Taxonomic study of sucrose-positive *Aeromonas jandaei*-like isolates from faeces, water and eels: emendation of *A. jandaei* Carnahan *et al.* 1992

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Fourteen sucrose-positive *Aeromonas jandaei*-like isolates from fresh water and reared European eels were subjected to a polyphasic study to determine their taxonomic position. Numerical taxonomy was used to analyse phenotypic data obtained for these isolates and 43 type and reference strains representative of recognized *Aeromonas* species. The *A. jandaei* cluster (phenon 1) was defined at 81.6 % similarity (S_j); this included the *A. jandaei*-like isolates, the sucrose-positive strain *Aeromonas veronii* biogroup sobria CECT 4910 and nearly all *A. jandaei* reference strains used in the study. Four other reference strains of *A. veronii* biogroup sobria and the type strain of *Aeromonas ichthiosmia* were peripheral to the *A. jandaei* cluster. The supra-group '*A. jandaei*–*A. veronii* biogroup sobria–*A. ichthiosmia*' was linked at 80.7 % similarity (S_j) and was clearly segregated from the phenotypic core of the *A. veronii* biogroup sobria species, which was related to the reference strain Popoff 224 (CECT 4835). DNA relatedness between strains grouped in the *A. jandaei* cluster (phenon 1) and *A. jandaei* CECT 4228^T ranged from 70 to 100 %, but was below 50 % when DNAs from *A. veronii* biogroup sobria CECT 4835, *A. veronii* biogroup veronii CECT 4257^T and *A. ichthiosmia* CECT 4486^T were used. In addition, DNA relatedness between peripheral *A. veronii* biogroup sobria strains and the species *A. jandaei* (CECT 4228^T), *A. veronii* (CECT 4257^T, CECT 4835) and *A. ichthiosmia* (CECT 4486^T) was always below 54 %, as it was between the species *A. ichthiosmia* (CECT 4486^T) and *A. veronii* (CECT 4257^T, CECT 4835). Emendation of *A. jandaei* is proposed; this taxon now includes sucrose-positive clinical and environmental strains as well as environmental isolates that are pathogenic for fish and humans. Other new traits for this species are the ability to grow at 4–42 °C, acid production from glycerol but not from lactose, D-melibiose or D-raffinose, the use of D-gluconate, L-glutamate or L-proline but not L-lactate, L-alanine, L-arabinose or L-arginine, hydrolytic activity against casein, elastin, starch and lecithin and the inability to lyse arbutin. The DNA G + C content of *A. jandaei* is also reported for the first time; it ranges from 58.1 to 61.1 mol%. On the other hand, the DNA relatedness data support the classification of peripheral reference strains of *A. veronii* biogroup sobria outside this taxon, indicating that biogroup sobria requires further revision.

INTRODUCTION

During the last 15 years, at least seven novel *Aeromonas* species have been isolated from clinical samples, including the taxa *Aeromonas veronii* and *Aeromonas jandaei* (Hickman-Brenner *et al.*, 1987, 1988; Schubert *et al.*, 1990a,

b; Carnahan *et al.*, 1991a, b; Martínez-Murcia *et al.*, 1992b). These two species show 99.5 % similarity in their complete 1502 bp 16S rDNA sequences (Martínez-Murcia *et al.*, 1992a), but they can clearly be segregated by DNA–DNA hybridization methods (Carnahan *et al.*, 1991a). In fact, the

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Abbreviation: DHG, DNA homology group.

Details of the strains used in this study, LD₅₀ values of *A. jandaei*-like strains and a dendrogram based on the S_{SM} /UPGMA analysis are available as supplementary material in IJSEM Online.

species *A. veronii* and *A. jandaei* are synonyms of DNA homology groups (DHG) 8/10 and 9, respectively, of the genus *Aeromonas* (Joseph & Carnahan, 2000).

The present taxonomic status of *A. veronii* is based mainly on genomic data (Hickman-Brenner *et al.*, 1987; Martínez-Murcia *et al.*, 1992a), since its biogroups *veronii* and *sobria* are not phenotypically related (Kämpfer & Altwegg, 1992; Valera & Esteve, 2002). In fact, *A. veronii* biogroup *sobria* displays closer phenotypic resemblance to *A. jandaei*, as phenotypic differentiation between these taxa is based only on acid production from sucrose, which is positive for *A. veronii* biogroup *sobria* but negative for *A. jandaei* (Carnahan *et al.*, 1991c; Abbot *et al.*, 1992). On the other hand, the limits of *A. veronii* biogroup *sobria* have recently been extended by the inclusion of the type strain of *Aeromonas ichthiosmia* (Huys *et al.*, 2001).

Although *A. jandaei* was formerly associated with clinical samples, the reported incidence of this taxon in human disease is extremely low (Janda *et al.*, 1994; Bravo *et al.*, 1995; Hänninen *et al.*, 1995; Kühn *et al.*, 1997; Borrell *et al.*, 1998; Ghenghesh *et al.*, 1999; Demarta *et al.*, 2000). By contrast, presumptive *A. jandaei* strains have frequently been isolated from cultured eels suffering from 'red fin disease' (Esteve *et al.*, 1993; Valera & Esteve, 2002). However, these environmental strains were somewhat related to *A. veronii* biogroup *sobria*, as they produced acid from sucrose. The aim of the present study was to determine the taxonomic position of these novel isolates in relation to reference strains representative of recognized *Aeromonas* species (*A. jandaei*, *A. veronii*, *Aeromonas sobria*, *A. ichthiosmia*, *Aeromonas enteropelogenes*, *Aeromonas trota*, *Aeromonas allosaccharophila* and *Aeromonas popoffii*).

