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Biochemical identification and numerical taxonomy of *Aeromonas* spp. isolated from food samples in Turkey

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Abstract: Numerical taxonomy was used to analyze phenotypic data obtained from 126 new isolates of *Aeromonas* strains taken from red meat, raw chicken, minced meat, and fish samples. Each strain was tested for 86 characters but only the final data including 63 characters were analysed using the S_{SM} coefficients and the UPGMA clustering algorithm. At S_{SM} values of ≥ 83%, the strains clustered into 10 aggregate groups consisting of 7 major (5 and up strains) and 3 minor (2-4 strains), and 5 single member clusters, each of which was identified as *A. hydrophila*, *A. caviae*, and *A. sobria*, respectively. It was proved that the food isolates showed a relative phenotypical distance and the groups of strains that had atypical profiles were compared with the type species by the present identification schemes. It was clearly seen that the phenetic approach was a necessary tool to delimitate and identify the *Aeromonas* species. Numerical taxonomy of *Aeromonas* strains isolated from different sources revealed the presence of potentially pathogenic *Aeromonas* spp., especially in food.

Key words: Numerical taxonomy, *Aeromonas*, biochemical identification

Türkiye'de gıda örneklerinden izole edilen *Aeromonas*ların biyokimyasal tanımlanması ve nümerik taksonomisi

Özet: Kırmızı et, çiğ tavuk, kıyma ve balıktan alınan *Aeromonas* suşlarına ait yeni 126 izolatın fenotipik verilerini analiz etmek için nümerik taksonomi kullanılmıştır. Her bir suş 86 karakter bakımından test edilmesine rağmen son veri seti 63 karakter ile S_{SM} benzerlik katsayısı ve UPGMA kümeleme algoritmasıyla analiz edilmiştir. Suşlar % 83'den büyük S_{SM} değerlerinde, 7 büyük (5 ve üzeri suş), 3 küçük (2-4 suş) ve 5 adet tek üyeli kümeden oluşan 10 agregat grup içinde toplanmış ve bu suşlar sırasıyla *A. hydrophila*, *A. caviae* ve *A. sobria* olarak tanımlanmışlardır. Gıdalardan elde edilen izolatların nisbi bir fenotipik aralık gösterdiği ve suş gruplarının mevcut tanımlama şemasındaki tip örnekleriyle mukayese edildiklerinde tipik olmayan profillere sahip olduğu kanıtlanmıştır. Fenetik yaklaşımın *Aeromonas* türlerini tanımlamada ve sınırlandırmada gerekli bir araç olduğu açıkça görülmüştür. Farklı kaynaklardan izole edilen *Aeromonas* suşlarının nümerik taksonomisi, özellikle gıdalarda potansiyel patojenik *Aeromonas*'ların varlığını ortaya çıkarmıştır.

Anahtar sözcükler: Nümerik taksonomi, *Aeromonas*, biyokimyasal tanımlama

Introduction

Members of this group are gram-negative, non-spore forming, rod shaped, oxidase and catalase positive, motile by polar flagellum, mesophilic and facultative anaerobic bacteria of family *Aeromonadaceae* (1,2). *Aeromonas* species are widely distributed in the aquatic environment, including raw and processed drinking water (3,4), and have been frequently isolated from various food products such as fish and shellfish, raw meat, vegetables, and raw milk (5,6). Additionally, in recent years aeromonads have been implicated as causative agents of human disease, ranging from gastroenteritis to wound infections (2,3,5-7).

The genus *Aeromonas* has undergone a number of taxonomic and nomenclature revisions over the past 20 years. Valera and Esteve (8) have reported that it currently constitutes a new family, *Aeromonadaceae* (1), and that the number of recognised species in the genus *Aeromonas* increased from 4 (9) to 16 (4,10-12). In spite of this progress, many questions concerning the taxonomy of this genus remain unresolved, among them the identification of new isolates to the species level (8).

Twenty years ago, only 5 species of *Aeromonas* were recognised (6), and, out of the concerned 5, 3 (*A. hydrophila, A. sobria,* and *A. caviae*) existed as phenospecies, which means a named species containing multiple DNA groups, and whose members could not be differentiated from one another using simple biochemical characteristics, which were no longer adequate. In recent times, molecular and chemotaxonomic methods have been devised in order to identify *Aeromonas* spp., and these represented some improvement (13-15). However, remarkable discrepancies were observed in association with DNA/DNA homology data and 16S rRNA sequencing data (16-18). On the other hand, there is still some confusion regarding the determination of the appropriate assignment of *Aeromonas* strains to the recognised species using biochemical characters, and further assessment is needed to overcome this confusion. It has been reported that the use of available diagnostic kits and phenotypic schemes was not advisable for making such precise identifications (19-21).

