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A Taxonomic Study of the *Aeromonas hydrophila–Aeromonas punctata* Group

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SUMMARY

A total of 203 characters has been determined for 68 strains of Aeromonas belonging to the *Aeromonas hydrophila–A. punctata* group. The results have been subjected to computer analysis using the coefficient of Jaccard-Sneath and the strains clustered by the method of aggregation according to the variance.

The 68 strains can be divided into two well-segregated classes on the basis of 59 variable characters, of which seven are of diagnostic value. The two classes are considered as two separate species. The first one (42 strains) is assigned to the type species of the genus, *A. hydrophila*, and it appears that the species name, *A. punctata*, is an illegitimate synonym for *A. hydrophila*. The second (26 strains) constitutes a new species for which the name *A. sobria* sp.nov. is proposed. The type strain of this new species has been deposited under the reference CIP7433 (our strain 208).

INTRODUCTION

The genus *Aeromonas* was proposed by Kluyver & van Niel (1936) to accommodate rod-shaped bacteria possessing the general properties of the enteric group, but motile by means of polar flagella. This genus is now included in the family of *Vibrionaceae* (Véron, 1966). In *Bergey's Manual of Determinative Bacteriology* (1974) the original definition has been amended to include the following salient properties: Gram-negative straight rods; polar flagellated (generally monotrichous) or immotile; facultative anaerobes, fermenting carbohydrates with formation of acid or acid and gas; oxidase positive; reducing nitrates to nitrites; insensitive to the vibriostatic compound 2,4-diamino-6,7-diisopropylpteridine (O/129); guanine–cytosine content of the DNA, 57 to 63 mol %.

Of the three species now recognized (*Bergey's Manual of Determinative Bacteriology*, 1974), the fish pathogen *Aeromonas salmonicida* is easily distinguishable by the following characters: non-motile; auxotrophy; no growth at 37 °C; producing a brown diffusible pigment on nutrient agar; indole not formed. Smith (1963) has even suggested removing this species from the genus *Aeromonas*, proposing the alternative name *Necromonas salmonicida*. The '*A. hydrophila–A. punctata*' group is more complex; Schubert (1967*a*, *b*, 1968) proposed a total of five sub-species, three assigned to *A. hydrophila* and two to *A. punctata*. Our attempts to classify newly-isolated strains of the hydrophila–punctata complex in terms of the specific and sub-specific criteria proposed by Schubert have indicated that this scheme is unsatisfactory. The present study attempts to clarify and simplify the classification of aeromonads not assignable to the species *A. salmonicida*.

Table 1. *Proposed taxonomic classification of the new isolates*

Assigned to	Source	Strain nos.
Aeromonas hydrophila (class X)	Man	102, 230, 231, 241, 246, 247, 268, 307, 308
	Fish	201, 203, 212, 213, 214, 216, 218, 219, 222, 223, 225, 226, 227, 229, 233, 234, 235, 238, 239
	Frog	249
	Fresh water	244
A. sobria sp.nov. (class Y)	Man	243
	Fish	202, 204, 205, 207, 208, 209, 210, 211, 215, 217, 221, 224, 228, 232, 236, 237, 240, 242
	Frog	245, 248, 250

METHODS

Origin of the strains. In addition to 16 named strains, a detailed study of 52 wild-type strains possessing the properties of the hydrophila–punctata complex was made. They were isolated between 1967 and 1974 from the following sources: human pathological specimens submitted to bacteriological examination (10 strains); infected fishes (37 strains); infected frogs (4 strains); fresh water (1 strain). In Table 1, the new isolates are listed in terms of the taxonomic classifications now proposed as a result of our analysis. The origins and assignments of the 16 named strains are listed in Table 2.

Properties examined. In addition to the basic characters quoted in the Aeromonas group definition (see above), we examined a total of 57 characters previously used in taxonomic studies of the Aeromonas group or which are standard procedures for the identification of the enteric group of bacteria. They are listed below; those for which no reference is given are described by Popoff (1969):

Morphological characters: capsule; motility.

Physiological characters: catalase production; growth in KCN medium; methyl red and Voges–Proskauer reactions; maximum temperature for growth in nutrient broth (30, 37 and 41 °C); growth factor requirements using a mineral–ammonium medium containing glucose or succinate as sole source of carbon and energy; growth in peptone water in the presence or absence of sodium chloride (Richard, Giammanco & Popoff, 1974).