METHODS

Aeromonas strains and culture conditions. The 14 sucrose-positive *A. jandaei*-like isolates recovered from fresh water and eels are detailed in Supplementary Table A (available in IJSEM Online). These environmental isolates were resistant to the following antimicrobials (mg l⁻¹ unless indicated): ampicillin (50), penicillin V (10), sulfanilamide (300), sulfadimethoxine (12) and trimethoprim (25); but they were sensitive to tetracycline (15), tobramycin (25), streptomycin (25), gentamicin (10), rifampin (30), amikacin (25), kanamycin (50), erythromycin (15), chloramphenicol (25), nitrofurantoin (10), nalidixic acid (50), oxolinic acid (10) and polymyxin B (300 UI l⁻¹) (Esteve, 1995). These strains, which were mainly associated with epizootic outbreaks that occurred in eel farms from 1987 to 1998, were stored long-term as freeze-dried cultures. The 43 reference strains used in the study are detailed in Supplementary Table B (available in IJSEM Online). Strains were grown overnight on tryptone water [1% (w/v) tryptone, 0.5% (w/v) NaCl in distilled water; pH 7.2] or tryptone soy agar (Oxoid) at 28 °C.

Phenotypic characterization and numerical taxonomy. Each strain (57 strains and six duplicate cultures) was examined for 121 physiological and biochemical traits, as described previously (Valera & Esteve, 2002). Production of β -haemolysins against human erythrocytes was tested on solid medium according to Esteve (1995). The tests results were scored as 1 for positive responses and 0 for negative responses; these data were examined with the NTSYS-pc

numerical taxonomy and multivariate analysis system (Rohlf, 1998). Similarities were calculated using both simple matching (S_{SM}) and Jaccard (S_J) coefficients; clustering was achieved by the UPGMA method and cophenetic correlation was calculated using the correlation coefficient r (Sneath & Sokal, 1973). Test error was calculated according to Sneath & Johnson (1972).

DNA base composition. Previously described procedures (Marmur, 1961; Johnson, 1994) were used to extract and purify DNAs from 29 *Aeromonas* strains (Table 1). The DNA G + C content of the strains was obtained from the midpoints (T_m) of the thermal denaturation profiles (Marmur & Doty, 1962; Ferragut & Leclerc, 1976; Owen & Hill, 1979). The T_m of DNA from *Escherichia coli* NCTC 9001^T was determined experimentally and was used as a reference.

DNA-DNA hybridization. We have used the competitive nitrocellulose filter method, which is a classical method to test DNA relatedness among bacterial strains (Johnson, 1994). DNAs from reference strains (CECT 4228^T, CECT 4486^T, CECT 4257^T and CECT 4835) were denatured and then bound to a nitrocellulose filter or labelled with [³H]dCTP (Amersham). Each hybridization mixture included the following: (i) filter containing unlabelled reference single-stranded (ss) DNA, (ii) labelled homologous ssDNA, (iii) unlabelled competitor ssDNA and (iv) 30% formamide (Sigma). The ratio of the concentrations of labelled homologous ssDNA and unlabelled competitor ssDNA was equal to or less than 1:150. DNA-DNA hybridizations were made at a reassociation temperature of 55.5–56.0 °C, depending on the values of DNA T_m . All hybridization experiments were performed in triplicate. In some cases, the results showed in Table 1 are values of DNA relatedness obtained from two different hybridization experiments. Percentage relatedness was calculated as described previously (Johnson, 1994). In addition, the SD was calculated for each hybridization experiment according to Sokal & Rohlf (1969).

Pathogenicity. Animal models were used to assess potential pathogenicity of *A. jandaei* DHG9 strains. Juvenile European eels (5–12 g) and 6-week-old mice (19–31 g) were used in the infection trials. For each strain, six animals were anaesthetized and challenged with the same bacterial dose (10⁸, 10⁷, 10⁶, 10⁵, 10⁴ or 10³ cells). Six animals injected with sterile PBS were used as a negative control. Mortalities in a 7 day period were only considered if the injected bacterium was reisolated as a pure culture from freshly dead animals. The LD₅₀ of bacterial cells was calculated for each strain (Reed & Muench, 1938).

RESULTS AND DISCUSSION

Phenotypic characterization and numerical taxonomy

We have used results obtained from duplicate cultures to calculate the test error, which is a methodological parameter. Thus, the mean probability (P) of an erroneous result for the biochemical tests was 0.9% ($S_n^2 = 0.0097$), which is an acceptable value according to Sneath & Johnson (1972). Consistent results were obtained from the phenotypic study, as nearly the same dendrogram was derived by the use of the two similarity coefficients (S_{SM} and S_J) and UPGMA clustering algorithm. In this study, S_J /UPGMA analysis was chosen because the correlation observed between the similarity matrix and the corresponding tree matrix ($r = 0.90$) was higher than that presented for S_{SM} /UPGMA analysis ($r = 0.86$). Seventy-seven per cent of strains were grouped by the S_J /UPGMA analysis into nine

Table 1. DNA base composition of *A. jandaei* strains and other *Aeromonas* strains and DNA–DNA relatedness among isolates

DNA–DNA relatedness percentages were obtained from hybridization experiments performed in triplicate. ND, Not determined.

