Aeromonas taiwanensis sp. nov. and *Aeromonas sanarellii* sp. nov., clinical species from Taiwan

Anabel Alperi,¹ Antonio J. Martínez-Murcia,² Wen-Chien Ko,³
Arturo Monera,² Maria J. Saavedra^{2,4} and Maria J. Figueras¹

Correspondence

Maria J. Figueras
mariajose.figueras@urv.cat

¹Unit of Microbiology, Rovira i Virgili University, IISPV, Sant Llorenç 21, 43201 Reus, Spain

²Molecular Diagnostics Center (MDC), Biomolecular Technologies SL, and Miguel Hernández University, 03300 Orihuela (Alicante), Spain

³Department of Medicine, National Cheng Kung University, Tainan, Taiwan ROC

⁴Department of Veterinary Sciences, CECAV – University of Trás-os-Montes e Alto Douro, Vila Real, Portugal

Two clinical *Aeromonas* strains (A2-50^T and A2-67^T) recovered from the wounds of two patients in Taiwan could not be assigned to any known species of this genus based on their 16S rRNA gene sequences, which showed similarities of 99.6–99.8% to those of the type strains of *Aeromonas caviae*, *A. trota* and *A. aquariorum*. The *rpoD* phylogenetic tree allocated these strains to two novel and independent phylogenetic lines, the neighbouring species being *A. caviae*, the type strain of which showed 93.2% similarity (56 bp differences) to strain A2-50^T and 92.2% (63 bp differences) to strain A2-67^T. A multilocus phylogenetic analysis of five housekeeping genes (*gyrB*, *rpoD*, *recA*, *dnaJ* and *gyrA*; 3684 bp) confirmed that the two strains formed independent phylogenetic lineages within the genus. These data, together with phenotypic characterization and DNA–DNA reassociation results, revealed that these strains represent novel *Aeromonas* species, for which the names *Aeromonas taiwanensis* sp. nov. (type strain A2-50^T = CECT 7403^T = LMG 24683^T) and *Aeromonas sanarellii* sp. nov. (type strain A2-67^T = CECT 7402^T = LMG 24682^T) are proposed.

The genus *Aeromonas* includes facultatively anaerobic, Gram-negative, non-spore-forming bacilli or coccobacilli that are generally motile, usually oxidase- and catalase-positive, able to reduce nitrate to nitrite and generally resistant to the vibriostatic agent O/129 (2,4-diamino-6,7-diisopropylpteridine) (Abbott *et al.*, 2003; Martin-Carnahan & Joseph, 2005). The genus belongs to the family *Aeromonadaceae*, order *Aeromonadales*, class *Gamma*proteobacteria (Martin-Carnahan & Joseph, 2005). At the time of writing, the genus *Aeromonas* includes 20 recognized species: *Aeromonas hydrophila* (the type species), *A. bestiarum*, *A. salmonicida*, *A. caviae*, *A. media*, *A. eucrenophila*, *A. sobria*, *A. veronii*, *A. jandaei*, *A. schubertii*, *A. trota*, *A. allosaccharophila*, *A.*

encheleia, *A. popoffii* (Martin-Carnahan & Joseph, 2005), *A. simiae* (Harf-Monteil *et al.*, 2004), *A. molluscorum* (Miñana-Galbis *et al.*, 2004), *A. bivalvium* (Miñana-Galbis *et al.*, 2007), *A. aquariorum* (Martínez-Murcia *et al.*, 2008), *A. tecta* (Demarta *et al.*, 2008) and the recently described *A. fluvialis*, isolated from river water (Alperi *et al.*, 2010). Furthermore, *Aeromonas piscicola*, isolated from diseased fish, has been proposed by our group (Beaz-Hidalgo *et al.*, 2009), as well as some reclassifications (Martínez-Murcia *et al.*, 2007, 2009). The taxonomy of this genus is complex because, despite each species apparently having specific phenotypic characteristics, biochemical identification is laborious and very imprecise, showing poor correlation with genotypic identification (Borrell *et al.*, 1998; Soler *et al.*, 2003; Figueras, 2005; Ormen *et al.*, 2005). The 16S rRNA gene sequence similarity among species of the genus is very high, ranging from 96.7 to 100% (Martínez-Murcia *et al.*, 1992, 2007; Saavedra *et al.*, 2006). Furthermore, this gene presents microheterogeneities (Figueras *et al.*, 2005; Martínez-Murcia *et al.*, 2005; Morandi *et al.*, 2005; Alperi *et al.*, 2008) that reduce its usefulness for identification (Alperi *et al.*, 2008); such was the case for *Aeromonas culicicola* (Figueras *et al.*, 2005; Alperi *et al.*, 2008), now considered a synonym of *A. veronii* (Huys *et al.*, 2005). However, several housekeeping genes have proven to have

Abbreviation: MLPA, multilocus phylogenetic analysis.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA, *gyrB*, *rpoD*, *recA*, *dnaJ* and *gyrA* gene sequences of strains A2-50^T and A2-67^T are respectively FJ230077 and FJ230076, FJ807272 and FJ807277, FJ472928 and FJ472929, FJ472930 and FJ47931, FJ807270 and FJ807279 and FJ807274 and FJ807276.

An unrooted 16S rRNA gene sequence-based maximum-parsimony phylogenetic tree, an unrooted MLPA minimum-evolution tree, details of differences in the 16S rRNA gene sequences of the novel strains and DNA–DNA hybridization results are available as supplementary material with the online version of this paper.

high discriminatory power for differentiating neighbouring species and have been used to clarify the phylogeny of *Aeromonas* (Yañez *et al.*, 2003; Soler *et al.*, 2004; Kpfer *et al.*, 2006; Nhung *et al.*, 2007; Sepe *et al.*, 2008).