Carbohydrate metabolism: production of acid and gas from glucose and glycerol; production of acid from L-arabinose, D-xylose, L-rhamnose, D-mannose, sorbose, D-cellobiose, D-lactose, D-maltose, melibiose, D-sucrose, D-trehalose, raffinose, erythritol, adonitol, D-mannitol, D-dulcitol, *meso*inositol, D-sorbitol, mucate, salicin; esculin hydrolysis; production of β-galactosidase and butanediol-dehydrogenase (Schubert & Kexel, 1964).

Metabolism of nitrogenous compounds: production of urease, lysine decarboxylase, ornithine decarboxylase, arginine dihydrolase, tryptophan deaminase, phenylalanine deaminase, tetrathionate reductase; indole production in peptone water; H_2S production on Kligler's medium and from cysteine on cysteine–iron agar (Gélose G.C.F.; Véron & Gasser, 1963).

Extracellular enzymes: production of gelatinase, elastase (Scharmann, 1972), pectinase (Davis & Ewing, 1964), lipase, DNAase and RNAase.

All cultures were incubated at 30 °C; the reactions were observed for 2 days for growth in KCN medium and for 1 to 4 days for the other tests.

In addition, strains were screened for their ability to use as principal sources of carbon and energy, the 146 organic compounds studied by Stanier, Palleroni & Doudoroff (1966), using the media and techniques described by Véron (1975). The mineral–ammonium medium

Table 2. *The sources and assignations of the* 16 *named strains*

Strain no.*	Received as	Source
Strains assigned to *A. hydrophila* (class X)		
267 (NCIB9234 = ATCC13136)	*A. formicans*	Oil emulsion (Pivnick & Sabina, 1957)
309 (CIP R307)	*A. punctata*	Man (Delabre *et al.*, 1973)
314 (Lille C105), 315 (Lille C106), 316 (Lille C102)	*A. dourgesi* var. *aerogenes*	Fresh water (Leclerc, 1962)
317 (Lille C94), 318 (Lille C95), 319 (Lille C98)	*A. dourgesi* var. *anaerogenes*	Fresh water (Leclerc, 1962)
543 (ATCC7966 = NCTC8049 = NCMB86 = NCIB9240 = CDC-RH35)	*A. hydrophila* subsp. *hydrophila*, neotype strain	Canned milk (Speck & Stark, 1942)
544 (ATCC15467)	*A. hydrophila* subsp. *anaerogenes*, type strain	Oil emulsion (Pivnick & Sabina, 1957)
545 (ATCC15468)	*A. punctata* subsp. *caviae*, type strain	Guinea pig (Scherago, 1936)
546 (NCMB74 = ATCC23309 = Schäperclaus strain B = CDC-RH63)	*A. punctata* subsp. *punctata*, *neotype strain*	Fresh water fish (Schäperclaus, 1930)
Strains assigned to *A. sobria* (class Y)		
310 (CIP6324 = CCEB36)	*A. punctata*	Unknown source (Lysenko, 1961)
311 (CIP5749 = NCTC7810 = ATCC9071)	*A. hydrophila*	Frog (Kulp & Borden, 1942)
320 (Lille C103)	*A. dourgesi* var. *anaerogenes*	Fresh water (Leclerc, 1962)
Strain excluded from the genus *Aeromonas*		
262 (NCMB1326 = ATCC 15338)	*A. proteolytica*, type strain	Marine crustacea (Merkel *et al.*, 1964)

* NCMB, National Collection of Marine Bacteria, Aberdeen; ATCC. American Type Culture Collection, Rockville, Maryland, U.S.A.; NCIB, National Collection of Industrial Bacteria, Aberdeen; CIP, Collection of the Institut Pasteur, Paris, France; CCEB, Culture Collection of Entomogenous Bacteria, Praha, Czechoslovakia; Lille, Collection of Professor H. Leclerc, Lille, France.

was supplemented with yeast extract (Difco; 0·1 g l^{-1}). For solid medium, Oxoid Ionagar no. 2 was used (1 %, w/v). The growth on the plates was read around each substrate spot after 3 and 5 days at 30 °C.