Our literature scan showed that no study has been conducted until now on the numerical taxonomy of *Aeromonas* in Turkey. Therefore, the present study was undertaken to identify *Aeromonas* strains isolated from different food samples and to cluster them by numerical taxonomy.

Materials and methods

Bacterial strains

Food samples were purchased from various markets, local bazaars, and butcher shops in Turkey. A total of 129 of motile aeromonads isolated from fish, chicken meat, red meat, and minced meat samples were used in this study (Table 1). From these isolates, 92 strains (73.0%), 25 strains (19.8%), and 9 (7.1%) strains were identified as *A. hydrophila* (obtained from 32 fish, 20 chicken meat, 27 minced meat, and 13 red meat), *Aeromonas caviae* (11 fish, 2 chicken meat, 10 minced meat, and 2 red meat) and *A. sobria* (3 fish, 4 chicken, and 2 minced meat), respectively.

Strains were grown on Tryptic Soy Agar (TSA, Oxoid) at 28 °C. Finally *A. hydrophila* ATCC 7966 (American Type Culture Collection), *Aeromonas caviae* ATCC 15468, and *A. sobria* ATCC 43979 microorganisms were included as type strains in this study. The test error was evaluated by examining 12 strains in duplicate (around 10% of the total strains), according to the method suggested by Sneath and Johnson (22).

Phenotypic characterization

Each strain was tested for 86 phenotypic properties. Unless otherwise stated, incubations were performed at 25 °C (23) and all media contained 1% (w/v) NaCl, provided as such or supplemented at the laboratory (24). Oxidase-positive, glucose-fermentative, gram-negative rods with non-swarming production, no sodium requirements, absence of growth at 6% NaCl, and resistance to vibriostatic agent O/129 were presumptively identified as *Aeromonadaceae* and stored in tryptone soy broth (TSB) with 20% (v/v) glycerol at –70 °C until further analyses were carried out. The oxidation-fermentation test was performed in O/F basal medium (Difco) supplemented with 1% (w/v) glucose following Hugh and Leifson (25).

The following tests were carried out as described elsewhere (26): cell shape; cytochrome oxidase;

catalase activity; swarming motility on tryptone soy agar (TSA) and citrate utilization (Simmons' citrate agar) after 7 days; gas production from D-glucose and β-hemolysis of sheep blood after 48 h; indole production, methyl red and Voges-Proskauer reactions, esculin (but with 0.1% esculin and 0.1% ferric ammonium citrate), starch hydrolyses, protease, Congo red uptake, crystal violet uptake, siderophore, acriflavine agglutination, and DNase test after 72 h. Motility was verified in overnight cultures in peptone water by microscopic examination when there were doubts. The following tests were performed as described elsewhere (27): the nitrate reduction test was carried out in nitrate broth after 48 h, the urease test (Rustigian and Stuart's urea broth) after 48 h and the lysine (LDC) and ornithine (ODC) decarboxylases and arginine dehydrolase (ADH) tests in Falkow decarboxylase broth after 4 days Arbutin hydrolysis was carried out as reported elsewhere (28). The salt tolerance test [0% and 6% (w/v) NaCl] was carried out following Twedt's method (29) after 72 h; acid production from 1% (w/v) of the following substrates, L-arabinose, D-lactose, D-mannose, D-mannitol, salicin, D-sorbitol, and sucrose, was determined after 7 days. The utilization of substrates as sole carbon sources was studied on M70 medium (30,31). The following substrates [0.2% (w/v) sugars, 0.1% (w/v) others] were filter sterilized: acetate, L-arabinose, L-arginine, L-histidine, and D-mannitol. Bacterial growth was examined for 14 days. Hydrogen sulphide from cysteine after 4 days and elastase production after 15 days were according to Popoff and Lallier (31). Susceptibility to the vibriostatic agent O/129 (150 µg; Oxoid) and the following antibiotics (Biomerieux, Marcy l'Etoile, France): cephalothine (30 µg), carbenicillin (30 µg), gentamicin (10 µg), penicillin (6 µg), neomycin (5 µg), gentamicin (10 µg), oxacillin (5 µg), chloramphenicol (30 µg), nitrofurantoin (300 µg), ampicillin (10 µg), and tetracyclin (30 µg) was tested by the disc diffusion method (32). After incubation at 28 °C for 24-28 h, the cultures were streaked on glutamate starch phenol red agar (GSP agar, Merck) (33) and incubated at 28 °C again for 24-48 h. Yellow colonies surrounded by yellow zone were picked and grown on a fresh GSP agar plate for reconfirmation. Additional phenotypic tests that have been associated with specific biotypes or used as potential virulence-associated markers were also evaluated. These were lysine decarboxylase production, Voges-Proskauer reaction, and hemolysis on TSA plates with 5% sheep blood by streaking and stabbing, acriflavine agglutination, siderophore, DNase, proteinase, and pyrazinamidase activities (19,34-36).

