., and E. J. Bottone.** 1990. *Aeromonas hydrophila* diarrhea in a long-term care setting. J. Am. Geriatr. Soc. **38:**804–806.
4. **Borrell, N., M. J. Figueras, and J. Guarro.** 1998. Phenotypic identification of *Aeromonas* genomospecies from clinical and environmental sources. Can. J. Microbiol. **44:**103–108.
5. **Carnahan, A. M., S. Behram, and S. W. Joseph.** 1991. Aerokey II: a flexible key for identifying clinical *Aeromonas* species. J. Clin. Microbiol. **29:**2843–2849.
6. **Carnahan, A. M., T. Chakraborty, G. R. Fanning, D. Verma, A. Ali, J. M. Janda, and S. W. Joseph.** 1991. *Aeromonas trota* sp. nov., an ampicillin-susceptible species isolated from clinical specimens. J. Clin. Microbiol. **29:**1206–1210.
7. **Carson, J., T. Wagner, T. Wilson, and L. Donachie.** 2001. Miniaturized tests for computer-assisted identification of motile *Aeromonas* species with an improved probability matrix. J. Appl. Microbiol. **90:**190–200.
8. **Colwell, R. R., M. T. MacDonell, and J. De Ley.** 1986. Proposal to recognize the family *Aeromonadaceae*. Int. J. Syst. Bacteriol. **36:**473–477.
9. **de la Morena, M., R. Van, K. Singh, M. Brian, B. E. Murray, and L. K. Pickering.** 1993. Diarrhea associated with *Aeromonas* species in children in day care centers. J. Infect. Dis. **168:**215–218.
10. **Donlon, J., S. McGettigan, P. O'Brien, and P. Ó'Carra.** 1983. Re-appraisal of the nature of the pigment produced by *Aeromonas salmonicida*. FEMS Microbiol. Lett. **19:**285–290.
11. **Duraka, E.** 1998. Aeromonas media strain that decarboxylates lysine. Alpe Adria Microbiol. J. **7:**197–201.
12. **Esteve, C., M. C. Gutiérrez, and A. Ventosa.** 1995. *Aeromonas encheleia* sp. nov., isolated from European eels. J. Clin. Microbiol. **45:**462–466.
13. **Ewing, W. H., and B. R. Davis.** 1981. Medium and tests for differentiation of *Enterobacteriaceae*. U.S. Department of Health and Human Services publication no. CDC 81–8236. U.S. Department of Health and Human Services, Washington, D.C.
14. **Hickman-Brenner, F. W., G. R. Fanning, M. J. Arduino, D. J. Brenner, and J. J. Farmer, I. I. I.** 1988. *Aeromonas schubertii*, a new mannitol-negative species found in human clinical specimens. J. Clin. Microbiol. **26:**1561–1564.
15. **Huys, G., P. Kämpfer, M. Altwegg, I. Kersters, A. Lamb, R. Coopman, J. Lüthy-Hottenstein, M. Vancanneyt, P. Janssen, and K. Kersters.** 1997. *Aeromonas popoffii* sp. nov., a mesophilic bacterium isolated from drinking water production plants and reservoirs. Int. J. Syst. Bacteriol. **47:**1165–1171.
16. **Janda, J. M.** 1985. Biochemical and exoenzymatic properties of *Aeromonas* species. Diagn. Microbiol. Infect. Dis. **3:**223–232.
17. **Janda, J. M.** 2001. *Aeromonas* and *Plesiomonas*, p. 1237–1270. *In* M. Sussman (ed.), Molecular medical microbiology, Academic Press, London, England.
18. **Janda, J. M., and S. L. Abbott.** 1998. Evolving concepts regarding the genus *Aeromonas*: an expanding panorama of species, disease presentations, and unanswered questions. Clin. Infect. Dis. **27:**332–344.
19. **Janda, J. M., S. L. Abbott, S. Khashe, G. H. Kellogg, and T. Shimada.** 1996. Further studies on biochemical characteristics and serologic properties of the genus *Aeromonas*. J. Clin. Microbiol. **34:**1930–1933.
