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Aeromonas encheleia sp. nov., Isolated from European Eels

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Four strains isolated from European eels in Valencia, Spain, were found to constitute a DNA relatedness group which is 0 to 50% related to the 13 species and DNA group 11 of the genus *Aeromonas*. Phenotypically, these strains have all of the properties that define the genus *Aeromonas*. However, they differ from the previously described *Aeromonas* species by three or more properties. The strains are positive for motility, growth at 37°C, indole production, and arginine dihydrolase activity. They exhibit negative reactions in tests for growth at 42°C and in thiosulfate-citrate-bile salts-sucrose medium (Oxoid), Simmons citrate tests, and tests for lysine and ornithine decarboxylase activities. They produce acid from salicin but not from L-arabinose, D-cellobiose, or lactose. All four strains hydrolyze esculin and arbutin but not elastin. They use L-serine as a sole carbon and energy source but cannot utilize L-arabinose, L-arginine, D-gluconate, or L-glutamine. The strains are resistant to ampicillin. The guanine-plus-cytosine content of the DNA is 59.4 to 60.8 mol%. The name *Aeromonas encheleia* sp. nov. is proposed for these strains; strain S181 (= CECT 4342) is the type strain. This new species is generally not pathogenic for eels or mice.

The genus *Aeromonas* was proposed by Kluyver and van Niel in 1936 (23) and comprises a collection of oxidase- and catalase-positive, glucose-fermenting, facultatively anaerobic, gram-negative, rod-shaped bacteria that are resistant to vibriostatic agent O/129 (2,4-diamino-6,7-diisopropyl pteridine) and generally are motile by means of polar flagella (32). *Bergey's Manual of Systematic Bacteriology* includes four species in the genus *Aeromonas*, *Aeromonas salmonicida*, *Aeromonas hydrophila*, *Aeromonas caviae*, and *Aeromonas sobria*, some of which are genotypically heterogeneous (32, 34). Recently, the number of recognized species in the genus *Aeromonas* has increased from 4 to 13; these species represent clearly differentiated DNA homology groups (2, 6, 7, 13, 14, 38–40). Furthermore, methods for identification of *Aeromonas* strains at the genus and species levels have undergone major improvements primarily as a result of chemotaxonomic studies (3, 4, 20).

During a survey to determine what bacteria are associated with European eels reared in a freshwater farm located in Valencia, Spain, four phenotypically related strains were isolated. These strains could not be identified as members of any previously described *Aeromonas* species. The purposes of this study were to determine, by DNA-DNA hybridization and extensive phenotypic tests, the taxonomic position of the four eel isolates and to assess by inoculation in animal models the pathological significance of these organisms. In this paper we describe a new species, *Aeromonas encheleia*, which is generally not pathogenic for eels or mice.

MATERIALS AND METHODS

Bacterial strains. The four strains of *A. encheleia* used in this study (S176, S177, S181^T [T = type strain], and S191) were recovered from healthy European eels reared in a freshwater farm located in Valencia, Spain. Fifty juvenile eels (average weight, 0.3 g) per tank were analyzed. The eels were washed by vigorous agitation in saline (Oxoid) and then homogenized in a fresh solution. Thus, strains S176, S177, S181^T, and S191 were originally isolated from fish homogenates on tryptic soy agar (TSA) (Oxoid) supplemented with 0.5% (wt/vol) NaCl. These four strains constituted a phenon defined at a level of similarity of 90.5% in a previous study (10), in which one of us (C.E.) used the simple matching coefficient followed by clustering of the operational taxonomic units into groups by unweighted pair group mathematical averaging. The strains were maintained at −80°C in a medium containing 1.7% (wt/vol) tryptone (Oxoid), 0.3% (wt/vol) soytone (Difco), 0.6% (wt/vol) yeast extract (Oxoid), 1% (wt/vol) NaCl (pH 7.3), and 20% (vol/vol) glycerol. The *Aeromonas* culture collection strains used in DNA relatedness experiments are listed in Table 1.