Source of unlabelled DNA	Phenon	G + C content (mol%)	³ H-labelled DNA from:			
			<i>A. jandaei</i> CECT 4228 ^T	<i>A. ichthiosmia</i> CECT 4486 ^T	<i>A. veronii</i> CECT 4257 ^T	<i>A. veronii</i> CECT 4835
<i>A. jandaei</i> CECT 4228 ^{T*}	1	58.8	100	28	18	23
<i>A. jandaei</i> CECT 4813*	1	59.4	100	31	29	41
<i>A. jandaei</i> CECT 4229*	1	58.8	94	10	33	15
<i>A. jandaei</i> CECT 4838*	1	60.4	85	13	24	15
<i>A. jandaei</i> CECT 4901*	1	61.1	80	25	42	30
<i>A. jandaei</i> CECT 4231*	1	59.6	71	19	20	8
<i>A. jandaei</i> -like E30	1	60.9	85†	20	12	46
<i>A. jandaei</i> -like TW69	1	58.3	85	45	32	18
<i>A. jandaei</i> -like SA253	1	60.0	85	21	32	21
<i>A. jandaei</i> -like AO60	1	60.0	83	50	16	17
<i>A. jandaei</i> -like R400	1	ND	82	11	37	25
<i>A. jandaei</i> -like TW155	1	60.0	80	27	25	50
<i>A. jandaei</i> -like E44	1	58.3	79	48	31	28
<i>A. jandaei</i> -like SO337	1	58.1	75	12	3	40
<i>A. jandaei</i> CECT 4815	1	59.2	74	35	18	42
<i>A. jandaei</i> -like TW9	1	58.3	72	30	23	29
<i>A. veronii</i> CECT 4910	1	61.1	70†	10	12	17
<i>A. jandaei</i> -like S345	1	59.2	70†	27	31	25
<i>A. jandaei</i> -like A26	1	60.7	70	5	32	40
<i>A. jandaei</i> -like E273	1	58.1	63	ND	11	43
<i>A. jandaei</i> -like AO62	1	59.6	62	9	19	33
<i>A. jandaei</i> -like M6	1	61.0	59	26	56	32
<i>A. veronii</i> CECT 4906	2	61.1	44	23	30	39
<i>A. veronii</i> CECT 4911	2	61.9	31	12	15	25
<i>A. veronii</i> CECT 4902	3	60.0	25	6	50	26
<i>A. veronii</i> CECT 4903	3	60.7	ND	18	41	51
<i>A. ichthiosmia</i> CECT 4486 ^T	–	60.3	40	100	43	52
<i>A. veronii</i> CECT 4257 ^T	4	59.4	45	45	100	83
<i>A. veronii</i> CECT 4835	6	60.3	34	45	ND	100

*Strain included in the original description of *A. jandaei* (Carnahan *et al.*, 1991a).

†Reciprocal hybridizations yielded values of DNA relatedness in the range 75–87 %.

phenons defined at or above 81.6 % similarity (Fig. 1). The $S_{SM}/UPGMA$ analysis yielded almost identical grouping at similarity values of ≥ 90 %, although the percentage of total clustered strains was slightly higher (81 %). A dendrogram based on the $S_{SM}/UPGMA$ analysis is shown in Supplementary Fig. A, available in IJSEM Online.

Phenon 1, which is formed at 81.6 % similarity (S_j), was identified as *A. jandaei*. This cluster included the strain *A. veronii* biogroup sobria CECT 4910, the 14 *A. jandaei*-like isolates and all reference strains of the species *A. jandaei* with the exception of CECT 4814, which was clustered in the '*A. trota* cluster' (phenon 9). The phenotypic characteristics of the '*A. jandaei* cluster' (phenon 1) showed discrepancies from the description of this species (Carnahan *et al.*, 1991a) regarding the high percentages of sucrose- and elastase-positive strains observed (Table 2). In

contrast to data reported previously (Hasan *et al.*, 1992), all *A. jandaei* strains displayed elastolytic activity on the medium of Hsu *et al.* (1981). In our opinion, this medium might enhance the elastolytic capability of strains as it contains large amounts of ground elastin (1 %, w/v) but very small amounts of tryptone (0.2 %, w/v) and it also contains micronutrients (L-cysteine and Ca^{2+} ions) that stabilize elastase structure and function. Moreover, the pH of the medium is buffered to 8.0, which is optimal for most reported elastases (Wretling & Wadström, 1977; Kothary & Kreger, 1985).

Four reference strains of *A. veronii* biogroup sobria (29 % of the total strains of this taxon), which were clustered in phenons 2 and 3, and the type strain of *A. ichthiosmia* were close to the '*A. jandaei* cluster' (Fig. 1). This supra-group, '*A. jandaei*–*A. veronii* biogroup sobria–*A. ichthiosmia*', was

Fig. 1. Simplified dendrogram based on the S_J coefficient and UPGMA clustering algorithm, showing the relationship between the nine phenons defined at or above 81.6% similarity. bg., Biogroup. A dendrogram based on the S_{SM} /UPGMA analysis is available in IJSEM Online.

linked at 80.7% similarity (S_J) and was clearly segregated from the other *Aeromonas* species (Fig. 1). In fact, we have observed that the phenotypic core of the species *A. veronii* biogroup *sobria* corresponded to phenons 5, 6 and 7 (Fig. 1). These clusters grouped 36% of the total *A. veronii* biogroup *sobria* strains, among them strain Popoff 224 (CECT 4835), which is a reference strain for the genospecies DHG8 (Joseph & Carnahan, 2000). Our results are in agreement with the previous numerical study by Kämpfer & Altwegg (1992), in which these reference strains of *A. veronii* biogroup *sobria* were grouped in two well-segregated clusters. Thus, diversity inside the taxon *A. veronii* biogroup *sobria* could be much higher than is currently recognized (Abbot *et al.*, 1992; Martinetti Lucchini & Altwegg, 1992; Huys *et al.*, 2001). In view of these overall results, for the genomic study, we used all strains that clustered in the supra-group '*A. jandaei*–*A. veronii* biogroup *sobria*–*A. ichthiosmia*' (Fig. 1) as well as the reference strains

of *A. veronii* (biogroup *veronii* CECT 4257^T; biogroup *sobria* CECT 4835).

As expected, the two biogroups of *A. veronii* were not related phenotypically, as all reference strains of *A. veronii* biogroup *veronii* were clustered in phenon 4 (Fig. 1). Moreover, our numerical analysis clearly segregated the species *A. allosaccharophila* (phenon 8) from the *A. veronii* taxon, in contrast to other studies (Huys *et al.*, 1996, 2001). Finally, we should note that all reference strains of the species *A. trota* were clustered in phenon 9, along with strain CECT 4814 (Fig. 1), which had been received as a reference strain for *A. jandaei*.