Aeromonas strains are primarily inhabitants of aquatic environments, often associated with fish and human diseases (Figueras, 2005; Martin-Carnahan & Joseph, 2005). The most common clinical presentation of *Aeromonas* is diarrhoea, followed by localized soft-tissue infections and bacteraemia, the prevailing associated species being *A. veronii*, *A. caviae* and *A. hydrophila* (Figueras, 2005). In recent years, numerous cases of *Aeromonas* infection have been described in Taiwan (Huang *et al.*, 2007; Wu *et al.*, 2007 and references therein). The present investigation was initiated to identify genetically a group of extraintestinal *Aeromonas* strains isolated in the National Cheng Kung University Hospital (Tainan, Taiwan) using a previously described 16S rRNA gene RFLP method (Figueras *et al.*, 2000). Two of these strains, A2-50^T and A2-67^T, received as *A. hydrophila* and *A. caviae*, respectively, showed atypical RFLP patterns similar to patterns observed for *A. caviae* strains that presented microheterogeneities in the 16S rRNA gene (Alperi *et al.*, 2008). However, the *rpoD* sequences showed that these strains might constitute novel and independent phylogenetic lines within *Aeromonas*.

In the present study, a polyphasic approach, based on 16S rRNA gene sequence analysis, multilocus phylogenetic analysis (MLPA) of five genes (*gyrB*, *rpoD*, *recA*, *dnaJ* and *gyrA*), DNA–DNA reassociation experiments and phenotypic analysis, was adopted to establish the taxonomic position of the clinical isolates A2-50^T and A2-67^T.

Strain A2-50^T, together with strains of *Enterobacter cloacae* and *Enterococcus* sp., was isolated on the tenth day of hospitalization from a burn wound of a 40-year-old male with flame burns involving 52 % of the body surface area. The patient also developed a concurrent *Aeromonas* and *Enterococcus* bacteraemia, but unfortunately the *Aeromonas* blood isolate was lost. Both the burn wound infection and the bacteraemia were considered nosocomial. Strain A2-67^T was isolated from a 70-year-old female with non-insulin-dependent diabetes mellitus. She had experienced trauma and had abrasion wounds over her right elbow and a right humeral fracture. On admission, a wound culture from her right elbow grew *Aeromonas*, *Pseudomonas aeruginosa* and *Bacillus* species. No bacteraemia was noted.

To perform the phylogenetic study, strains A2-50^T and A2-67^T were cultured on sheep-blood agar at 30 °C and DNA was extracted from single colonies using InstaGene Matrix (Bio-Rad) following the manufacturer's instructions in order to sequence the 16S rRNA (1503 bp) and *rpoD* (820 bp) genes. Primers and conditions for amplification and sequencing have been described previously (Martnez-Murcia *et al.*, 1992; Soler *et al.*, 2004).

The MLPA was performed on the basis of *gyrB* (923 bp), *rpoD* (652 bp), *recA* (600 bp), *dnaJ* (800 bp) and *gyrA*

(709 bp) gene sequences, as described elsewhere (A. J. Martnez-Murcia, A. Monera, R. Oncina, M. Lopez-Alvarez, E. Lara, M. J. Saavedra & M. J. Figueras, unpublished results).

The sequences obtained were aligned with the sequences of type and reference strains of all members of the genus *Aeromonas* that were available in GenBank (<http://www.ncbi.nlm.nih.gov/sites/entrez?db=nucleotide>) by using the CLUSTAL W program, version 1.83 (Thompson *et al.*, 1994). Genetic distances and clustering were obtained using Kimura's two-parameter model (Kimura, 1980) and phylogenetic trees were reconstructed by the neighbour-joining, maximum-parsimony and minimum-evolution methods (Saitou & Nei, 1987) using the MEGA4 program (Tamura *et al.*, 2007). Stability of relationships was assessed by bootstrapping (1000 replicates).

16S rRNA gene phylogenetic trees (Fig. 1 and Supplementary Fig. S1, available in IJSEM Online) showed strains A2-50^T and A2-67^T as independent phylogenetic lines within the genus *Aeromonas*. The strains clustered in the 16S rRNA gene trees with *A. caviae*, *A. trota* and *A. aquariorum*, with robust bootstrap values of 99 % when the phylogeny was inferred with the neighbour-joining algorithm (Fig. 1) and 96 % with the maximum-parsimony algorithm (Supplementary Fig. S1). The two strains were highly related to each other, with just three bp differences (99.8 %) between their 16S rRNA gene sequences. The nearest type strains to strains A2-50^T and A2-67^T on the basis of the 16S rRNA gene sequence were those of *A. trota* (99.7 and 99.6 % similarity, four and five bp differences, respectively), *A. caviae* (99.6 and 99.7 % similarity, five and four bp differences) and the recently described *A. aquariorum* (99.8 and 99.6 % similarity, two and five bp differences). Chromatogram analysis of the 16S rRNA gene sequences (1503 bp) of strains A2-50^T and A2-67^T revealed the existence of microheterogeneities in eight and three positions, respectively (Supplementary Table S1), representing variability of 0.53 % and 0.2 %, respectively. Microheterogeneities were located within the V3 and V6 regions, which contain signature nucleotides that enable identification of most *Aeromonas* species (Martnez-Murcia *et al.*, 1992; Saavedra *et al.*, 2006), thereby hampering proper identification (Alperi *et al.*, 2008). Strains A2-50^T and A2-67^T showed 99 % similarity to the type strains of *A. caviae*, *A. trota* and *A. aquariorum* using BLAST (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). This high similarity is in agreement with generally observed similarity within this genus. In fact, two type strains, those of *A. salmonicida* and *A. bestiarum*, possess identical 16S rRNA gene sequences (Martnez-Murcia *et al.*, 1992, 2005), and the other type strains possess similarities that range from 96.8 % (48 bp differences between *A. simiae* and *A. bestiarum*) to 99.8 % (three bp differences between *A. molluscorum* and *A. encheleia* and between *A. hydrophila* and *A. media*). Consequently, the 16S rRNA gene sequence similarity cut-off value of 97 % proposed for species differentiation (Stackebrandt & Goebel, 1994; Stackebrandt *et al.*, 2002)