Numerical taxonomy. A total of 203 simple, qualitative characters was codified in binary form. The coefficient D used for taxonomic distance was given by $D = 1 - S$, S being the similarity coefficient of Jaccard-Sneath (Sokal & Sneath, 1963). Strains clusters were set up by the method of aggregation according to the variance (Delabre, Bianchi & Véron, 1973). Classes were delimited by computing the coefficient of distinctness (previously termed 'acuteness coefficient'; Véron, 1974). The diagnostic value of the characters was determined by the method of Gyllenberg (1965). The characters of greatest differential value were selected to define ideal phenotypes (Stanier *et al.*, 1966). The centrotype of a taxon was defined as the strain of minimum average distance among the strains of this taxon (Silvestri *et al.*, 1962).

Programmes and computations were made on a Wang 2200 computer.

DNA base compositions. The mole per cent guanine plus cytosine (mol % GC) in the DNAs of representative strains was determined by the method of Marmur & Doty (1962). The thermal denaturation profiles were determined in 0·1 × SSC (standard saline citrate:

0·15 M-NaCl, 0·015 M-sodium citrate) at pH 7·0, on a Gilford 240 spectrophotometer fitted with a temperature programmer. The mol % GC was calculated by the method of De Ley (1970) using *Escherichia coli* K12 (CIP54117) as the standard.

RESULTS

Characters either positive or negative for all strains examined

A total of 28 characters, positive for all strains, included: the ability to ferment mannitol, maltose and trehalose; the production of catalase, DNAase, RNAase, gelatinase, lipase and arginine dihydrolase; the utilization, as principal carbon and energy sources, of 19 substrates, namely D-ribose, D-fructose, D-galactose, D-glucose, D-maltose, D-trehalose, D-gluconate, starch, caprylate, pelargonate, caprate, succinate, fumarate, DL-glycerate, L-malate, glycerol, D-mannitol, L-aspartate, L-glutamate.

Characters negative for all strains were: capsule; pectinase, lysine and ornithine decarboxylases; tryptophan and phenylalanine deaminases; fermentation of xylose, sorbose, erythritol, adonitol, dulcitol, inositol and mucate; production of H_2S on Kligler medium. In addition, 95 of the substrates tested as principal sources of carbon and energy were never utilized. These can be ascertained from the list of substrates given by Stanier *et al.* (1966), but eliminating the 19 utilized by all our strains as well as certain others used by some of the strains examined (quoted in Tables 3, 4 and 6).

Numerical analysis

Among the 64 variable characters, it appeared that fermentation of L-arabinose, L-rhamnose, D-sucrose and D-lactose was redundant with utilization of these four substrates as principal energy sources; in addition, growth on D-α-alanine was also redundant with L-α-alanine. Consequently these five characters were omitted from numerical analyses, which were finally performed with 59 characters (see Tables 3 to 6).

Figure 1 shows the similarity relationships between the 68 strains examined. A primary division of these strains into two groups is obvious. The highest values of the coefficient of distinctness K, for the hierarchic levels H, are: $K = 36{\cdot}52$ for $H = 0{\cdot}15$; $K = 38{\cdot}08$ for $H = 0{\cdot}24$; and $K = 131{\cdot}28$ for $H = 0{\cdot}99$.

The best cutting level for the dendrogram is therefore at $H_{opt} = 0{\cdot}99$. At this level, there are two well separated primary classes, X and Y, containing 42 and 26 strains respectively. The strains in each class are shown in Fig. 1.

The coefficients of distinctness within each primary class (K_X and K_Y) were calculated for every hierarchic level less than or equal to H_{opt}. The value of the coefficient K within both classes is maximal for the level $H = 0{\cdot}99$. Within each class, the two highest values of the coefficient K occurred at the following levels: $K_X = 61{\cdot}95$ for $H = 0{\cdot}99$ and $K_X = 24{\cdot}41$ for $H = 0{\cdot}39$ in class X; and $K_Y = 69{\cdot}33$ for $H = 0{\cdot}99$ and $K_Y = 27{\cdot}42$ for $H = 0{\cdot}24$ in class Y. The ratios of the two K values are 0·40 and 0·39, respectively, for X and Y. The low values of these ratios suggests that the primary classes, X and Y, must not be divided into secondary classes (Véron, 1974). However, from the disposition of clusters in Fig. 1, the subdivision of class X into two biovars, X_1 (13 strains) and X_2 (29 strains), may be considered.