Genomic study: DNA base composition and DNA–DNA relatedness

The DNA G+C contents of strains included in the '*A. jandaei* cluster' (phenon 1) ranged from 58.1 to 61.1 mol%

Table 2. Variable characteristics of *A. jandaei* and their distribution depending on strain origin

Clinical strains tested were CECT 4228^T, CECT 4229, CECT 4813, CECT 4815, CECT 4838, CECT 4901 and CECT 4910. Environmental strains tested were CECT 4231, TW9, A26, E30, E44, AO60, TW69, TW155, SA253, SO337, S345 and R400. LDC, Lysine decarboxylase (Moeller's medium).

Characteristic	Phenotype*		Positive strains (%)	Reaction of strain CECT 4228 ^T
	Clinical	Environmental		
LDC	9/10†	10/12	86	+
Growth at pH 4.5	2/7	4/12	32	—
Acid production from:				
Sucrose	1/10†	11/12	55‡	—
D-Cellobiose	0/10†	3/12	14	—
Maltose	6/7	11/12	90	+
Hydrolysis of:				
SDS	3/7	9/12	63	+
Elastin	7/10†	10/12	77‡	+‡
Tween 80	5/7	5/12	53	+
Utilization of:				
Acetate	6/7	9/12	79	+
DL-Glycerate	1/7	3/12	21	—
Citrate	9/10†	10/12	86	+
L-Histidine	2/7	6/12	42	—
L-Serine	7/7	10/12	90	+
D-Glucosamine	5/7	12/12	90	—
Virulence for:§				
Eels	0/2	4/8	40	—
Mice	4/5	4/8	62	+

*No. positive/no. tested.

†Results reported by Carnahan *et al.* (1991a) for strains AS-206, AS-235 and AS-176 are also included.

‡Reported elsewhere as negative (Carnahan *et al.*, 1991a; Hasan *et al.*, 1992).

§Strains that exhibited an LD₅₀ of less than 10^{7.5} c.f.u.

||Reported by Janda & Kokka (1991) for clinical *A. jandaei* strains.

(Table 1). This range of three units is similar to that described among bacterial strains belonging to a single *Aeromonas* species (Popoff *et al.*, 1981; Hickman-Brenner *et al.*, 1987; Esteve *et al.*, 1995b). The DNA G + C contents of strains clustered in phenons 2 and 3 were respectively within the ranges 61.1–61.9 mol% and 60.0–60.7 mol% (Table 1). It is noteworthy that the DNA G + C contents of all these strains are being reported for the first time, even those of strains included in the original description of the species *A. jandaei* (Carnahan *et al.*, 1991a). As expected, the overall values obtained for DNA G + C content (Table 1) were within the range reported for the genus *Aeromonas* (57.0–63 mol%) (Popoff *et al.*, 1981; Popoff, 1984; Allen *et al.*, 1983; Hickman-Brenner *et al.*, 1987; Schubert & Hegazi, 1988; Schubert *et al.*, 1990a, b; Esteve *et al.*, 1995a, b; Huys *et al.*, 1997).

Except for isolates E273, AO62 and M6, DNA relatedness among strains grouped in the '*A. jandaei* cluster' (phenon 1) and the type strain, *A. jandaei* CECT 4228^T, ranged from 70 to 100 % (Table 1), values currently accepted for the

definition of a bacterial species (Stackebrandt & Goebel, 1994). In addition, reciprocal DNA–DNA hybridizations performed between some strains clustered in phenon 1 (E30, CECT 4910 and S345) and the type strain, CECT 4228^T, yielded values in the range 75–87 %. Thus, these strains, which include many sucrose-positive *A. jandaei*-like isolates and the clinical strain CECT 4910, should be classified as members of genospecies 9 (DHG9), and they therefore belong to the *A. jandaei* taxon. As expected, DNA relatedness between *A. jandaei* DHG9 strains and representative strains of *A. ichthiosmia* (CECT 4486^T) and *A. veronii* (CECT 4257^T, CECT 4835) was well under 70 % (Table 1). Thus, DNA–DNA hybridizations clearly segregated *A. jandaei* DHG9 from the taxa *A. ichthiosmia* and *A. veronii*, although they are close relatives on the basis of 16S rDNA sequence comparison (Martínez-Murcia *et al.*, 1992a; Collins *et al.*, 1993) and phenotypic features (Fig. 1).

Reciprocal DNA–DNA hybridizations performed between *A. ichthiosmia* CECT 4486^T and reference strains of *A. veronii* (biogroup *veronii* CECT 4257^T; biogroup *sobria*

CECT 4835) yielded values of around 45 % DNA relatedness (Table 1). In fact, our data agree with the value of 60 % DNA relatedness originally reported for the pair *A. ichthiosmia* CECT 4486^T/*A. veronii* CECT 4835 (Schubert *et al.*, 1990b) by using the spectrophotometric method (De Ley *et al.*, 1970). In contrast, they contradict the value of 96 % DNA relatedness recently reported for the pair *A. ichthiosmia* CECT 4486^T/*A. veronii* CECT 4257^T (Huys *et al.*, 2001) by using the micro-well hybridization method of Goris *et al.* (1998). It is worth noting that Huys *et al.* (2001) used these DNA relatedness data to propose that *A. ichthiosmia* is a later synonym of *A. veronii*. However, in our opinion, this proposal should be reconsidered on the basis of the striking lack of concordance between these hybridization studies (Huys *et al.*, 2001; Schubert *et al.*, 1990b; Table 1).

On the other hand, the peripheral *A. veronii* biogroup sobria strains (phenons 2 and 3) were related to the species *A. jandaei*, *A. ichthiosmia* and *A. veronii* at levels of DNA relatedness below 50 % (Table 1), and they therefore do not belong to any of these taxa. It is worth noting that DNA relatedness between strains CECT 4902, CECT 4903, CECT 4906 and CECT 4911 and *A. veronii* (CECT 4257^T, CECT 4835) is reported here for the first time (Table 1), even though these four strains have been widely used as reference strains for *A. veronii* biogroup sobria DHG8 (Huys *et al.*, 1996, 2001). However, according to our results, these four strains should not be assigned to the species *A. veronii*, indicating that the biogroup sobria is a heterogeneous taxon that requires further revision. In fact, strain CECT 4911 has recently been classified as *A. allosaccharophila* on the basis of *gyrB* gene sequencing data (Yáñez *et al.*, 2003).