Physiological and biochemical characterization. Unless otherwise indicated, cultures were incubated at 28°C. Cell shape and the Gram stain (9) reaction were determined after 24 h of incubation on TSA. Production of a diffusible brown pigment was determined with 7-day cultures on TSA plates. Motility was determined by microscopically examining 18-h cultures in tryptic soy broth (Oxoid). The following tests were performed by using previously described methods (41): tests to determine Kovács cytochrome oxidase activity, reduction of nitrate and nitrite, catalase activity, gelatin liquefaction (method 1), and swarming. The tests used to determine susceptibility to vibriostatic agent O/129 (150-μg discs; Oxoid), acetoin production (Voges-Proskauer test), lysine and ornithine decarboxylase and arginine dihydrolase activities (Moeller's method), salt tolerance (0, 3, 6, 8, and 10% [wt/vol] NaCl), and hydrolysis of esculin and arbutin were performed as described previously (24). Arginine dihydrolase activity was also tested by Thornley's method (42). The oxidation-fermentation test was performed by the method of Hugh and Leifson (15) in O/F basal medium (Difco) supplemented with 1% (wt/vol) glucose. Production of gas from glucose was determined on tryptic soy broth, as described by Lee et al. (25). Production of indole and production of hydrogen sulfide were determined on sulfide indole motility medium (Difco) after 24 h. Peptone water (1% [wt/vol] peptone [Oxoid]) supplemented with 0.5% (wt/vol) tryptophan was also used to test indole production (41). Growth at different temperatures was determined in tryptic soy broth after 24 h for 37 and 40°C, after 15 days for 15°C, and after 21 days for 4°C. Growth at pH 4.5 and 9.0 was determined in tryptic soy broth after 48 h. Growth on MacConkey medium (Oxoid) and growth on thiosulfate-citrate-bile salts-sucrose medium (Oxoid) were determined after 48 h. Urease activity was determined in urea broth (Difco) incubated for 48 h. Citrate utilization was determined on Simmons citrate agar (Oxoid). DNase activity was determined on DNase agar (Oxoid). The test to determine hydrolysis of chondroitin sulfate (Sigma) was performed by using previously described methods (21). Acid production from carbohydrates was determined on nutrient agar (Oxoid) supplemented with 0.001% (wt/vol) bromocresol purple and one of the following substrates (Sigma) at a concentration of 1% (wt/vol): L-arabinose, salicin, sucrose, D-cellobiose, D-xylose, maltose, D-melibiose, D-trehalose, D-galactose, lactose, D-raffinose, D-mannose, L-rhamnose, *myo*-erythritol, dulcitol, glycerol, *myo*-inositol, D-sorbitol, and D-mannitol. A yellow color around the growth after 48 h was recorded as a positive reaction. The tests to determine hydrolysis of starch, casein, and Tween 80 were performed as described previously (41). Hydrolysis of chitin (Sigma) was examined by the method of Reichenbach and Dworkin (36). The test to determine hydrolysis of collagen (Sigma) was performed in the medium of Gauthier et al. (12). The tests to determine lecithinase activity and hydrolysis of fibrinogen (Sigma) were performed as described by Janda and Bottone (17). Alkyl sulfatase activity was examined on TSA supplemented with 0.2% (wt/vol) sodium dodecyl sulfate (SDS) (Sigma); the method used was similar to that described by Kitaura et al. (22). Hydrolysis of mucin (Sigma) and hydrolysis of keratin were determined as described previously (29, 44). The test to determine hydrolysis of elastin (Sigma) was performed on modified Scharman medium (16). Hemolytic activity against human erythrocytes was determined on TSA supplemented with 5%

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TABLE 1. Levels of DNA relatedness of strains S181^T and S176 to other *A. encheleia* strains, strains of other *Aeromonas* species, and related bacteria

Source of unlabeled DNA[a]	% Homology with ^{3}H-labeled DNA from:	
	A. encheleia S181^T	*A. encheleia* S176
A. encheleia strains		
S181^T	100	70
S176	86	100
S177	70	88
S191	88	92
A. hydrophila ATCC 7966^T (DNA group 1)	27	25
A. salmonicida subsp. *salmonicida* (DNA group 3) strains		
NCIMB 1102^T	49	46
ATCC 14174	45	0
A. caviae ATCC 15468^T (DNA group 4)	29	ND[b]
A. media ATCC 33907^T (DNA group 5b)	6	50
A. eucrenophila NCIMB 74^T (DNA group 6)	7	ND
A. sobria CIP 74.33^T (DNA group 7)	48	3
A. veronii bv. sobria (DNA group 8/10) strain ATCC 9071	40	6
A. veronii bv. veronii (DNA group 8/10) strain ATCC 35624^T	19	34
A. jandaei ATCC 49568^T (DNA group 9)	0	2
Aeromonas sp. strain ATCC 35941 (DNA group 11)	12	37
A. schubertii ATCC 43700^T (DNA group 12)	0	0
A. trota ATCC 49657^T (DNA group 13)	0	0
Aeromonas sp. strain ATCC 43946 (enteric group 501)	3	16
A. ichthiosmia DSM 6393^T	9	20
A. enteropelogenes DSM 6394^T	3	7
A. allosaccharophila strains		
CECT 4199^T	0	6
290	17	0
ATCC 35942	1	24