For the three taxa, the centrotypes are strains 218 (X_1), 239 (X_2) and 208 (Y), which have been deposited in the Collection of the Institut Pasteur (Paris) under the numbers CIP7430, CIP7432 and CIP7433. Within their own taxa, these centrotypes show an average coefficient of similarity of 0·770 (X_1), 0·711 (X_2), and 0·691 (Y).

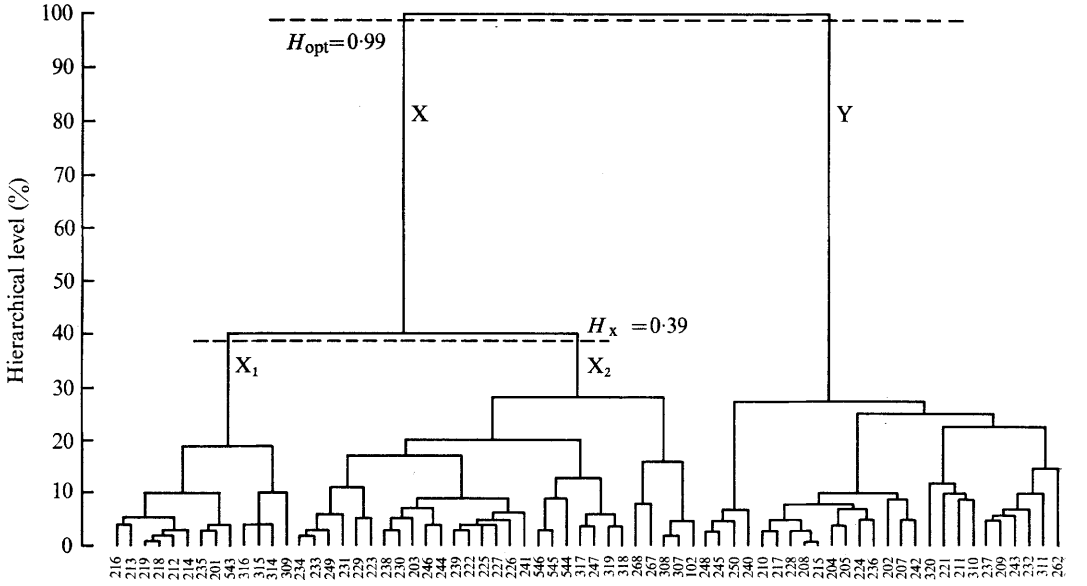

Fig. 1. Numerical analysis of strains.

Table 3. *Characters of value for differentiation of classes X and Y*

	Class X (42 strains)			Class Y (26 strains)		
	Positive strains			Positive strains		
Designation of the characters	Total	%	Negative strains	Total	%	Negative strains
Esculin hydrolysis	42	100	None	5	19	All except 204, 221, 240, 310, 320
Fermentation of salicin	36	86	201, 268, 307, 308, 543, 544	5	19	All except 211, 221, 240, 310, 320
H_2S production in GCF agar*	13	31	All except 201, 212, 213, 214, 216, 218, 219, 229, 235, 315, 316, 544, 545	25	96	262
Growth on KCN medium	41	98	319	5	19	All except 205, 209, 243, 310, 320
Growth on L-arabinose	39	93	223, 318	5	19	All except 209, 236, 310, 311, 320
Growth on salicin	33	79	102, 201, 227, 267, 268, 307, 308, 543, 544	6	23	All except 211, 221, 240, 250, 311, 320
Growth on L-arginine	31	74	201, 203, 223, 229, 231, 235, 268, 309, 314, 315, 316	2	8	All except 223, 250
Growth on L-histidine	41	98	229	2	8	All except 243, 320

* Character also of value for differentiating between the biovars of class X (see Table 7).