Numerical taxonomy: The sources and taxonomic histories of the 126 *Aeromonas* isolates and 3 type cultures are given in Table 1.

Table 1. Isolates and type strains used in this study.

Cluster	Species	Strain	Source
1	*A. hydrophila*	20	fish, chicken meat, red meat
2	*A. hydrophila*	48	fish, chicken meat, minced meat
3	*A. hydrophila*	8	fish, minced meat
4	*A. caviae*	3	red meat
5	*A. caviae*	2	fish
6	*A. hydrophila*	10	chicken meat, minced meat
7	*A. hydrophila*	7	minced meat, ATCC7966
8	*A. caviae*	13	red meat
9	*A. caviae*	4	fish
10	*A. sobria*	9	chicken meat
SMC1	*A. caviae*	1	fish
SMC2	*A. caviae*	1	fish
SMC3	*A. caviae*	1	ATCC15468
SMC4	*A. sobria*	1	ATCC43979
SMC5	*A. caviae*	1	minced meat

ATCC7966, *A. hydrophila;* ATCC15468, *A. caviae;* ATCC43979, *A. sobria;* **SMC**, Single Member Cluster; **ATCC,** American Type Culture Collection

Coding data: Nearly all of the characters existed in one or two mutually exclusive states and scored positive (+) or negative (-). Qualitative multistate characters, such as some of pigmentation and morphological tests, were coded as several independent characters and were scored present (1)

for the character state shown and absent (0) for all alternatives. Some of the tests, notably tolerance to antibiotics and chemical inhibitors, were coded using an additive method (37).

Computation: The binary test data were typed in a +/- format and simple matching coefficient (S_{SM}) values were obtained using X-Taxon program (38). Based on the S_{SM} coefficient Unweighted Pair Group Method with Arithmetic Mean (UPGMA) clustering was achieved using the NTSYS-pc statistical program (39).

Test reproducibility: A total of 129 *Aeromonas* strains, including 12 duplicated strains and 3 *Aeromonas* type strains were tested for a total of 86 unit characters in numerical studies. The average probability of error (P) was calculated as 3.10% from the pooled variance (= 0.050 mean) of 86 unit characters (22). The present investigation also showed that the taxonomic structure was not markedly affected by the value of test error (P), a figure well within the 10% guideline recommended by Sneath and Johnson (22). There was no excluded test from the final data matrix for their high test variances (variances above 0.1).

Results and discussion

Identification and distribution of *Aeromonas* species

All *Aeromonas* strains were positive for rod morphology, glucose oxidation–fermentation, oxidase, O/129 resistance, nitrate reduction, growth at 0% and 6% NaCl, and 37 °C growth on GSP agar and acid production from mannitol and negative for Gram reaction, growth at 6% NaCl, swarming motility, and urea hydrolysis. The results of the remaining tests are shown in Table 2. Phenotypic tests (marked with an asterisk in Table 2) used in this study allowed the identification of the genus *Aeromonas* obtained from different foods. Most species were differentiated by 3 or more tests, although there were some exceptions.

Clustering of strains using the S_{SM} coefficient with the UPGMA algorithm

The classification based on the S_{SM} - UPGMA analysis is described in detail as it gave the most compact aggregate groups and clusters together with a suitable high cophenetic correlation value (0.832). The 86 test strains were assigned to 10 cluster groups, at the 83% similarity (S) level.