[a] ATCC, American Type Culture Collection, Rockville, Md.; CECT, Colección Española de Cultivos Tipo, Valencia, Spain; CIP, Collection de l'Institut Pasteur, Paris, France; DSM, Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany; NCIMB, National Collection of Industrial and Marine Bacteria, Aberdeen, Scotland.
[b] ND, not determined.

(vol/vol) washed blood (43). Utilization of several carbon sources was examined by using the basal medium described by Lee et al. (25) supplemented with 1% (wt/vol) purified agar (Oxoid) and either a sugar at a concentration of 0.2% (wt/vol) or another substrate at a concentration of 0.1% (wt/vol). The carbon sources which we used were obtained from Sigma and included L-arabinose, salicin, D-cellobiose, sucrose, L-rhamnose, lactose, maltose, D-mannose, D-trehalose, D-galactose, D-raffinose, L-histidine, L-arginine, L-citrulline, L-leucine, L-alanine, glycine, L-proline, L-serine, L-glutamine, L-tyrosine, L-glutamate, L-aspartate, DL-3-hydroxybutyrate, propionate, γ-aminobutyrate, D-gluconate, D-glucuronate, L-malate, α-ketoglutarate, fumarate, succinate, ethanol, *myo*-inositol, D-mannitol, *myo*-erythritol, dulcitol, putrescine, and glycerol. Susceptibility to antibacterial compounds was determined on TSA supplemented with the following substances: amikacin (25 mg/liter), streptomycin (25 mg/liter), gentamicin (10 mg/liter), kanamycin (50 mg/liter), tobramycin (25 mg/liter), penicillin V (10 mg/liter), ampicillin (50 mg/liter), amoxicillin (50 mg/liter), carbenicillin (100 mg/liter), erythromycin (15 mg/liter), nitrofurantoin (10 mg/liter), oxolinic acid (10 mg/liter), nalidixic acid (50 mg/liter), polymyxin B (300 U/liter), rifampin (30 mg/liter), sulfanilamide (300 mg/liter), sulfadimethoxine (12 mg/liter), tetracycline (15 mg/liter), chloramphenicol (25 mg/liter), phosphomycin (50 mg/liter), novobiocin (5 mg/liter), and trimethoprim (25 mg/liter).

Pathogenicity. The virulence of strains S181^T, S176, and S191 for eels and mice was also examined. Juvenile European eels (5 to 12 g) and 6-week-old mice (19 to 31 g) were used in the infection trials. For each strain, six animals were injected intraperitoneally with 0.1 ml of a suspension containing from 10^{10} to 10^4 cells per ml in phosphate-buffered saline (PBS) (Oxoid), and then they were kept separately in a laboratory. Six animals injected with 0.1 ml of PBS were kept under the same conditions. Mortality was recorded daily for 7 days. The 50% lethal doses (LD_{50}) of bacterial cells were calculated as described previously (35). Strains that had an LD_{50} of $\geq 10^8$ cells per fish or mouse were considered avirulent, as described by Santos et al. (37) and Janda and Kokka (18).

TABLE 2. T_m values and DNA base compositions of *A. encheleia* strains and *E. coli* NCTC 9001[a]

Strain	T_m (°C)[b]	G+C content (mol%)
A. encheleia S181^T	79.0 ± 0.3	60.0
A. encheleia S176	78.8 ± 0.5	59.5
A. encheleia S177	78.7 ± 0.2	59.4
A. encheleia S191	79.4 ± 0.1	60.8
E. coli NCTC 9001	74.6 ± 0.05[c]	50.9[d]

[a] NCTC, National Collection of Type Cultures, Colindale, London, England.
[b] Mean ± standard deviation. The means are averages of the values from three or more separate determinations in 0.1× SSC buffer.
[c] The previously reported T_m value is 74.6°C (31).
[d] The previously reported G+C content is 50.9 mol% (31).