The genomic relationship between the reference strains of *A. veronii* biogroup sobria DHG8 (CECT 4835) and *A. veronii* biogroup veronii DHG10 (CECT 4257^T) was closer (Table 1) than the current limit for species definition (70 %) and our data are therefore in agreement with previous reports that classified them in the *A. veronii* taxon (Hickman-Brenner *et al.*, 1987; Martínez-Murcia *et al.*, 1992a; Yáñez *et al.*, 2003).

The overall SD of DNA relatedness determined in our competitive hybridization study, at around 6 %, is comparable to other studies based on the micro-well (Goris *et al.*, 1998; Christensen *et al.*, 2000) and spectrophotometric methods (Angen *et al.*, 1999). Moreover, we have observed that the mean SD of high DNA similarity data (≥ 70 %) was around 2 %, this value being significantly below the 7 % recorded as the mean SD for low DNA similarity data (< 70 %).

In summary, the present phenotypic and genotypic data support the classification of the *A. jandaei*-like strains, as well as strain CECT 4910, previously assigned to *A. veronii* biogroup sobria, in the species *A. jandaei*. Thus, we propose the emendation of the species *A. jandaei* to include these novel clinical and environmental isolates.

Phenotypic differentiation of the species *A. jandaei*

A. jandaei can be differentiated easily from the species *A. veronii* biogroup veronii, *Aeromonas hydrophila*, *Aeromonas bestiarum*, *Aeromonas eucrenophila* and *Aeromonas encheleia* by the absence of production of acid from salicin and its inability to hydrolyse aesculin and arbutin. In addition, *A. jandaei* can be segregated from *Aeromonas caviae* and *Aeromonas media* by its production of gas from glucose and its positive response to Voges–Proskauer and gluconate oxidation tests. *A. jandaei* and *Aeromonas schubertii* can be separated on the basis of the negative responses displayed by the latter for the production of indole, acid from D-mannitol and gas from glucose. The key reactions that differentiate *A. jandaei* from phenotypically related species and reference strains of mesophilic *Aeromonas* species are detailed in Table 3. It is worth noting that *Aeromonas culicicola* and *A. jandaei* cannot be differentiated on the basis of the phenotypic profile reported recently by Pidiyar *et al.* (2002).

Prevalence and pathogenicity of *A. jandaei*

The fact that the species *A. jandaei* includes sucrose-positive strains may be highly significant in establishing the prevalence of this taxon in clinical samples, as similar sucrose-positive *Aeromonas* isolates from human sources have until now been largely identified as *A. veronii* biogroup sobria (Carnahan *et al.*, 1991c; Abbott *et al.*, 1992). In fact, the present study has demonstrated clearly that one of these previously identified *A. veronii* biogroup sobria isolates, CECT 4910, is a bona fide *A. jandaei* strain. Thus, such misidentifications could account for the scanty reports on *A. jandaei* in relation to human infectious diseases (Janda & Abbott, 1998). Moreover, the prevalence of *A. jandaei* in aquatic environments (fresh water and fish) is reported for the first time, as only one environmental strain, CECT 4231, has so far been isolated, from a prawn (Carnahan *et al.*, 1991a). It is worth noting that environmental *A. jandaei* strains present LD₅₀ values for mice similar to those reported for clinical *A. jandaei* strains (Supplementary Table A), and they can therefore be classified into the ‘high’ (LD₅₀ $< 10^{7.0}$ c.f.u.) or ‘moderate’ (LD₅₀ $10^{7.0}–10^{7.5}$ c.f.u.) virulence categories described previously by Janda & Kokka (1991). Furthermore, nearly all *A. jandaei* strains have been able to degrade elastin, elastolytic activity currently being related to pathogenic significance in *Aeromonas* (Hsu *et al.*, 1981). Thus, the clinical significance of the species *A. jandaei* and its importance in public health issues need to be reviewed in the light of the present findings.

Emended description of *Aeromonas jandaei* Carnahan *et al.* 1992

Gram-negative, straight rods that are motile by a single polar flagellum. Colonies develop within 24 h at 28 °C on TSA (Oxoid) and are not pigmented. No brown water-soluble pigment is produced. Growth occurs on MacConkey agar

Table 3. Key tests for phenotypic differentiation of *A. jandaei*

Species: 1, *A. jandaei* (strains TW9, A26, E30, E44, AO60, TW69, TW155, SA253, SO337, S345, R400 and CECT strains 4228^T, 4229, 4231, 4813, 4815, 4838, 4901 and 4910); 2, *Aeromonas* sp. (CECT strains 4902, 4903, 4906 and 4911); 3, *A. ichthiosmia*; 4, *A. veronii* biogroup *veronii*; 5, *A. veronii* biogroup *sobria* (CECT strains 4251, 4835, 4907–4909 and 4912); 6, *A. allosaccharophila*; 7, *A. trota*; 8, *A. enteropelogenes*; 9, *A. popoffii*; 10, *A. sobria*. Data were taken from this study; Valera & Esteve (2002) and Kämpfer & Altwegg (1992). Percentages of positive strains are indicated as: +, 100–86%; v+, 85–80%; –, 14–0%; numbers indicate other percentages.