Fig. 1. Unrooted neighbour-joining phylogenetic tree derived from 16S rRNA gene sequences (1503 nt) showing the relationships of strains A2-50^T (*Aeromonas taiwanensis* sp. nov.) and A2-67^T (*Aeromonas sanarellii* sp. nov.) to currently known species of *Aeromonas*. Numbers at nodes indicate bootstrap values (percentages of 1000 replicates). Bar, 0.002 substitutions per nucleotide position.

cannot be applied to *Aeromonas*, nor can the new proposed value of 98.7–99% (Stackebrandt & Ebers, 2006). This statement agrees with conclusions derived from an early 16S rRNA gene sequence phylogeny published by Martínez-Murcia *et al.* (1992), where absolute values for species delineation in bacteria were not recommended because of different rates of sequence divergence.

The *rpoD* phylogenetic tree showed strains A2-50^T and A2-67^T as two independent phylogenetic lines within *Aeromonas* (Fig. 2), showing 92.7% similarity (59 bp differences) between their sequences. The nearest type strain on the basis of the *rpoD* gene was that of *A. caviae*, showing sequence similarities of 93.2% (56 bp differences) to A2-50^T and 92.2% (63 bp differences) to A2-67^T. These values are below the mean intraspecies similarity of 97% established previously for the *rpoD* gene in the genus *Aeromonas* (Soler *et al.*, 2004) and confirmed in our laboratory after sequencing this gene in more than 149 strains of different species (not shown).

The MLPA tree showed, in concordance with the *rpoD* phylogeny and in contrast to the 16S rRNA gene, that strains A2-50^T and A2-67^T were not phylogenetically related to *A. trota* or *A. aquariorum* (Fig. 3 and Supplementary Fig. S2), but appeared in the same cluster that included *A. media* and *A. caviae*, forming independent branches. Species delineation based on the analysis of five housekeeping genes has been recommended (Stackebrandt *et al.*, 2002), although only the recently described *A. fluvialis* (Alperi *et al.*, 2010) and the two species described in this study comply with this recommendation in the genus *Aeromonas*.

To establish DNA–DNA reassociation values, DNA was extracted using Marmur's method (Marmur, 1961). Reassociation experiments were conducted using the methodology of Ziemke *et al.* (1998) and Urdiain *et al.* (2008). Reassociation was performed under optimal conditions at 70 °C, reassociation values were determined at least three times (direct and reciprocal reactions, e.g. A × B and B × A) for any given strain pair and results are

Fig. 2. Unrooted neighbour-joining phylogenetic tree derived from *rpoD* gene sequences (720 nt) showing the relationships of strains A2-50^T (*A. taiwanensis* sp. nov.) and A2-67^T (*A. sanarellii* sp. nov.) to currently known species of *Aeromonas*. Numbers at nodes indicate bootstrap values (percentages of 1000 replicates). Bar, 0.02 substitutions per nucleotide position.

Fig. 3. Unrooted neighbour-joining phylogenetic tree derived from MLPA (*gyrB*, *rpoD*, *recA*, *dnaJ* and *gyrA*) showing the relationships of strains A2-50^T (*A. taiwanensis* sp. nov.) and A2-67^T (*A. sanarellii* sp. nov.) to currently known species of *Aeromonas*. The phylogenetic tree was constructed with a concatenated sequence of 3684 nt. Numbers at nodes indicate bootstrap values (percentages of 1000 replicates). Bar, 0.02 substitutions per nucleotide position.

expressed as means $\pm$ SD, as described previously (Alperi *et al.*, 2010). The mean DNA–DNA reassociation values between strains A2-50^T and A2-67^T and *A. caviae* CECT 838^T and *A. media* CECT 4232^T, the most closely related type strains on the basis of MLPA, were 59.9 and 64.7 %, respectively, with *A. caviae* CECT 838^T and 54.5 and 29.4 %, respectively, with *A. media* CECT 4232^T; the two novel strains showed 63.8 % relatedness (Supplementary Table S2). These values were all below the 70 % established for species delineation (Wayne *et al.*, 1987; Stackebrandt & Goebel, 1994; Stackebrandt *et al.*, 2002; Rosselló-Móra, 2006). Although DNA–DNA hybridization values >60 % may be considered borderline, these values are quite common for different *Aeromonas* species (Nhung *et al.*, 2007; Alperi *et al.*, 2010). This also happens among species of the neighbouring genus *Vibrio* (Gómez-Gil *et al.*, 2003; Thompson *et al.*, 2003).

The G + C content of the DNA of strains A2-50^T and A2-67^T was determined by three independent analyses by the HPLC technique at the BCCM/LMG Identification Service (Universiteit Gent, Belgium) following the procedures described by Miñana-Galbis *et al.* (2004, 2007). The DNA G + C content of strains A2-50^T and A2-67^T was 62.8 and 63.1 mol%, respectively, within the range described for the genus *Aeromonas* (57–63 mol%; Martin-Carnahan & Joseph, 2005).