DNA-DNA hybridization. Previously described procedures were used to extract, purify, and shear unlabeled DNAs (19, 26). The competitive nitrocellulose filter method was used for DNA hybridization (19). Experiments were performed three times. DNAs from strains S181^T and S176 were nick translated with [^{3}H]dCTP (catalog no. RPN 1601Y; Amersham International, Amersham, United Kingdom) and reacted with unlabeled competitor DNAs from other *Aeromonas* strains at a reassociation temperature of 55.5 to 56.0°C. The hybridization mixtures contained 30% formamide (Sigma). The ratio of the concentration of competitor DNA to concentration of labeled DNA was at least 150:1. The percentages of homology were calculated as described previously (19).

DNA base composition. The G+C contents of the DNAs of strains S181^T, S176, S177, and S191 were determined from the midpoints (T_m) of the thermal denaturation profiles (11, 27, 30). The T_m of *Escherichia coli* NCTC 9001 DNA was determined experimentally and was used as a reference.

RESULTS AND DISCUSSION

The phenotypic properties common to strains S181^T, S176, S177, and S191 are given in the species description below.

Identification to the genus level. The four strains isolated from eels have the phenotypic characteristics of the genus *Aeromonas* (32). The cells are gram negative, motile, and rod shaped with rounded ends, and they occur singly, in pairs, or in short chains. The strains are facultative anaerobes, and most strains produce acid and gas from glucose under anaerobic conditions (the exception is strain S177, which does not produce gas from glucose). The strains are oxidase and catalase positive, reduce nitrate to nitrite, and are resistant to vibriostatic agent O/129. The strains use D-mannitol and exhibit gelatinase, DNase, and Tween 80 esterase activities. The strains do not produce acid from xylose, dulcitol, and *myo*-inositol. The strains do not produce hydrogen sulfide from thiosulfate and do not require sodium ions for growth. The G+C contents of the DNAs of strains S181^T, S176, S177, and S191 range from 59.4 to 60.8 mol% (Table 2). These values are within the range reported for the genus *Aeromonas* (57 to 59 mol% for *A. salmonicida* [32] and 58 to 63 mol% for the mesophilic *Aeromonas* species [2, 14, 32, 38–40]).

DNA relatedness. The levels of DNA relatedness of strains S181^T and S176 to three other strains isolated from eels, 13 *Aeromonas* species, the *Aeromonas* DNA group 11 reference strain, and *Aeromonas* sp. strain ATCC 43946 (enteric group 501) are shown in Table 1. The *Aeromonas* species and hybridization groups included in the study were 0 to 50% related to strains S181^T and S176. The four strains isolated from eels (S181^T, S176, S177, and S191) constituted a tight DNA genomic species (levels of DNA homology, ≥70%) distinct from previously described *Aeromonas* species.

Pathogenicity. Strains S181^T, S176, S177, and S191 were isolated in December 1987 from healthy eels. The previous and subsequent epizootic outbreaks at the eel farm occurred in August 1987 and March 1988, respectively. Infection trials in

TABLE 3. Characteristics useful for distinguishing *A. encheleia* from previously described mesophilic *Aeromonas* species

Species	Motility	Indole	Gas produced from glucose	Acid produced from:		Ornithine decarboxylase activity	Use of:				Growth at 42°C	Hydrolysis of:		
				Salicin	Cellobiose		Gluconate	Arginine	Glutamine	Arabinose		Elastin	Esculin	Arbutin
A. encheleia	+[a]	+	d	+	−	−	−	−	−	−	−	−	+	+
A. hydrophila[b]	+	+	+	d	d	−	+	+	d	+	d	+	+	+
A. caviae[b]	+	+	−	+	d	−	+	+	+	+	d	−	+	+
A. media[c]	−	+	−[d]	+	+	−	+	+	+	d	d	−	+	+
A. eucrenophila[e]	+	+[d]	+	+[d]	+[d]	−[d]	+[d]	+	+[d]	+[d]	−	+	+	−[d]
A. sobria[b]	+	+	+	−	d	−	+	−	−[d]	−	d	d	d	−
A. veronii bv. *veronii*[f]	+	+	+	+	d	+	+	+	+	−	+	−	+	d
A. veronii bv. *sobria*[g]	d	+	+	−	ND	−	ND	ND	ND	ND	+	ND	−	−
A. jandaei[h]	+	+	+	−	d	−	d	d	d	−	+	d	−	−
A. trota[i]	+	+	+	−	+	−	+[d]	+[d]	+[d]	−[d]	+	−	−	d
A. allosaccharophila[j]	+	+	+	−	+	d	+	+	d	+	+	−	d	−
A. enteropelogenes[k]	+	+	+	ND	ND	−	ND	ND	ND	ND	ND	ND	−	−
A. ichthiosmia[l]	+	+	+	ND	d	−	ND	ND	ND	ND	−	ND	−	−
A. schubertii[m]	+	−	−	−	−	−	+[d]	+[d]	ND	−[d]	+	+[d]	−	−