Characteristic	1	2	3	4	5	6	7	8	9	10
Acid production from:										
Salicin	–	–	–	+	–	–	–	–	–	–
L-Arabinose	–	25	–	–	33	67	–	–	–	–
D-Cellobiose	–*	+	–	80	67	+	+	+	–	+
Voges–Proskauer	+	+	+	+	–†	–	–	–	+	+
LDC	+	+	–	+	33	+	+	+	–	+
Growth at 42 °C	+	–	+	†	80	–	+	60	+	–
Hydrolysis of:										
Arbutin	–	–	–	+	–	–	–	–	–	–
SDS	63	50	–	+	–	–	+	+	+	+
Use of:										
L-Lactate	–	–	–	–	–	–	+	–	–†	–
Acetate	v+	50	+	+	–	+	+	+	–	–
Citrate	+	+	–	80	50	67	+	+	+	+
L-Glutamate	+	+	+	+	+	+	+	+	+	–
D-Gluconate	+	+	+	+	+	+	+	+	+	–
L-Alanine	–	–	–	40	–	–	+	+	–†	–
L-Arginine	–	–	–	–	–	+	v+	–	–	–
L-Proline	+	+	+	+	+	+	+	+	–	–
Glycine	–	–	–	–	–	–	+	–	–	–
L-Arabinose	–	–	–	–	–	+	–	–	–	–

*Results reported by Carnahan *et al.* (1991a) for strains AS-206, AS-235 and AS-176 are also included.

†Different response reported elsewhere (Schubert *et al.*, 1990b; Huys *et al.*, 1997, 2001).

and on TCBS agar (Oxoid). Chemo-organotrophic, with both oxidative and fermentative metabolism. Acid and gas are produced from glucose. Kovacs' cytochrome oxidase- and catalase-positive. Reduces nitrate to nitrite. Does not swarm on solid media (1.5% agar, w/v) and is resistant to vibriostatic agent O/129 (150 µg). Growth occurs in the presence of 0–3% (w/v) NaCl, at temperatures between 4 and 42 °C and under alkaline (pH 8.5) conditions. All strains produce H₂S from L-cysteine. Positive for indole, Moeller's arginine dihydrolase, Thornley's arginine, gluconate oxidation and Voges–Proskauer test but negative for Moeller's ornithine and glutamine decarboxylases. All strains produce acid from fructose, D-galactose, glycerol, D-mannose, D-mannitol, D-ribose and D-trehalose. None of the strains produces acid from adonitol, D-amylgdalin,

D-arabinose, L-arabinose, arbutin, dulcitol, 2-deoxy-D-glucose, *myo*-inositol, lactose, D-melibiose, D-raffinose, D-rhamnose, salicin, D-sorbitol, L-sorbose, xylitol or xylose. All strains use the following substrates as sole carbon and energy sources: *N*-acetylglucosamine, fumarate, D-gluconate, L-glutamate, L-glutamine, malate (alkalinization), L-proline and succinate. None of the strains uses *trans*-aconitate, adenine, L-alanine, γ -aminobutyrate, L-arabinose, L-arginine, L-asparagine, L-aspartate, benzoate, L-carnosine, L-citrulline, L-cysteine, D-galacturonate, D-glucuronate, glutarate, glutathione, glycine, guanine, L-isoleucine, L-lactate, L-leucine, L-lysine, maleate (alkalinization), malonate (alkalinization), L-methionine, mucate, L-ornithine, L-phenylalanine, propionate, putrescine, sarcosine, L-tartrate, L-threonine, L-tryptophan, L-tyrosine, L-valine or xanthine. Strains hydrolyse casein and gelatin but not alginate, arbutin or aesculin. All strains display amylase and lecithinase activities and excrete β -haemolysins against human red blood cells. All strains are resistant to ampicillin and penicillin but sensitive to amikacin, gentamicin, tetracycline and tobramycin (Carnahan *et al.*, 1991a; Esteve 1995). Additional characteristics that vary among strains are shown in Table 2. The DNA G + C content ranges from 58.1 to 61.1 mol%, as determined by the *T_m* method.

Isolated from clinical samples, fresh water, prawns and eels (*Anguilla anguilla*). Mostly virulent for eels and mice; the latter animal model is currently used to assess pathogenic significance for humans.

The type strain is ATCC 49568^T (= CECT 4228^T); it presents all of the properties given above for the species. In addition, it is positive for Moeller's lysine decarboxylase, produces acid from maltose, hydrolyses elastin, SDS and Tween 80 and uses acetate, citrate and L-serine (Table 2). However, it is negative for acid production from sucrose and D-cellobiose, does not grow at pH 4.5 and does not use DL-glycerate, L-histidine or D-glucosamine. Isolated from faeces. The DNA G + C content of the type strain is 58.8 mol%.

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REFERENCES

- Abbot, S. L., Cheung, W. K. W., Kroske-Bystrom, S., Malekzadeh, T. & Janda, J. M. (1992). Identification of *Aeromonas* strains to the genospecies level in the clinical laboratory. *J Clin Microbiol* **30**, 1262–1266.
- Allen, D. A., Austin, B. & Colwell, R. R. (1983). *Aeromonas media*, a new species isolated from river water. *Int J Syst Bacteriol* **33**, 599–604.
- Angen, Ø., Møtters, R., Caugant, D. A., Olsen, J. E. & Bisgaard, M. (1999). Taxonomic relationships of the [*Pasteurella*] *haemolytica* complex as evaluated by DNA–DNA hybridizations and 16S rRNA sequencing with proposal of *Mannheimia haemolytica* gen. nov., comb. nov., *Mannheimia granulomatis* comb. nov., *Mannheimia*