Table 4. *Characters almost always positive or negative for the 68 strains examined*

Characters	Positive strains: Total	% (X)	% (Y)	Negative strains
Minimal concentration of NaCl = 0 %	67	100	96	262
Minimal concentration of NaCl = 0·5 %	1	0	4	All except 262
Maximal concentration of NaCl = 3 %	67	100	96	262
Maximal concentration of NaCl = 10 %	1	0	4	All except 262
β-Galactosidase production	67	100	96	262
Growth factors required	5	5	12	All except 202, 211, 227, 230, 236
Fermentation of mannose	62	88	96	230, 249, 267, 268, 309, 317
Fermentation of raffinose	2	0	8	All except 236, 320
Fermentation of glycerol	61	86	100	230, 238, 244, 307, 308, 310, 545
Fermentation of melibiose	4	8	4	All except 211, 223, 225, 228
Indole production	63	95	88	202, 207, 232, 267, 268
Growth on rhamnose	5	12	0	All except 212, 214, 218, 219, 225
Growth on mannose	62	88	96	231, 249, 267, 268, 309, 320
Growth on sucrose	62	100	77	205, 242, 245, 248, 250, 262
Growth on D-sorbitol	3	8	0	All except 201, 227, 235
Growth on isobutyrate	5	8	8	All except 202, 203, 224, 227, 239
Growth on DL-3-hydroxybutyrate	1	2	0	All except 203
Growth on D-malate	2	5	0	All except 216, 222
Growth on laevulinate	2	0	7	All except 245, 250
Growth on L-proline	6	11	4	All except 203, 221, 226, 236, 245, 543

Description of the taxa

Using the separation coefficient of Gyllenberg (1965), we determined the most significant characters for the differentiation of classes X and Y.

Among the 59 variable characters used for classification, eight are significant for differentiating classes X and Y (see Table 3). The remainder are without differential value, either because they are positive for at least 90 % or less than 10 % of strains (see Table 4), or because their occurrence is not statistically significant (see Tables 5 and 6).

One character useful for differentiating classes X and Y (see footnote, Table 3) as well as three other variable characters without primary differential value (see footnote, Table 5) make it possible to differentiate between the two biovars of class X.

We have been unable to confirm the value of one pair of 'classical' differential characters proposed by Schubert: gas production with glucose and with glycerol. In our tests, all but one strain gave results which were either both positive or both negative with these two carbohydrates.

Table 7 summarizes all the characters of value in differentiating between species, i.e. it gives the ideal phenotypes of these taxa. The proportion of strains possessing all or all but one of the characters of the ideal strain was as follows: 70 % (X_1), 76 % (X_2), and 66 % (Y).

The halophilic character has not been included in the list of those used for discriminating between strains, because only one strain (strain 262) has this property; this strain requires at least 100 mM-NaCl for growth in peptone water, and differs from the ideal phenotype of class Y in only two characters: elastase and H_2S from cysteine.

Table 5. *Variable characters without value for differentiation of classes X and Y*

Characters	Positive strains: Total	% (X)	% (Y)	Negative strains
Motility	56	(74)	(96)	102, 246, 307, 308, 309, 314, 315, 316, 317, 318, 319, 320
Maximum temperature for growth = 28 °C	21	(9)	(65)	All except 102, 202, 204, 205, 207, 208, 210, 211, 215, 217, 221, 224, 228, 236, 242, 262, 267, 268, 307, 310, 320
Maximum temperature for growth = 37 °C	47	(90)	(34)	102, 202, 204, 205, 207, 208, 210, 211, 215, 217, 221, 224, 228, 236, 242, 262, 267, 268, 307, 310, 320
Gas production from glucose*	39	(38)	(89)	102, 203, 211, 222, 225, 226, 227, 230, 231, 232, 233, 237, 238, 239, 241, 244, 246, 249, 267, 268, 307, 308, 309, 317, 318, 319, 320, 544, 545
Gas production from glycerol	38	(36)	(89)	Idem + 546
Tetrathionate reductase	21	(47)	(4)	All except 203, 221, 222, 225, 226, 227, 230, 231, 233, 238, 239, 241, 244, 247, 267, 317, 318, 543, 544, 545, 546
Fermentation of cellobiose	50	(71)	(73)	209, 212, 213, 218, 219, 221, 230, 237, 243, 262, 267, 268, 309, 311, 315, 316, 320, 543
Fermentation of sorbitol	11	(19)	(11)	All except 211, 235, 241, 246, 247, 262, 309, 310, 314, 315, 316
Acetoin production*	28	(31)	(57)	All except 201, 202, 204, 205, 207, 208, 209, 211, 212, 213, 214, 215, 216, 217, 218, 219, 228 235, 242, 243, 262, 311, 314, 315, 316, 320, 543, 544
Methyl red	53	(76)	(81)	201, 204, 205, 209, 212, 214, 216, 218, 219, 235, 262, 311, 314, 543, 544
Urease	7	(16)	(0)	All except 102, 231, 267, 268, 307, 308, 309
Elastase*	15	(28)	(11)	All except 201, 212, 213, 214, 216, 218, 219, 235, 262, 310, 314, 315, 316, 320, 543
Butanediol dehydrogenase	48	(55)	(96)	102, 203, 221, 222, 226, 227, 229, 230, 233, 238, 239, 241, 244, 246, 249, 307, 308, 309, 545, 546