[a] +, 90% or more of the strains are positive; −, 90% or more of the strains are negative; d, 11 to 89% of the strains are positive; ND, no data available.
[b] Data from references 5, 8, 10, and 33.
[c] Data from references 2, 5, and 10.
[d] Results obtained with the type strain of the species.
[e] Data from references 10 and 38.
[f] Data from references 8, 10, and 14.
[g] Data from references 1 and 8.
[h] Data from references 7, 8, and 10.
[i] Data from references 6, 8, and 10.
[j] Data from reference 28.
[k] Data from reference 39.
[l] Data from reference 40.
[m] Data from references 8, 10, and 13.

which we injected bacterial cells intraperitoneally into healthy eels showed that strains S181^T, S177, and S191 were avirulent for eels (LD_{50}, 4.5×10^8 to 6.3×10^8 cells per fish), whereas strain S176 was weakly virulent for eels (LD_{50}, 1.7×10^7 cells per fish). The fact that strains S181^T, S176, S177, and S191 were not related to epizootic outbreaks and the fact that they were avirulent or weakly virulent for eels suggest that they are members of the saprophytic microbial community associated with European eels. On the other hand, all of these strains were avirulent for mice (LD_{50}, 1.0×10^8 to 1.8×10^8 cells per mouse).

Phenotypic differentiation of the new genomic species. The strains isolated from eels (S181^T, S176, S177, and S191) are readily distinguished from *A. salmonicida* by their motility and their ability to grow in broth at 37°C. The major characteristics that differentiate the new genomic species from other mesophilic *Aeromonas* species are shown in Table 3. The eel isolates differ from the previously described mesophilic *Aeromonas* species by their inability to use D-gluconate as a sole carbon and energy source. All of the esculin-positive *Aeromonas* species (*A. hydrophila*, *A. caviae*, *Aeromonas media*, *Aeromonas eucrenophila*, and *Aeromonas veronii* bv. veronii) are able to use L-arginine and L-glutamine, but the strains isolated from eels are not. In addition, *A. hydrophila* hydrolyzes elastin; *A. media* is nonmotile and produces acid from D-cellobiose; *A. eucrenophila* hydrolyzes elastin but not arbutin and produces acid from D-cellobiose; and *A. veronii* bv. veronii exhibits ornithine decarboxylase activity and grows in broth at 42°C. *A. veronii* bv. sobria, *Aeromonas jandaei*, *Aeromonas trota*, *Aeromonas enteropelogenes*, *Aeromonas ichthiosmia*, and *Aeromonas schubertii* differ from the eel isolates by failing to hydrolyze esculin and arbutin. In addition, *A. veronii* bv. sobria and *A. jandaei* grow at 42°C but do not produce acid from salicin; *A. trota* also produces acid from D-cellobiose; and *A. schubertii* is also elastase positive. *Aeromonas allosaccharophila* is easily distinguished from the eel isolates by the following characteristics: acid is produced from salicin and D-cellobiose, arbutin is hydrolyzed, L-arginine and L-arabinose are utilized, and growth occurs at 42°C. *A. sobria* differs from the eel isolates by its inability to hydrolyze arbutin and its inability to produce acid from salicin.

Since strains S181^T, S176, S177, and S191 constitute a genomic species that can be identified by phenotypic properties, they represent a new species (45), for which we propose the name *Aeromonas encheleia.*

Description of *Aeromonas encheleia* sp. nov. *Aeromonas encheleia* (en.che′le.ia. Gr. n. *enchelys*, eel; M. L. adj. *encheleia*, from eels). Gram-negative, straight, motile rods. Colonies develop within 24 h at 28°C on TSA (Oxoid) and are not pigmented. Old cultures (10 to 15 days), however, contain colonies with light brown pigmented centers. No brown water-soluble pigment is produced. Growth occurs on MacConkey agar but not on thiosulfate-citrate-bile salts-sucrose agar. Chemoorganotrophic, with both oxidative and fermentative metabolism. Acid is produced from glucose. Three of the four strains produce gas from glucose (Table 4). Oxidase and catalase positive. Reduces nitrate to nitrite. Resistant to vibriostatic agent O/129. Growth occurs in the presence of 0 to 3% (wt/vol) NaCl, at temperatures between 4 and 37°C, and under alkaline (pH 9.0) conditions. Arginine dihydrolase and indole positive. H_2S and lysine and ornithine decarboxylase negative. Three of the four strains are Voges-Proskauer negative (Table 4).