- glucosida* sp. nov., *Mannheimia ruminalis* sp. nov. and *Mannheimia varigena* sp. nov. *Int J Syst Bacteriol* **49**, 67–86.
- Borrell, N., Figueras, M. J. & Guarro, J. (1998). Phenotypic identification of *Aeromonas* genomospecies from clinical and environmental sources. *Can J Microbiol* **44**, 103–108.
- Bravo, L., Monte, R. J., Alfonso, V., Cabrera, N., Gómez, M., Hernández, R. & García, B. (1995). New species of *Aeromonas* isolated in Cuba. *Rev Cubana Med Trop* **47**, 215–216 (in Spanish).
- Carnahan, A., Fanning, G. R. & Joseph, S. W. (1991a). *Aeromonas jandaei* (formerly genospecies DNA group 9 *A. sobria*), a new sucrose-negative species isolated from clinical specimens. *J Clin Microbiol* **29**, 560–564.
- Carnahan, A. M., Chakraborty, T., Fanning, G. R., Verma, D., Ali, A., Janda, J. M. & Joseph, S. W. (1991b). *Aeromonas trota* sp. nov., an ampicillin-susceptible species isolated from clinical specimens. *J Clin Microbiol* **29**, 1206–1210.
- Carnahan, A. M., Behram, S. & Joseph, S. W. (1991c). Aerokey II: a flexible key for identifying clinical *Aeromonas* species. *J Clin Microbiol* **29**, 2843–2849.
- Christensen, H., Angen, Ø., Mutters, R., Olsen, J. E. & Bisgaard, M. (2000). DNA–DNA hybridization determined in micro-wells using covalent attachment of DNA. *Int J Syst Evol Microbiol* **50**, 1095–1102.
- Collins, M. D., Martinez-Murcia, A. J. & Cai, J. (1993). *Aeromonas enteropelogenes* and *Aeromonas ichthiosmia* are identical to *Aeromonas trota* and *Aeromonas veronii*, respectively, as revealed by small-subunit rRNA sequence analysis. *Int J Syst Bacteriol* **43**, 855–856.
- De Ley, J., Cattoir, H. & Reynaerts, A. (1970). The quantitative measurement of DNA hybridization from renaturation rates. *Eur J Biochem* **12**, 133–142.
- Demarta, A., Tonolla, M., Caminada, A., Beretta, M. & Peduzzi, R. (2000). Epidemiological relationships between *Aeromonas* strains isolated from symptomatic children and household environments as determined by ribotyping. *Eur J Epidemiol* **16**, 447–453.
- Esteve, C. (1995). Numerical taxonomy of *Aeromonadaceae* and *Vibrionaceae* associated with reared fish and surrounding fresh and brackish water. *Syst Appl Microbiol* **18**, 391–402.
- Esteve, C., Biosca, E. G. & Amaro, C. (1993). Virulence of *Aeromonas hydrophila* and some other bacteria isolated from European eels *Anguilla anguilla* reared in freshwater. *Dis Aquat Org* **16**, 15–20.
- Esteve, C., Gutiérrez, M. C. & Ventosa, A. (1995a). DNA relatedness among *Aeromonas allosaccharophila* strains and DNA hybridization groups of the genus *Aeromonas*. *Int J Syst Bacteriol* **45**, 390–391.
- Esteve, C., Gutiérrez, M. C. & Ventosa, A. (1995b). *Aeromonas encheleia* sp. nov., isolated from European eels. *Int J Syst Bacteriol* **45**, 462–466.
- Ferragut, C. & Leclerc, H. (1976). Étude comparative des méthodes de détermination du T_m de l'ADN bactérien. *Ann Microbiol* **127**, 223–235.
- Ghenghesh, K. S., Bara, F., Bukris, B., el-Surmani, A. & Abeid, S. S. (1999). Characterization of virulence factors of *Aeromonas* isolated from children with and without diarrhoea in Tripoli, Libya. *J Diarrhoeal Dis Res* **17**, 75–80.
- Goris, J., Suzuki, K., De Vos, P., Nakase, T. & Kersters, K. (1998). Evaluation of a microplate DNA–DNA hybridization method compared with the initial renaturation method. *Can J Microbiol* **44**, 1148–1153.
- Hänninen, M.-L., Salmi, S., Mattila, L., Taipainen, R. & Siitonen, A. (1995). Association of *Aeromonas* spp. with travellers' diarrhoea in Finland. *J Med Microbiol* **42**, 26–31.
- Hasan, J. A. K., Macaluso, P., Carnahan, A. M. & Joseph, S. W. (1992). Elastolytic activity among *Aeromonas* spp. using a modified bilayer plate assay. *Diagn Microbiol Infect Dis* **15**, 201–206.
- Hickman-Brenner, F. W., MacDonald, K. L., Steigerwalt, A. G., Fanning, G. R., Brenner, D. J. & Farmer, J. J., III (1987). *Aeromonas veronii*, a new ornithine decarboxylase-positive species that may cause diarrhea. *J Clin Microbiol* **25**, 900–906.
- Hickman-Brenner, F. W., Fanning, G. R., Arduino, M. J., Brenner, D. J. & Farmer, J. J., III (1988). *Aeromonas schubertii*, a new mannitol-negative species found in human clinical specimens. *J Clin Microbiol* **26**, 1561–1564.
- Hsu, T. C., Waltman, W. D. & Shotts, E. B. (1981). Correlation of extracellular enzymatic activity and biochemical characteristics with regard to virulence of *Aeromonas hydrophila*. *Dev Biol Stand* **49**, 101–111.
- Huys, G., Coopman, R., Janssen, P. & Kersters, K. (1996). High-resolution genotypic analysis of the genus *Aeromonas* by AFLP fingerprinting. *Int J Syst Bacteriol* **46**, 572–580.
- Huys, G., Kämpfer, P., Altwegg, M. & 7 other authors (1997). *Aeromonas popoffii* sp. nov., a mesophilic bacterium isolated from drinking water production plants and reservoirs. *Int J Syst Bacteriol* **47**, 1165–1171.
- Huys, G., Kämpfer, P. & Swings, J. (2001). New DNA–DNA hybridization and phenotypic data on the species *Aeromonas ichthiosmia* and *Aeromonas allosaccharophila*: *A. ichthiosmia* Schubert *et al.* 1990 is a later synonym of *A. veronii* Hickman-Brenner *et al.* 1987. *Syst Appl Microbiol* **24**, 177–182.
- Janda, J. M. & Abbott, S. L. (1998). Evolving concepts regarding the genus *Aeromonas*: an expanding panorama of species, disease presentations, and unanswered questions. *Clin Infect Dis* **27**, 332–344.
- Janda, J. M. & Kokka, R. P. (1991). The pathogenicity of *Aeromonas* strains relative to genospecies and phenospecies identification. *FEMS Microbiol Lett* **90**, 29–34.
- Janda, J. M., Guthertz, L. S., Kokka, R. P. & Shimada, T. (1994). *Aeromonas* species in septicemia: laboratory characteristics and clinical observations. *Clin Infect Dis* **19**, 77–83.
- Johnson, J. L. (1994). Similarity analysis of DNAs. In *Methods for General and Molecular Bacteriology*. pp. 655–682. Edited by P. Gerhardt, R. G. E. Murray, W. A. Wood & N. R. Krieg. Washington, DC: American Society for Microbiology.
- Joseph, S. W. & Carnahan, A. M. (2000). Update of the genus *Aeromonas*. *ASM News* **66**, 218–223.
- Kämpfer, P. & Altwegg, M. (1992). Numerical classification and identification of *Aeromonas* genospecies. *J Appl Bacteriol* **72**, 341–351.
- Kothary, M. H. & Kreger, A. S. (1985). Production and partial characterization of an elastolytic protease of *Vibrio vulnificus*. *Infect Immun* **50**, 534–540.
- Kühn, I., Albert, M. J., Ansaruzzaman, M. & 8 other authors (1997). Characterization of *Aeromonas* spp. isolated from humans with diarrhea, from healthy controls, and from surface water in Bangladesh. *J Clin Microbiol* **35**, 369–373.
- Marmur, J. (1961). A procedure for the isolation of deoxyribonucleic acid from microorganisms. *J Mol Biol* **3**, 208–218.
- Marmur, J. & Doty, P. (1962). Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. *J Mol Biol* **5**, 109–118.
- Martinetti Lucchini, G. & Altwegg, M. (1992). rRNA gene restriction patterns as taxonomic tools for the genus *Aeromonas*. *Int J Syst Bacteriol* **42**, 384–389.