Seven major (5 and up), 3 minor (2-4 strains), and 5 single membered clusters were circumscribed at or above the 83% similarity level (Figure). These clusters were assigned names according to the distribution of type and type strains. The characteristics of the major and minor clusters are given in Table 2.

It was interesting that in the present numerical taxonomic study, 29 strains out of the total 129 isolated strains were differently clustered from type strains. These organisms were assigned to 4 major (Cluster 3, 7, 8, and 9), 4 minor (Cluster 4 and 5), and 3 single membered clusters (KA052, KA081 and KA051). This means that numerical taxonomies also need to be evaluated in the light of additional information derived from the application of independent taxonomic methods, notably by the use of chemotaxonomic and molecular systematic techniques.

Numerical taxonomy

Each of the 126 food strains was tested for the 86 phenotypic characters. The same characters were also tested in the type strains of the following relevant species: *A hydrophila, A. sobria*, and *A. caviae*. Considering the results of the relevant test for species identification according to Popoff and Lallier's (31) identification scheme, all the type strains were correctly identified and, among the food strains, 25 (19.8%) were identified as *A. caviae*, 9 (7.1%) as *A. sobria*, and 92 (73%) as *A. hydrophilia*. The strains were grouped by the S_{SM} -UPGMA analysis into 10 clusters, defined at or above the 83% similarity S_{SM} (Figure). The S_{SM}-UPGMA analysis yielded a very similar dendrogram, grouping the strains into 10 clusters at similarity values of 83%. The dendrogram obtained by S_{SM}-UPGMA analysis is shown in the Figure. Sixty-three of the 86 phenotypic characters evaluated in this study appeared to be variable among different strains and were used to perform numerical taxonomy analysis.

The phenospecies *A. hydrophila* is represented by several clusters: The phenotypic profile of clusters 1, 2, 3, 6, and 7 agreed well with the description of *A. hydrophila* (31). They are formed at 93% S_{SM} and 98%