In order to determine colony characteristics, i.e. size, colour and production of brown diffusible pigment, strains A2-50^T and A2-67^T were grown at 30 °C for 24 h on TSA and on blood agar to determine the existence of haemolysis. Optimal growth temperature and pH were determined on TSB after 24 h by following optical density. Twenty-eight phenotypic tests, selected from Abbott *et al.* (2003) and listed in a previous study (Alperi *et al.*, 2010), were used for the characterization of strains A2-50^T and A2-67^T. These tests were performed three times and were incubated at 30 °C and also at 36 °C, due to the clinical origin of the strains. Test results were read every 24 h for up to 7 days. Some tests were confirmed in parallel using commercial identification kits (API 20NE and API 20E; bioMérieux). Additional tests from the latter kits, together with acid production from/hydrolysis of 49 carbohydrates using API 50CH (bioMérieux), were also considered. The type strains of all *Aeromonas* species were evaluated, under conditions identical to those used for A2-50^T and A2-67^T, for all differential tests included in Table 1.

Strains A2-50^T and A2-67^T showed relatively similar biochemical profiles, but they could be differentiated from each other because A2-50^T was able to use citrate and to produce acid from raffinose, whereas strain A2-67^T was not. In addition, the latter was able to grow at 45 °C on sheep-blood agar, whereas strain A2-50^T was not (Table 1). No differences were observed between biochemical profiles at 30 and 36 °C. Strains A2-50^T and A2-67^T showed distinctive characters that separated them from other species (*A. hydrophila*, *A. veronii* bv. *Sobria* and *A. caviae*)

Table 1. Key tests for phenotypic differentiation of strains A2-50^T (*A. taiwanensis* sp. nov.) and A2-67^T (*A. sanarellii* sp. nov.) from other *Aeromonas* species

Taxa: A, *A. taiwanensis* sp. nov. (A2-50^T); B, *A. sanarellii* sp. nov. (A2-67^T); 1, *A. hydrophila*; 2, *A. bestiarum*; 3, *A. salmonicida*; 4, *A. caviae*; 5, *A. media*; 6, *A. eucrenophila*; 7, *A. sobria*; 8, *A. veronii* bv. *Sobria*; 9, *A. jandaei*; 10, *A. veronii* bv. *Veronii*; 11, *A. schubertii*; 12, *A. trota*; 13, *A. encheleia*; 14, *A. allosaccharophila*; 15, *A. popoffii*; 16, *A. simiae* (tested at 30 °C; data from Harf-Monteil *et al.*, 2004); 17, *A. molluscorum* (Miñana-Galbis *et al.*, 2007); 18, *A. bivalvium* (tested at 25–30 °C; Miñana-Galbis *et al.*, 2004); 19, *A. aquariorum* (tested at 30 °C; Martínez-Murcia *et al.*, 2008); 20, *A. tecta* (tested at 30 °C; Demarta *et al.*, 2008); 21, *A. fluvialis* (tested at 30 °C; Alperi *et al.*, 2010). +, 85–100 % of strains positive; v, 16–84 % of strains positive; –, 0–15 % of strains positive (results obtained in this study for type strains are given in parentheses). Data in columns 1–15 were obtained from Abbott *et al.* (2003), who performed tests at 35 °C, with the exception of *A. popoffii* and *A. sobria*, which were tested at 25 °C, as did Miñana-Galbis *et al.* (2007). Tests carried out in this study for type strains as well as for the novel strains were performed at 36 and 30 °C, with the exception of *A. salmonicida*, which was also tested at room temperature. Test results were read every 24 h for up to 7 days. ND, No data available from the cited study; VP, Voges–Proskauer reaction; LDH, lysine decarboxylase.

Characteristic	A	B	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
VP	–	–	+(+)	v(+)	v(–)	–(–)	–(–)	–(–)	–(–)	+(+)	+(+)	v(–)	v(–)	–(–)	–(–)	–(–)	+(+)	–(–)	–(–)	–(–)	–(–)	v(+)	–(–)
LDH	–	–	+(+)	v(–)	v(+)	–(–)	–(–)	–(–)	+(+)	+(+)	+(+)	+(+)	v(–)	+(+)	–(–)	+(+)	–(–)	+(+)	–(–)	+(+)	+(+)	v(+)	–(–)
Gas from glucose	–	–	+(+)	v(+)	v(–)	–(–)	–(–)	v(+)	v(+)	+(+)	+(+)	+(+)	–(–)	v(+)	v(+)	+(+)	+(+)	–(–)	–(–)	–(–)	+(+)	+(+)	+(+)
Hydrolysis of aesculin	+	+	+(+)	v(+)	+(+)	v(+)	v(+)	v(+)	–(–)	–(–)	–(–)	+(+)	–(–)	–(–)	v(+)	v(+)	–(–)	v(–)	+(+)	+(+)	+(+)	v(–)	–(–)
Citrate utilization	+	–	+(+)	–(–)	+(+)	+(+)	v(+)	–(–)	+(+)	v(–)	+(+)	+(+)	v(+)	+(+)	–(–)	v(–)	+(+)	ND(–)	+(+)	+(+)	ND(+)	v(+)	+(+)
Growth at 45 °C on sheep-blood agar	–	+	+(+)	–(–)	–(–)	+(+)	–(–)	–(–)	–(–)	–(–)	+(+)	+(+)	+(+)	–(–)	–(–)	–(–)	–(–)	ND(+)	–(–)	–(–)	ND(–)	–(–)	–(–)
Acid from:																							
Amygdalin	+	+	–(–)	–(–)	–(–)	–(–)	–(–)	–(–)	–(–)	–(–)	–(–)	–(–)	–(–)	–(–)	–(–)	–(–)	–(–)	–(–)	ND(–)	ND(–)	–(–)	ND(–)	–(–)
Cellobiose	–	–	–(–)	v(–)	v(–)	+(+)	+(+)	v(+)	+(+)	v(–)	–(–)	v(+)	–(–)	+(+)	–(–)	+(+)	–(–)	+(+)	v(+)	ND(–)	–(–)	–(–)	+(+)
Raffinose	+	–	–(–)	–(–)	–(–)	–(–)	–(–)	–(–)	–(–)	–(–)	–(–)	–(–)	–(–)	–(–)	–(–)	v(–)	–(–)	–(–)	–(–)	–(–)	–(–)	ND(–)	–(–)
L-Arabinose	+	+	v(+)	+(+)	+(+)	+(+)	+(+)	v(+)	–(–)	–(–)	–(–)	–(–)	–(–)	–(–)	–(–)	v(+)	v(+)	–(–)	+(+)	+(+)	–(–)	–(–)	–(–)
Glycerol	+	+	+(+)	+(+)	+(+)	v(–)	v(+)	–(–)	+(+)	+(+)	+(+)	+(+)	–(–)	v(+)	+(+)	+(+)	+(+)	+(+)	+(+)	+(+)	+(+)	+(+)	+(+)
D-Mannose	–	+	+(+)	+(+)	+(+)	v(–)	+(+)	+(+)	+(+)	+(+)	+(+)	+(+)	+(+)	+(+)	+(+)	+(+)	+(+)	+(+)	+(+)	–(–)	+(+)	ND(+)	+(+)
Salicin	+	+	v(–)	v(+)	v(–)	v(+)	v(+)	v(–)	–(–)	–(–)	–(–)	+(+)	–(–)	–(–)	–(–)	–(–)	–(–)	–(–)	ND(+)	+(+)	+(+)	v(–)	+(+)