* Characters of value for differentiating between the two biovars of X (see Table 7).

DNA base compositions

The average value of the mol % GC content in the DNA of representative strains of each class (Table 8) is $61{\cdot}5 \pm 1{\cdot}7$. However, if we consider the five strains of class X and the three of class Y separately, the averages for these two classes ($62{\cdot}6 \pm 0{\cdot}4$ and $59{\cdot}6 \pm 1{\cdot}0$, respectively) differ significantly ($P = 0{\cdot}001$).

DISCUSSION

Since the description by Zimmerman (1890) of *Bacillus punctatus* (*A. punctata*) and by Sanarelli (1891) of *B. hydrophilus fuscus* (*A. hydrophila*), a number of additional species of motile aeromonads has been proposed (summarized in Ewing, Hugh & Johnson, 1961). Ewing *et al.* (1961) recognized only three valid species, *A. salmonicida*, *A. shigelloides* and *A. hydrophila*, of which only *A. hydrophila* is included in the present study.

The most recent taxonomic studies on the hydrophila–punctata group, summarized in Table 9, give quite discordant results. The proposals of Schubert have been adopted in the most recent (8th) edition of *Bergey's Manual of Determinative Bacteriology* (1974), which lists characters used for the differentiation of these five taxa.

Table 6. *Variable nutritional characters without value for differentiation of classes X and Y*

Ability to grow on:	Positive strains			Negative strains
	Total	% (X)	% (Y)	
D-Cellobiose	46	(71)	(61)	207, 209, 211, 212, 213, 216, 218, 219, 221, 231, 232, 237, 243, 262, 267, 268, 309, 311, 315, 316, 320, 543
D-Lactose	17	(36)	(8)	All except 102, 240, 244, 247, 267, 307, 308, 309, 314, 315, 316, 317, 318, 319, 320, 544, 546
Acetate	35	(59)	(39)	204, 205, 208, 209, 211, 212, 213, 214, 215, 216, 217, 221, 222, 223, 224, 225, 226, 227, 228, 229, 231, 232, 233, 237, 242, 249, 262, 267, 268, 310, 315, 320, 544
Propionate	15	(24)	(19)	All except 102, 202, 203, 230, 238, 239, 240, 241, 244, 245, 246, 248, 250, 317, 319
Butyrate	22	(38)	(23)	All except 202, 203, 222, 225, 227, 230, 238, 239, 240, 241, 244, 245, 246, 248, 250, 311, 314, 315, 317, 543, 545, 546
n-Valerate	28	(55)	(23)	All except 202, 203, 213, 214, 216, 218, 219, 222, 225, 227, 231, 232, 233, 234, 238, 239, 240, 241, 244, 245, 246, 248, 249, 250, 316, 317, 545, 546
n-Caproate	48	(76)	(61)	207, 209, 211, 221, 223, 228, 237, 242, 309, 310, 311, 314, 315, 318, 319, 320, 543, 544, 545, 546
Heptanoate	47	(76)	(58)	202, 207, 209, 211, 221, 223, 228, 229, 237, 241, 242, 303, 310, 311, 318, 319, 320, 543, 544, 545, 546
DL-Lactate	23	(45)	(15)	All except 102, 212, 213, 221, 231, 233, 234, 244, 245, 246, 247, 248, 249, 250, 267, 308, 309, 317, 318, 319, 543, 544, 545
Pyruvate	59	(88)	(84)	245, 268, 307, 308, 309, 310, 311, 315, 320
Citrate	48	(82)	(50)	202, 203, 204, 205, 207, 211, 221, 224, 227, 230, 235, 236, 240, 242, 244, 250, 268, 310, 311, 546
L-α-Alanine D-α-Alanine	34	(76)	(8)	All except 102, 201, 203, 212, 213, 216, 218, 221, 222, 225, 227, 231, 233, 234, 235, 239, 241, 244, 246, 247, 250, 268, 307 308, 309, 314, 315, 316, 317, 318, 319, 543, 545, 546
L-Ornithine	22	(45)	(15)	All except 203, 213, 214, 222, 225, 226, 227, 230, 238, 239, 241, 245, 247, 248, 250, 317, 318, 320, 543, 544, 545, 546
L-Serine	53	(83)	(69)	102, 202, 210, 211, 217, 233, 234, 244, 262, 267, 307, 308, 310, 311, 545
L-Threonine	16	(31)	(11)	All except 201, 203, 213, 216, 221, 225, 230, 238, 242, 244, 246, 317, 318, 543, 545, 546
L-Tyrosine	21	(40)	(12)	All except 102, 203, 216, 217, 218, 222, 232, 238, 241, 307, 308, 309, 311, 314, 315, 318, 319, 320, 543, 545, 546
Putrescine	63	(100)	(81)	202, 204, 211, 215, 228
Spermine	20	(43)	(8)	All except 102, 201, 222, 230, 238, 247, 267, 307, 308, 309, 310, 314, 316, 317, 318, 319, 320, 544