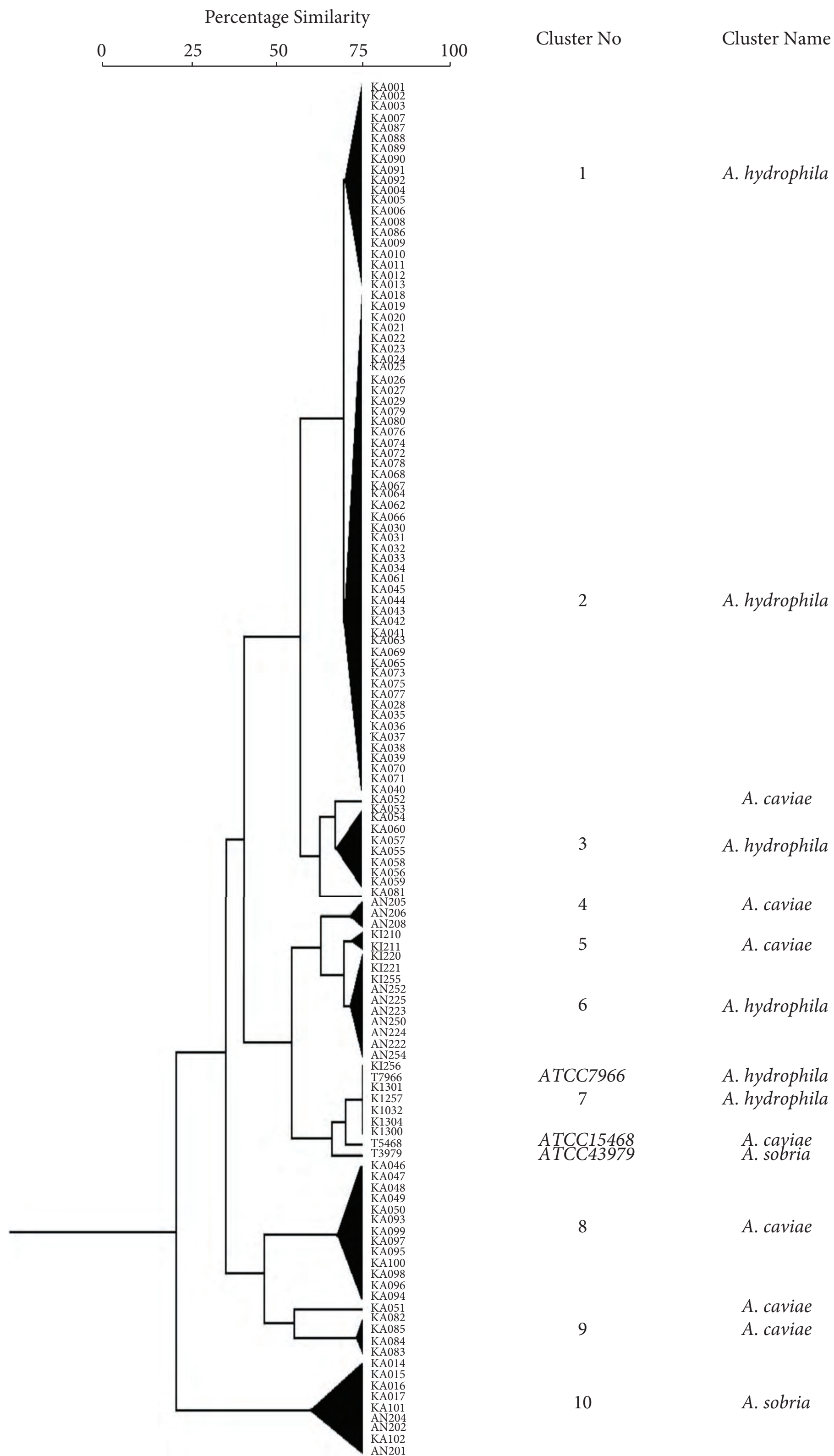

Figure. Abridged dendrogram showing relationships between the representative isolates and marker strains belonging to *Aeromonas* based on S_{SM}-UPGMA analysis.

Table 2. Biochemical properties of *Aeromonas* species.

Characteristics	Results[a] for:			References
	***A. hydrophila* (n = 92)**	***A. caviae* (n = 25)**	***A. sobria* (n = 9)**	
Motility	+	+	+	8,15,23,24,40
Catalase	+	+	+	9,15,23,24,26
Oxidase	+	+	+	9,15,23,24,26
Gas from glucose*	+	-	d	8,15,26, 40,41
Methyl red	+	-	d	9,15,26,41
Voges–Proskauer*	+	-	-	8,15,40,41,42
Lysine decarboxylase*	+	-	+	8,15,28,41
Ornithine decarboxylase*	-	-	-	8,15,28,41
Vibriostatic 0/129 (150 μg)	+	+	+	15,23,24,32
Production of:				
Indole*	+	+	+	8,15,26,40
Urease	-	-	-	15,28,40
Nitrate	+	+	+	9,27,41
Congo red	+	+	-	41
H_2S from L-cysteine*	+	-	-	8,20,31,40
Growth:				
0% NaCl	+	+	+	9,15,29,41
6% NaCl	-	-	-	9,15,29,41
KCN broth	+	+	-	9,41
Pyrazinamidase[c]	+	+	-	20,36,41
Hydrolysis of:				
Arbutin hydrolysis*	+	+	nd	8,15,28,40
Inulin	-	-	-	9,40,41
DNase	+	+	-	9,26,41,41
Elastin*	+	-	-	8,15, 40,41
Esculin*	+	+	-	8,15, 40,41
Starch*	+	+	+	15,26, 40,41
Gelatin	+	+-	-	8,9, 40,41
Beta hemolysis[b]	+	-	-	20,26,34,35,40
Alpha hemolysis	+	-	-	9,34,43,44
Prolin	+	+	+	8,9, 40,41
Acid from:				
Adonitol	-	-	-	8,9, 40,41
D-Arabitol	-	-	-	8,9, 40,41
L-Arabitol	-	-	-	8,9, 40,41
L-Arabinose*	+	-	-	8,15,29,30,31
D-Arabinose	d	+	-	8,29, 40,41
D-Fucose	-	-	-	8,9, 40,41
L-Fucose	-	-	-	8,9, 40,41
Galactose	+	+	+	8,9, 40,41
Gluconate	d	-	-	8,20,41,43
Dulcitol	-	-	-	8,9,40,41
Lactose	d	d	-	8,15,29,41,43
D-Mannitol*	+	+	+	8,29,30,31,41
Maltose	+	+	+	8,9,41,42
Melibiose	-	-	-	8,9,40,41
Inositol	-	-	-	8,9, 40,41
D-Mannose	+	d	+	8,15,20,41
Salicin	d	+	-	8,29,41,43
Malonate	-	-	-	9,40,41

Table 2. (Continued).