Acid is produced from salicin, maltose, D-mannose, D-trehalose, D-galactose, and D-mannitol, but not from L-arabinose, D-cellobiose, lactose, D-xylose, D-melibiose, D-raffinose, *myo*-erythritol, dulcitol, *myo*-inositol, or D-sorbitol. Three of the four strains produce acid from sucrose and L-rhamnose but not from glycerol (Table 4).

TABLE 4. Characteristics of *A. encheleia* which differ from strain to strain

Characteristic	No. of strains positive	Reaction of strain S181^T
Gas produced from glucose	3 (75)[a]	+[b]
Voges-Proskauer reaction	1 (25)	−
Acid produced from:		
Sucrose	3 (75)	+
L-Rhamnose	3 (75)	+
Glycerol	1 (25)	−
Utilization of[c]:		
D-Galactose	1 (25)	−
L-Histidine	3 (75)	+
L-Glutamate	1 (25)	−
γ-Aminobutyrate	1 (25)	+
Succinate	2 (50)	+
Glycerol	2 (50)	+
Hydrolysis of:		
Mucin	1 (25)	−
Fibrinogen	3 (75)	+
Starch	3 (75)	+

[a] The numbers in parentheses are percentages.
[b] +, positive; −, negative.
[c] Utilization as sole sources of carbon and energy.

The strains hydrolyze esculin, arbutin, gelatin, casein, collagen, chitin, Tween 80, egg yolk, and DNA, but not chondroitin sulfate, elastin, keratin, or urea. SDS-alkyl sulfatase negative. Three of the four strains hydrolyze fibrinogen and starch but not mucin (Table 4). Human erythrocytes are hemolyzed.

All *A. encheleia* strains use the following substrates as sole carbon and energy sources: sucrose, salicin, maltose, D-mannose, D-trehalose, L-proline, L-serine, L-malate, fumarate, and D-mannitol. Three of the four strains utilize L-hystidine (Table 4). None of the strains uses the following substrates as sole carbon and energy sources: L-arabinose, D-cellobiose, L-rhamnose, lactose, D-raffinose, L-arginine, L-citrulline, L-leucine, L-alanine, glycine, L-glutamine, L-tyrosine, citrate, L-aspartate, DL-3-hydroxybutyrate, propionate, D-gluconate, D-glucuronate, α-ketoglutarate, *myo*-inositol, *myo*-erythritol, dulcitol, ethanol, and putrescine. Three of the four strains do not utilize L-glutamate and D-galactose (Table 4).

Susceptible to amikacin, streptomycin, gentamicin, kanamycin, tobramycin, erythromycin, nitrofurantoin, nalidixic acid, oxolinic acid, polymyxin B, rifampin, tetracycline, and chloramphenicol. Resistant to penicillin V, ampicillin, amoxicillin, carbenicillin, sulfanilamide, sulfadimethoxine, phosphomycin, novobiocin, and trimethoprim. Additional characteristics which vary among strains are shown in Table 4.

The G+C content is 59.4 to 60.8 mol% as determined by the T_m method.

Isolated from healthy European eels (*Anguilla anguilla*) reared in a freshwater farm located in Valencia, Spain. Mostly avirulent for eels. Not pathogenic for mice, which are currently used to assess pathological significance for humans.

The type strain is strain S181.

Description of the type strain. Strain S181^T has all of the properties given above for the species. In addition, gas is produced from glucose, acid is produced from sucrose and L-rhamnose, and the organism utilizes L-histidine, γ-aminobutyrate, succinate, and glycerol and hydrolyzes starch and fibrinogen (Table 4). Also, the Voges-Proskauer reaction is negative, acid is not produced from glycerol, mucin is not hydrolyzed, and D-galactose and L-glutamate are not utilized (Table 4). The G+C content of the DNA is 60.0 mol%. This strain has been deposited in the Colección Española de Cultivos Tipo, Valencia, Spain, as strain CECT 4342.

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