Our study shows clearly the existence of two main clusters, X and Y, for which we propose the rank of separate species. The differentiation of these two species is clear-cut if we consider the first seven characters of Table 7, since the degree of strain conformity to their ideal phenotypes is satisfactory: 81 % of X strains and 70 % of Y strains are in agreement with all or all but one characters of the ideal phenotypes.

In differentiating between these two species, no significant diagnostic value was found in gas production from glycerol (which is redundant with gas production from glucose) or in butanediol dehydrogenase production. Hence we consider that *A. hydrophila* cannot be distinguished from *A. punctata*, and these two specific epithets must be considered as synonyms. This opinion is reinforced by the fact that the neotype strains of *A. hydro-*

Table 7. *Ideal phenotypes of classes X and Y, and of biovars X_1 and X_2*

The feature of the ideal phenotype is given, with (in parentheses) the percentage of strains having the same feature as the ideal phenotype.

Characters	Class X (42 strains)		Class Y (26 strains)
Growth on L-histidine	+ (98)		− (92)
Esculin hydrolysis	+ (100)		− (81)
Growth on KCN medium	+ (98)		− (81)
Growth on L-arabinose	+ (93)		− (81)
Fermentation of salicin	+ (86)		− (81)
Growth on L-arginine	+ (74)		− (92)
Growth on salicin	+ (79)		− (77)
	Biovar X_1	Biovar X_2	
Elastase production	+ (92)	− (100)	− (88)
Acetoin from glucose	+ (92)	− (97)	±(+,58)
Gas from glucose	+ (92)	− (86)	+ (88)
H_2S in 'Gélose G.C.F.'	+ (85)	− (93)	+ (96)

+, Character usually positive; −, character usually negative; ±, non-significant frequency, the commonest result being shown in parentheses.

Table 8. *DNA base compositions of some strains*

Strain	Class	T_m (°C)	% GC
102	X	79·7	62·9
218	X	79·3	61·9
239	X	79·5	62·5
246	X	79·7	62·9
307	X	79·6	62·7
208	Y	78·0	58·8
209	Y	78·2	59·2
245	Y	78·8	60·7
262	Y	74·8	50·9
Escherichia coli K12	—	74·7	50·7

Table 9. *Summary of the most recent taxonomic studies on the hydrophila–punctata group*

Ewing *et al.* (1961)	Eddy (1960, 1962) Leclerc (1962) Merkel *et al.* (1964)	Schubert (1969*a*, *b*)
One species *A. hydrophila*	Four species *A. punctata* *A. caviae* *A. dourgesi* *A. proteolytica*	Two species *A. hydrophila* (three subspecies) *A. punctata* (two subspecies)

phila subsp. *hydrophila* and of *A. punctata* subsp. *punctata*, and the type strains of *A. hydrophila* subsp. *anaerogenes* and of *A. punctata* subsp. *caviae*, are all found in the species X.