Characteristics	Results[a] for:			References
	***A. hydrophila* (n = 92)**	***A. caviae* (n = 25)**	***A. sobria* (n = 9)**	
D-Sorbitol*	-	-	-	15,29,41
L-sorbose	-	-	-	8,9, 40
Saccharose (sucrose)*	+	+	+	15,29,41,44
D-Glucose	+	+	+	8,9,40,41
D-Trehalose	+	+	+	8,9,40,41
D-Rafinose	-	-	-	8,9,40,41
L-Rhamnose	d	-	-	8,9,40,41
Glycerol	+	d	+	8,9,40,41
Ribose	+	+	+	8,9,40,41
D-xylose	-	+	+	8,9,40,41
L-xylose	-	+	+	8,9,40,41
Tryptophane	-	-	-	8,9,40,41
Glycogen	+	+	+	8,9,40,41
Malate	+	+	-	8,9,40,41
Erythritol	-	-	-	8,9,40,41
D-Togotose	-	-	-	8,9,40,41
Utilisation of:				
Acetate	+	+	-	30,31,40,41
Arginine dihydrolase	+	+	-	8,15,28,30,31,40
Histidine	+	+	+	8,15,30,31,40,41
Lysine	+	+	+	8,9,40,41
Casein	+	-	-	8,52,40,41
Citrate	d	+	+	15,26,40,41
Protease	+[a]	+	-[a]	45
Haemagglutination	+	-	-	9,31,34,45
Cytotoxin	+	-	-	9,31,34,45
Enterotoxin	+	-	-	9,31,34,45
Siderophore	+	+	-	9,45
Crystal violet uptake	+	+	-	9,31
Calcium dependency	+	-	-	9,31
Acriflavine agglutination	+	-	-	9,34
Resistance to:				
Ampicillin (10 µg)	R[e]	R	R	15,20,32
Cephalothin (30 µg)	R	R	S[e]	15,20,32
Gentamicin (10 µg)	R	R	R	9,32
Penicillin (6 µg),	R	R	R	9,32
Tetracyclin (30 µg)	S	S	S	9,15,32
Neomycin (5 µg)	S	S	S	9,32
Carbenicillin (30 µg)	R	R	S	9,32
Oxacillin (5 µg)	S	S	S	9,32
Chloramphenicol (30 µg)	S	S	S	9,32
Nitrofurantoin (300 µg)	S	S	S	9,32

Abbreviations:

*The 14 basic tests used to identify mesophilic *Aeromonas* species.

[a]Symbols:+, > 90%; -, < 10%; d, 89% positive with incubation at 28 °C for 7 days.

[b]Hemolysis detected on TSA on 5% sheep blood agar.

[c]Pyrazinamidase activity slants were incubated for only 48 h.

[d]Data from Esteve et al. (10) and Huys et al. (39); analyses were performed at 28 °C unless otherwise indicated.

R[e], resistant, S[e], Susceptible.

nd, not determined.

S_{SM}, respectively. The phenotypic characteristics of this cluster agreed well with those previously reported for *A. hydrophila* (31). Cluster 1 contained isolates 20 strains and all strains belonged to *A. hydrophila* (Figure). Cluster 2, defined at 95% S_{SM}, contained 48 strains, all strains identified as *A. hydrophila*. Cluster 3, defined at 93% S_{SM}, contained 8 strains, all strains identified as *A. hydrophila*, and Cluster 6, defined at 98% S_{SM}, contained 10 strains isolated from food. All strains were identified as *A. hydrophila*. Cluster 7, defined at 100% similarity level with S_{SM} coefficient, contained 7 *A. hydrophila* and also including the type strain of *A. hydrophila* ATCC 7966.

Biochemically, *A. hydrophila* hydrolyzes esculin; has a positive Voges-Proskauer test; displays pyrazinamidase activity; produces acid from D-mannitol and sucrose and variably from arabinose; is resistant to ampicillin and cephalothin. It decarboxylates lysine but not ornithine; produces indole, H_2S, and gas from D-glucose; and undergoes β-hemolysis on TSA with 5% sheep blood agar. The species belonging to *A. hydrophila* were differentiated on the basis of sorbitol fermentation. Differential and descriptive tests to aid in the identification of *A. hydrophila* from all validly named motile species are presented in Table 2.