- Martínez-Murcia, A. J., Benlloch, S. & Collins, M. D. (1992a).** 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.
- Martínez-Murcia, A. J., Esteve, C., Garay, E. & Collins, M. D. (1992b).** *Aeromonas allosaccharophila* sp. nov., a new mesophilic member of the genus *Aeromonas*. *FEMS Microbiol Lett* **91**, 199–206.
- Owen, R. J. & Hill, L. R. (1979).** The estimation of base composition, base pairing and genome size of bacterial deoxyribonucleic acids. In *Identification Methods for Microbiologists*, 2nd edn, pp. 217–298. Edited by F. A. Skinner & D. W. Lovelock. London: Academic Press.
- Pidiyar, V., Kaznowski, A., Narayan, N. B., Patole, M. & Shouche, Y. S. (2002).** *Aeromonas culicicola* sp. nov., from the midgut of *Culex quinquefasciatus*. *Int J Syst Evol Microbiol* **52**, 1723–1728.
- Popoff, M. (1984).** Genus III. *Aeromonas* Kluver and Van Niel 1936, 398^{AL}. In *Bergey's Manual of Systematic Bacteriology*, vol. 1, pp. 545–548. Edited by N. R. Krieg & J. G. Holt. Baltimore: Williams & Wilkins.
- Popoff, M. Y., Coynault, C., Kiredjian, M. & Lemelin, M. (1981).** Polynucleotide sequence relatedness among motile *Aeromonas* species. *Cur Microbiol* **5**, 109–114.
- Reed, M. J. & Muench, M. (1938).** A simple method for estimating fifty percent endpoints. *Am J Hyg* **27**, 493–497.
- Rohlf, F. J. (1998).** *NTSYS-pc. Numerical Taxonomy and Multivariate Analysis System*, version 2.0. New York: Exeter Software.
- Schubert, R. H. W. & Hegazi, M. (1988).** *Aeromonas eucrenophila* species nova *Aeromonas caviae* a later and illegitimate synonym of *Aeromonas punctata*. *Zentbl Bakteriol Mikrobiol Hyg A* **268**, 34–39.
- Schubert, R. H. W., Hegazi, M. & Wahlig, W. (1990a).** *Aeromonas enteropelogenes* species nova. *Hyg Med* **15**, 471–472.
- Schubert, R. H. W., Hegazi, M. & Wahlig, W. (1990b).** *Aeromonas ichthiosmia* species nova. *Hyg Med* **15**, 477–479.
- Sneath, P. H. A. & Johnson, R. (1972).** The influence on numerical taxonomic similarities of errors in microbiological tests. *J Gen Microbiol* **72**, 377–392.
- Sneath, P. H. A. & Sokal, R. R. (1973).** *Numerical Taxonomy. The Principles and Practice of Numerical Classification*. Edited by D. Kennedy & R. B. Park. San Francisco: W. B. Freeman.
- Sokal, R. R. & Rohlf, F. J. (1969).** *Biometry, the Principles and Practice of Statistics in Biological Research*. Ames, IA: Iowa State University Press.
- Stackebrandt, E. & Goebel, B. M. (1994).** Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int J Syst Bacteriol* **44**, 846–849.
- Valera, L. & Esteve, C. (2002).** Phenotypic study by numerical taxonomy of strains belonging to the genus *Aeromonas*. *J Appl Microbiol* **93**, 77–95.
- Wretling, B. & Wadström, T. (1977).** Purification and properties of a protease with elastase activity from *Pseudomonas aeruginosa*. *J Gen Microbiol* **103**, 319–327.
- Yáñez, M. A., Catalán, V., Apráiz, D., Figueras, M. J. & Martínez-Murcia, A. J. (2003).** Phylogenetic analysis of members of the genus *Aeromonas* based on *gyrB* gene sequences. *Int J Syst Evol Microbiol* **53**, 875–883.