According to Rule 42 of the International Code of Nomenclature of Bacteria (Lapage *et al.*, 1973), when two taxa of equal rank are united, 'the oldest legitimate name or epithet is retained'. *Aeromonas hydrophila* was first legitimately published as *B. hydrophilus* by Chester (1901) and *A. punctata* by Snieszko in the 7th edition of *Bergey's Manual of Determinative Bacteriology* (1957) because the description of *B. punctatus* by Zimmerman (1890) is unrecognizable (Ewing *et al.*, 1961). Therefore, we propose to retain the name *A. hydrophila* for the species X, with the proposed neotype strain ATCC7966 as type strain (Opinion

47 of the Code), and to give an amended definition of this species on the basis of the characters of cluster X.

The species X, *A. hydrophila*, could be divided into two biovars. In the first one (subclass X_1), most strains produce butanediol dehydrogenase (92 % of the strains), gas and acetoin from glucose (see Table 7), and correspond to biotype 1 of *A. hydrophila* subsp. *hydrophila* as defined by Schubert in *Bergey's Manual of Determinative Bacteriology* (1974). The second biovar (subclass X_2) does not produce gas or acetoin from glucose, gives variable results for the butanediol dehydrogenase test, and it corresponds to the anaerogenic strains of Aeromonas. Thus the subspecific epithet '*anaerogenes*' could be retained for this biovar.

The reference strain of *A. hydrophila* subsp. *proteolytica* (strain 262) happens to be included in class Y, but this strain differs from the others as follows: (i) phenotypically, in its sodium requirement – a fundamental physiological character reflecting a specific sodium requirement for a number of essential cellular processes (MacLeod, 1968); (ii) genotypically, by the GC content of its DNA (50·9 mol %) – a result agreeing with that of Baumann, Baumann & Mandel (1971), but which falls outside the range of values for the GC content of the DNA quoted for the genus *Aeromonas* in *Bergey's Manual of Determinative Bacteriology* (1974). The peritrichous flagellation appearing when the strain is grown on solid medium (Baumann *et al.*, 1971; McCarthy, 1975), the sodium requirement, the existence of species unique to the ocean (supported by an extensive study by Reichelt & Baumann, 1974) and the GC ratio are convincing reasons for excluding *A. hydrophila* subsp. *proteolytica* from the genus *Aeromonas*. The taxonomic assignment of this bacterium requires the study of more strains, because only one strain is actually known (NCMB1326 = ATCC15338).

With exception of this strain, 262, all the others in class Y belong to the genus *Aeromonas* because they possess all the fundamental properties of the Aeromonas definition, and have an average GC value close to that of *A. hydrophila* (class X). But these strains do not correspond to any previously described species, and we propose that class Y should be designated *A. sobria* sp.nov. because of the comparatively small number of substrates that support growth. It can be distinguished from the first biovar of *A. hydrophila* by eight characters and from the second one by nine characters (Table 7).

The proposed emendation of the description of the type species *A. hydrophila* (syn. *A. punctata*) is as follows: It has the basic morphological and physiological characters of the genus *Aeromonas* given in *Bergey's Manual of Determinative Bacteriology* (1974); utilizes as sole source of carbon and energy, L-histidine, L-arabinose, L-arginine and salicin; hydrolyses esculin; grows on KCN medium; ferments salicin. This species can be divided into two biovars: (i) *A. hydrophila* biovar *hydrophila* which produces gas and acetoin from glucose, H_2S from cysteine, and elastase (reference strain: ATCC7966 = NCTC8049 = NCMB86 = NCIB9240 = CDC-RH35); (ii) *A. hydrophila* biovar *anaerogenes* which does not produce gas and acetoin from glucose, H_2S from cysteine, and elastase (reference strain ATCC15468).

The formal description of *A. sobria* sp.nov. is as follows: possesses the basic morphological and physiological characters of the genus *Aeromonas* given in *Bergey's Manual of Determinative Bacteriology* (1974); does not utilize as sole source of carbon, L-histidine, L-arabinose, L-arginine and salicin; does not grow on KCN medium, or hydrolyse esculin, or ferment salicin; produces gas from glucose and H_2S from cysteine; elastase negative. Type strain (holotype): strain 208 = CIP7433.

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