20. **Janda, J. M., S. L. Abbott, S. Khashe, and T. Robin.** 1996. Biochemical investigations of biogroups and subspecies of *Morganella morganii*. J. Clin. Microbiol. **34:**108–113.
21. **Janda, J. M., and P. S. Duffey.** 1988. Mesophilic aeromonads in human disease: current taxonomy, laboratory identification, and infectious disease spectrum. Rev. Infect. Dis. **10:**980–997.
22. **Janda, J. M., M. Reitano, and E. J. Bottone.** 1984. Biotyping of *Aeromonas* isolates as a correlate to delineating a species-associated disease spectrum. J. Clin. Microbiol. **19:**44–47.
23. **Joseph, S. W., and A. M. Carnahan.** 2000. Update on the genus *Aeromonas*. ASM News **66:**218–223.
24. **Konig-Ward, T. F., and R. M. Robins-Browne.** 1995. Contribution of urease to acid tolerance in *Yersinia enterocolitica.* Infect. Immun. **63:**3790–3795.
25. **Martinez-Murcia, A. J., S. Benlloch, and M. D. Collins.** 1992. Phylogenetic interrelationships of members of the genera *Aeromonas* and *Plesiomonas* as determined by 16S ribosomal DNA sequencing: lack of congruence with results of DNA-DNA hybridizations. Int. J. Syst. Bacteriol. **42:**412–421.
26. **Oakey, H. J., J. T. Ellis, and L. F. Gibson.** 1996. A biochemical protocol for the differentiation of current genomospecies of *Aeromonas*. Zentralbl. Bakteriol. **284:**32–46.
27. **Okpokwasili, G. C.** 1991. *Aeromonas hydrophila:* variability in biochemical characteristics of environmental isolates. J. Basic Microbiol. **3:**169–176.

28. **Popoff, M., and M. Véron.** 1976. A taxonomic study of the *Aeromonas hydrophila-Aeromonas punctata* group. J. Gen. Microbiol. **94:**11–22.
29. **Rautelin, H., M. L. Hänninen, A. Sivonen, U. Turunen, and V. Valtonen.** 1995. Chronic diarrhea due to a single strain of *Aeromonas caviae*. Eur. J. Clin. Microbiol. Infect. Dis. **14:**51–53.
30. **Renaud, F., J. Freney, J.-M. Boeufgras, D. Monget, A. Sedaillain, and J. Fleurette.** 1988. Carbon substrate assimilation patterns of clinical and environmental strains of *Aeromonas hydrophila, Aeromonas sobria* and *Aeromonas caviae* with a micromethod. Zentralbl. Bakteriol. **269:**323–330.
31. **Ruimy, R., V. Breittmayer, P. Elbaze, B. Lafay, O. Boussemart, M. Gauthier, and R. Christen.** 1994. Phylogenetic analysis and assessment of the genera *Vibrio, Photobacterium, Aeromonas,* and *Plesiomonas* deduced from small-subunit rRNA sequences. Int. J. Syst. Bacteriol. **44:**416–426.
32. **Sherlock, C. H., D. R. Burdge, and J. A. Smith.** 1987. Does *Aeromonas hydrophila* preferentially colonize the bowels of patients with hematologic malignancies? Diagn. Microbiol. Infect. Dis. **7:**63–68.
33. **Sugita, H., T. Nakamura, K. Tanaka, and Y. Deguchi.** 1994. Identification of O/129-sensitive *Aeromonas* by the microplate hybridization method. Fish. Sci. **60:**351–352.
34. **Véron, M.** 1965. La position taxonomique des *Vibrio* et de certaines bactéries comparables. C. R. Acad. Sci. Paris **261:**5243–5246.
35. **Wang, G., K. D. Tyler, C. K. Munro, and W. M. Johnson.** 1996. Characterization of cytotoxic, hemolytic *Aeromonas caviae* clinical isolates and their identification by determining presence of a unique hemolysin gene. J. Clin. Microbiol. **34:**3203–3205.