commonly encountered in clinical samples (Table 1). Interestingly, both strains were able to produce acid from amygdalin, a characteristic only described so far in nine *Aeromonas* strains within a study performed by Abbott *et al.* (2003), who evaluated the atypical phenotypic properties of the genus. In that study, they encountered four *A. caviae* strains that produced acid from amygdalin and from raffinose, which coincides with the profile of A2-50^T (Table 1). Other traits that differentiated A2-50^T and A2-67^T from other species were their capacity to produce acid from L-arabinose, glycerol and salicin as well as to hydrolyse aesculin, and their negative results for the Voges–Proskauer test, lysine decarboxylase activity and production of gas from glucose and acid from cellobiose and D-mannose (Table 1). Interestingly, strain A2-67^T displayed two colony types of the same size (4–5 mm in diameter). One was opaque, circular and beige in colour, while the other was translucent on TSA after 48 h at 30 °C. These two morphologies were also observed on blood agar. The two colony types produced the same biochemical responses and showed identical ERIC-PCR profiles (not shown).

Cell sizes and morphologies and the presence of flagella were determined by electron microscopy following procedures described previously (Collado *et al.*, 2009).

The susceptibilities of strains A2-50^T and A2-67^T against 27 antibiotics were tested, as described previously (Alperi *et al.*, 2010). Both strains showed resistance to amoxicillin, amoxicillin plus clavulanic acid, ticarcillin, ticarcillin plus clavulanic acid, cephalothin and erythromycin; A2-50^T was intermediately susceptible to streptomycin, while A2-67^T showed resistance to cefoxitin. Both strains were susceptible to the other antimicrobials tested.

The 16S rRNA gene sequences, MLPA, phenotypic characterization and DNA–DNA reassociation results all clearly separated strain A2-50^T from A2-67^T and both strains from the other *Aeromonas* species.

Description of *Aeromonas taiwanensis* sp. nov.

Aeromonas taiwanensis (tai.wan.en'sis. N.L. masc. adj. *taiwanensis* of Taiwan, where the type strain was isolated).

Cells are straight, Gram-negative, non-spore-forming, non-encapsulated, motile rods, 0.8–1 µm wide and 1.8–2.5 µm long. Oxidase- and catalase-positive, reduces nitrate to nitrite and resistant to vibriostatic agent O/129. Colonies on TSA are 2–5 mm in diameter, opaque, circular and beige in colour after 48 h at 30 °C. No brown diffusible pigment is produced on TSA. Optimal growth occurs at 30 °C. Growth is observed at 36 °C but not at ≥40 or 4 °C after 24 h on sheep-blood agar. No β-haemolysis is observed on sheep-blood agar at 30 or 36 °C. Grows on MacConkey agar and in the absence of NaCl but not at 6 % NaCl after 24 h on TSA. Optimal growth occurs at pH 8.5–9.5 after 24 h on TSA; does not grow at pH 4.5. Produces indole from tryptophan and arginine dihydrolase activity,

is positive for the ONPG test (β-galactosidase activity) and hydrolysis of aesculin, gelatin, DNA and L-tryptophan and is able to use citrate. Negative for the Voges–Proskauer test, hydrolysis of elastin and urea, production of hydrogen sulfide from cysteine and gas from glucose, swarming and for lysine decarboxylase and ornithine decarboxylase tests. Hydrolyses starch, arbutin and aesculin. Uses D-glucose, L-arabinose, D-mannitol, maltose, N-acetylglucosamine, potassium gluconate, capric acid, malic acid, glycogen and potassium gluconate as sole carbon and energy sources, but not potassium 2-ketogluconate, potassium 5-ketogluconate, D-mannose, adipic acid, phenylacetic acid or trisodium citrate. Acid is produced from D-glucose, sucrose, L-arabinose, raffinose, D-ribose, amygdalin, salicin, D-galactose, D-fructose, D-mannitol, glycerol, N-acetylglucosamine, maltose and trehalose, but not from cellobiose, D-arabinose, L-rhamnose, myo-inositol, erythritol, L- or D-xylose, D-adonitol, methyl β-D-xylopyranoside, D-mannose, L-sorbose, L-rhamnose, dulcitol, inositol, D-sorbitol, methyl α-D-mannopyranoside, methyl α-D-glucopyranoside, lactose, melibiose, inulin, melizitol, xylitol, gentiobiose, turanose, D-lyxose, D-tagatose, L- or D-fucose or L- or D-arabitol. The API 20E and API 20NE codes for the type strain are 3266127 and 7575754, respectively. Resistant to amoxicillin, amoxicillin plus clavulanic acid, ticarcillin, ticarcillin plus clavulanic acid, cephalothin and erythromycin, shows intermediate resistance to streptomycin and is susceptible to piperacillin, piperacillin plus tazobactam, cefoxitin, cefotaxime, cefoperazone, ceftazidime, ceftriaxone, cefepime, aztreonam, imipenem, gentamicin, kanamycin, tobramycin, amikacin, tetracycline, ciprofloxacin, nalidixic acid, fosfomycin, trimethoprim-sulfamethoxazole and chloramphenicol.