The phenospecies *A. caviae* is represented by several clusters: The phenotypic profile of Clusters 4, 5, 8, and 9 agreed well with the description of *A. caviae* (31). The only discrepancy related to the typical reactions described for *A. caviae* (31) was the low percentage of strains that produce acid from salicin (Table 2). Cluster 4, defined at 98% S_{SM}, contained 3 strains, all strains identified as *A. caviae*. Cluster 5 defined at 99% S_{SM}, included 2 strains, all strains identified as *A. caviae*. Cluster 8, defined at 94% S_{SM}, contained 13 *A. caviae*. Cluster 9, defined at 99% S_{SM}, contained 4 *A. caviae*. It does not produce gas during D-glucose fermentation, is Voges–Proskauer negative, is lysine decarboxylase negative, and H_2S is not produced from L-cysteine. It utilizes D-lactate and citrate as the sole source of carbon. Differential tests to aid in the identification of *A. caviae* from all motile species are listed in Table 2.

The phenospecies *A. sobria* is represented by several clusters: The phenotypic profile of Cluster 10 agreed well with the description of *A. sobria* (31). They are motile, produce gas from glucose, hydrogen sulphide from cysteine, and acid from sucrose (100% positive responses), and grow at 37 °C. They do not hydrolyze esculin or acid from salicin and they use neither L-arabinose nor L-arginine (Table 2).

Cluster 10, defined at 86% S_{SM}, contained nine *A. sobria*. Biochemical results were obtained at 37 °C after 2 days of incubation. Produces acid and gas from glucose, acid from mannitol and sucrose, and is weakly positive Voges-Proskauer reaction. Lysine decarboxylase is weakly positive after 2 days; arginine dihydrolase and ornithine decarboxylase are not produced. It does not produce acid from L-arabinose. It does not hydrolyze esculin or produce gas from glucose at 37 °C after 48 h.

Distribution of mesophilic *Aeromonas* spp. has been previously reported in a wide range of samples as fresh waters, vegetables, meats and milk products, fish, shellfish, seawater, and clinical (3-7).

Our results showed that 99.9% of *Aeromonas* isolates was identified at species level. In different foods, *A. hydrophila* was the dominant species, followed by *A. caviae* and *A. sobria*. More recent investigations on the prevalence of *Aeromonas* species in environmental, clinical, food, and veterinary origin sources have focused on 3 mesophilic species: namely *A. hydrophila*, *A. caviae*, and *A. sobria* and these microorganisms are the most frequently isolated species (45-48), which is consistent with our results.

Numerical taxonomy studies of the genus *Aeromonas* have been previously published (10,15,16). However, our study presents some differences in the numerical analysis of the data. First, the final data matrix did not include those tests that gave positive or negative responses for all strains, in contrast to analyses of Kämpfer and Altwegg (44) and Noterdaeme et al. (41). Second, we have defined phenons at or above 83% S by using the S_{SM} coefficient, whereas most previous reports either delineated phenons at lower similarity level (70%-80% S) or used the simple matching (S_{SM}) coefficient, which is less restrictive (10,44,46). Moreover, the methodological parameters of the present study showed acceptable values (22,49). In conclusion, the present phenotypic study is a powerful taxonomic tool to delimitate and identify *Aeromonas* species. In

fact, a good correlation was mostly observed between this phenotypic clustering and previous genomic and phylogenetic data. Moreover, this approach has indicated some valuable traits for identifying *Aeromonas* as well as the possible existence of new *Aeromonas* species or biotypes. Nevertheless, the use of genomic studies based on classical DNA–DNA hybridisation methods is necessary to determine what the taxonomic position of these isolates is.

Biochemical tests used in this study positively helped in the isolation and identification of *A. hydrophila*, *A. caviae*, and *A. sobria*, which are likely to be in foods. However, these tests did not show a positive effect on identification of the others *Aeromonas* species such as *A. schubertii*, *A. jandei*, *A. veronii*, and *A. veronii* bv. *sobria*. As pointed out before, to determine the appropriate assignment of *Aeromonas* strains the molecular and chemotaxonomic methods must be required besides biochemical ones.

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