The type strain, A2-50^T (=CECT 7403^T =LMG 24683^T), was isolated from a wound infection from a patient at Tainan, Taiwan.

Description of *Aeromonas sanarellii* sp. nov.

Aeromonas sanarellii [sa.na.rell'i.i. N.L. gen. masc. n. *sanarellii* of Sanarelli, named in honour of G. Sanarelli, for his contribution to our knowledge of *Aeromonas*, having described the first member of *Aeromonas* as *Bacillus hydrophilus fuscus* in 1891 (Martin-Carnahan & Joseph, 2005)].

Cells are straight, non-spore-forming, non-encapsulated, motile rods, 0.7 µm wide and 2.5 µm long. Gram-negative, oxidase- and catalase-positive, reduces nitrate to nitrite and resistant to vibriostatic agent O/129. Displays two colony types on TSA and blood agar. Colonies on TSA are 4–5 mm in diameter; one colony type is opaque, circular and beige in colour, while the other is translucent, after 48 h at 30 °C. No brown diffusible pigment is produced on TSA. Optimal growth occurs at 30 °C; growth is observed up to 45 °C but not at 50 °C after 48 h or at 4 °C after 7 days on sheep-blood agar. No β-haemolysis is observed on sheep-blood agar at 30 or 36 °C. Able to grow on MacConkey and in the absence of NaCl but not at 6 % NaCl on TSA.

Optimal growth occurs at pH 9 after 24 h on TSA; does not grow at pH 4.5. Produces indole from tryptophan and arginine dihydrolase activity and is positive for the ONPG test (β -galactosidase) and hydrolyses arbutin, starch, aesculin, gelatin, DNA and L-tryptophan. Negative for the Voges–Proskauer test, utilization of citrate, hydrolysis of elastin and urea, production of hydrogen sulfide from cysteine and gas from glucose, swarming and for lysine decarboxylase and ornithine decarboxylase tests. Able to use potassium gluconate, D-glucose, L-arabinose, D-mannitol, maltose, N-acetylglucosamine, potassium gluconate, capric acid and malic acid as sole carbon and energy sources but not potassium 2-ketogluconate, potassium 5-ketogluconate, D-mannose, adipic acid, phenylacetic acid or trisodium citrate. Acid is produced from D-glucose, sucrose, L-arabinose, D-ribose, amygdalin, salicin, D-galactose, D-fructose, D-mannitol, glycerol, N-acetylglucosamine, maltose, trehalose and glycogen but not from cellobiose, raffinose, D-arabinose, L-rhamnose, myo-inositol, erythritol, L- or D-xylose, D-adonitol, methyl β -D-xylopyranoside, D-mannose, L-sorbose, L-rhamnose, dulcitol, inositol, D-sorbitol, methyl α -D-mannopyranoside, methyl α -D-glucopyranoside, lactose, melibiose, inulin, melezitose, xylitol, gentiobiose, turanose, D-lyxose, D-tagatose, L- or D-fucose or L- or D- arabitol. The API 20E and API 20NE codes for the type strain are 3066127 and 7575754, respectively. Resistant to amoxicillin, amoxicillin plus clavulanic acid, ticarcillin, ticarcillin plus clavulanic acid, cephalothin, cefoxitin and erythromycin and susceptible to piperacillin, piperacillin plus tazobactam, cefotaxime, cefoperazone, ceftazidime, ceftriaxone, cefepime, aztreonam, imipenem, gentamicin, kanamycin, tobramycin, amikacin, streptomycin, tetracycline, ciprofloxacin, nalidixic acid, fosfomycin, trimethoprim-sulfamethoxazole and chloramphenicol.

The type strain, A2-67^T (=CECT 7402^T =LMG 24682^T), was isolated from a wound culture from a patient at Tainan, Taiwan.

Acknowledgements

We are grateful to M^a Isabel Inza for her help in the phenotypic characterization and to Catalina Nuñez for her excellent technical assistance. We are also grateful to the Pharmacology Unit of the Rovira i Virgili University for the use of their Bio Whittaker Kinetic-QCL Microplate Reader. MDC work has been partly supported by grant IMIDTA/2007/68 from IMPIVA, Generalitat Valenciana, Spain. Part of this work was also supported by funds from the European Commission for the HEALTHY WATER Project (FOOD-CT-2006-036306). The authors are solely responsible for the content of this publication and it does not represent the opinion of the European Commission. The European Commission is not responsible for any use that might be made of data appearing therein.

References

Abbott, S. L., Cheung, W. K. & Janda, J. M. (2003). The genus *Aeromonas*: biochemical characteristics, atypical reactions, and phenotypic identification schemes. *J Clin Microbiol* **41**, 2348–2357.

Alperi, A., Figueras, M. J., Inza, I. & Martínez-Murcia, A. J. (2008). Analysis of 16S rRNA gene mutations in a subset of *Aeromonas* strains and their impact in species delineation. *Int Microbiol* **11**, 185–194.

Alperi, A., Martínez-Murcia, A. J., Monera, A., Saavedra, M. J. & Figueras, M. J. (2010). *Aeromonas fluvialis* sp. nov., isolated from a Spanish river. *Int J Syst Evol Microbiol* **60**, 72–77.

Beaz-Hidalgo, R., Alperi, A., Figueras, M. J. & Romalde, J. L. (2009). *Aeromonas piscicola* sp. nov., isolated from diseased fish. *Syst Appl Microbiol* **32**, 471–479.

Borrell, N., Figueras, M. J. & Guarro, J. (1998). Phenotypic identification of *Aeromonas* genomospecies from clinical and environmental sources. *Can J Microbiol* **44**, 103–108.

Brosius, J., Palmer, M. L., Kennedy, P. J. & Noller, H. F. (1978). Complete nucleotide sequence of a 16S ribosomal RNA gene from *Escherichia coli*. *Proc Natl Acad Sci U S A* **75**, 4801–4805.

Collado, L., Cleenwerck, I., Van Trappen, S., De Vos, P. & Figueras, M. J. (2009). *Arcobacter mytili* sp. nov., an indoxyl acetate-hydrolysis-negative bacterium isolated from mussels. *Int J Syst Evol Microbiol* **59**, 1391–1396.

Demarta, A., Küpfer, M., Riegel, P., Harf-Monteil, C., Tonolla, M., Peduzzi, R., Monera, A., Saavedra, M. J. & Martínez-Murcia, A. J. (2008). *Aeromonas tecta* sp. nov., isolated from clinical and environmental sources. *Syst Appl Microbiol* **31**, 278–286.

Figueras, M. J. (2005). Clinical relevance of *Aeromonas* sM503. *Rev Med Microbiol* **16**, 145–153.

Figueras, M. J., Soler, L., Chacón, M. R., Guarro, J. & Martínez-Murcia, A. J. (2000). Extended method for discrimination of *Aeromonas* spp. by 16S rDNA RFLP analysis. *Int J Syst Evol Microbiol* **50**, 2069–2073.

Figueras, M. J., Suarez-Franquet, A., Chacón, M. R., Soler, L., Navarro, M., Alejandre, C., Grasa, B., Martínez-Murcia, A. J. & Guarro, J. (2005). First record of the rare species *Aeromonas culicicola* from a drinking water supply. *Appl Environ Microbiol* **71**, 538–541.

Gómez-Gil, B., Thompson, F. L., Thompson, C. C. & Swings, J. (2003). *Vibrio rotiferianus* sp. nov., isolated from cultures of the rotifer *Brachionus plicatilis*. *Int J Syst Evol Microbiol* **53**, 239–243.

Harf-Monteil, C., Flèche, A. L., Riegel, P., Prévost, G., Bermond, D., Grimont, P. A. D. & Monteil, H. (2004). *Aeromonas simiae* sp. nov., isolated from monkey faeces. *Int J Syst Evol Microbiol* **54**, 481–485.

Huang, H. C., Yu, W. L., Huan, K. H., Cheng, K. C. & Chuang, Y. C. (2007). *Aeromonas sobria* prostatitis and septic shock in a healthy man with chronic alcoholic consumption. *Jpn J Infect Dis* **60**, 400–401.

Huys, G., Cnockaert, M. & Swings, J. (2005). *Aeromonas culicicola* Pidiyar *et al.* 2002 is a later subjective synonym of *Aeromonas veronii* Hickman-Brenner *et al.* 1987. *Syst Appl Microbiol* **28**, 604–609.

Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* **16**, 111–120.

Küpfer, M., Kuhnert, P., Korczak, B. M., Peduzzi, R. & Demarta, A. (2006). Genetic relationships of *Aeromonas* strains inferred from 16S rRNA, *gyrB* and *rpoB* gene sequences. *Int J Syst Evol Microbiol* **56**, 2743–2751.

Marmur, J. (1961). A procedure for the isolation of deoxyribonucleic acid from microorganisms. *J Mol Biol* **3**, 208–218.

Martin-Carnahan, A. & Joseph, S. W. (2005). Order XII. *Aeromonadales* ord. nov. In *Bergey's Manual of Systematic Bacteriology*, 2nd edn, vol. 2B, p. 556. Edited by D. J. Brenner, N. R. Krieg, J. T. Staley & G. M. Garrity. New York: Springer.

Martínez-Murcia, A. J., Benlloch, S. & Collins, M. D. (1992). Phylogenetic interrelationships of members of the genera *Aeromonas* and *Plesiomonas* as determined by 16S ribosomal DNA

sequencing: lack of congruence with results of DNA-DNA hybridizations. *Int J Syst Bacteriol* **42**, 412–421.

Martínez-Murcia, A. J., Soler, L., Saavedra, M. J., Chacón, M. R., Guarro, J., Stackebrandt, E. & Figueras, M. J. (2005). Phenotypic, genotypic, and phylogenetic discrepancies to differentiate *Aeromonas salmonicida* from *Aeromonas bestiarum*. *Int Microbiol* **8**, 259–269.

Martínez-Murcia, A. J., Figueras, M. J., Saavedra, M. J. & Stackebrandt, E. (2007). The recently proposed species *Aeromonas sharmiana* sp. nov., isolate GPTSA-6^T, is not a member of the genus *Aeromonas*. *Int Microbiol* **10**, 61–64.

Martínez-Murcia, A. J., Saavedra, M. J., Mota, V. R., Maier, T., Stackebrandt, E. & Cousin, S. (2008). *Aeromonas aquariorum* sp. nov., isolated from aquaria of ornamental fish. *Int J Syst Evol Microbiol* **58**, 1169–1175.

Martínez-Murcia, A. J., Monera, A., Alperi, A., Figueras, M. J. & Saavedra, M. J. (2009). Phylogenetic evidence suggests that strains of *Aeromonas hydrophila* subsp. *dhakensis* belong to the species *Aeromonas aquariorum* sp. nov. *Curr Microbiol* **58**, 76–80.

Miñana-Galbis, D., Farfán, M., Fusté, M. C. & Lorén, J. G. (2004). *Aeromonas molluscorum* sp. nov., isolated from bivalve molluscs. *Int J Syst Evol Microbiol* **54**, 2073–2078.

Miñana-Galbis, D., Farfán, M., Fusté, M. C. & Lorén, J. G. (2007). *Aeromonas bivalvium* sp. nov., isolated from bivalve molluscs. *Int J Syst Evol Microbiol* **57**, 582–587.

Morandi, A., Zhaxybayeva, O., Gogarten, J. P. & Graf, J. (2005). Evolutionary and diagnostic implications of intragenomic heterogeneity in the 16S rRNA gene in *Aeromonas* strains. *J Bacteriol* **187**, 6561–6564.

Nhung, P. H., Hata, H., Ohkusu, K., Noda, M., Shah, M. M., Goto, K. & Ezaki, T. (2007). Use of the novel phylogenetic marker *dnaJ* and DNA-DNA hybridization to clarify interrelationships within the genus *Aeromonas*. *Int J Syst Evol Microbiol* **57**, 1232–1237.

Ormen, O., Granum, P. E., Lassen, J. & Figueras, M. J. (2005). Lack of agreement between biochemical and genetic identification of *Aeromonas* spp. *APMIS* **113**, 203–207.

Rosselló-Móra, R. (2006). DNA-DNA reassociation applied to microbial taxonomy and their critical evaluation. In *Molecular Identification, Systematics, and Population Structure of Prokaryotes*, p. 105. Edited by E. Stackebrandt. Berlin & Heidelberg: Springer.

Saavedra, M. J., Figueras, M. J. & Martínez-Murcia, A. J. (2006). Updated phylogeny of the genus *Aeromonas*. *Int J Syst Evol Microbiol* **56**, 2481–2487.

Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.

Sepe, A., Barbieri, P., Peduzzi, R. & Demarta, A. (2008). Evaluation of *recA* sequencing for the classification of *Aeromonas* strains at the genotype level. *Lett Appl Microbiol* **46**, 439–444.

Soler, L., Marco, F., Vila, J., Chacón, M. R., Guarro, J. & Figueras, M. J. (2003). Evaluation of two miniaturized systems, MicroScan W/A and

BBL Crystal E/NF, for identification of clinical isolates of *Aeromonas* spp. *J Clin Microbiol* **41**, 5732–5734.

Soler, L., Yañez, M. A., Chacón, M. R., Aguilera-Arreola, M. G., Catalán, V., Figueras, M. J. & Martínez-Murcia, A. J. (2004). Phylogenetic analysis of the genus *Aeromonas* based on two housekeeping genes. *Int J Syst Evol Microbiol* **54**, 1511–1519.

Stackebrandt, E. & Ebers, J. (2006). Taxonomic parameters revisited: tarnished gold standards. *Microbiol Today* **33**, 152–155.

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.

Stackebrandt, E., Frederiksen, W., Garrity, G. M., Grimont, P. A. D., Kämpfer, P., Maiden, M. C. J., Nesme, X., Rosselló-Mora, R., Swings, J. & other authors (2002). Report of the ad hoc committee for the re-evaluation of the species definition in bacteriology. *Int J Syst Evol Microbiol* **52**, 1043–1047.

Tamura, K., Dudley, J., Nei, M. & Kumar, S. (2007). MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. *Mol Biol Evol* **24**, 1596–1599.

Thompson, J. D., Higgins, D. G. & Gibson, T. J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* **22**, 4673–4680.

Thompson, F. L., Thompson, C. C., Li, Y., Gómez-Gil, B., Vandenbergh, J., Hoste, B. & Swings, J. (2003). *Vibrio kanaloae* sp. nov., *Vibrio pomeroyi* sp. nov. and *Vibrio chagasii* sp. nov., from sea water and marine animals. *Int J Syst Evol Microbiol* **53**, 753–759.

Urdiain, M., López-López, A., Gonzalo, C., Busse, H. J., Langer, S., Kämpfer, P. & Rosselló-Móra, R. (2008). Reclassification of *Rhodobium marinum* and *Rhodobium pfennigii* as *Azifella marina* gen. nov. comb. nov. and *Azifella pfennigii* comb. nov., a new genus of photoheterotrophic *Alphaproteobacteria* and emended descriptions of *Rhodobium*, *Rhodobium orientis* and *Rhodobium gokarnense*. *Syst Appl Microbiol* **31**, 339–351.

Wayne, L. G., Brenner, D. J., Colwell, R. R., Grimont, P. A. D., Kandler, O., Krichevsky, M. I., Moore, L. H., Moore, W. E. C., Murray, R. G. E. & other authors (1987). International Committee on Systematic Bacteriology. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int J Syst Bacteriol* **37**, 463–464.

Wu, C. J., Wu, J. J., Yan, J. J., Lee, H. C., Lee, N. Y., Chang, C. M., Shih, H. I., Wu, H. M., Wang, L. R. & Ko, W. C. (2007). Clinical significance and distribution of putative virulence markers of 116 consecutive clinical *Aeromonas* isolates in southern Taiwan. *J Infect* **54**, 151–158.

Yañez, M. A., Catalán, V., Apraiz, 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.

Ziemke, F., Höfle, M. G., Lalucat, J. & Rosselló-Mora, R. (1998). Reclassification of *Shewanella putrefaciens* Owen's genomic group II as *Shewanella baltica* sp. nov. *Int J Syst Bacteriol* **48**, 179–186.