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Genetic relationships of *Aeromonas* strains inferred from 16S rRNA, *gyrB* and *rpoB* gene sequences

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Genetic relationships among bacterial strains belonging to the genus *Aeromonas* were inferred from 16S rRNA, *gyrB* and *rpoB* gene sequences. Twenty-eight type or collection strains of the recognized species or subspecies and 33 *Aeromonas* strains isolated from human and animal specimens as well as from environmental samples were included in the study. As reported previously, the 16S rRNA gene sequence is highly conserved within the genus *Aeromonas*, having only limited resolution for this very tight group of species. Analysis of a 1·1 kb *gyrB* sequence confirmed that this gene has high resolving power, with maximal interspecies divergence of 15·2 %. Similar results were obtained by sequencing only 517 bp of the *rpoB* gene, which showed maximal interspecies divergence of 13 %. The topologies of the *gyrB*- and *rpoB*-derived trees were similar. The results confirm the close relationship of species within the genus *Aeromonas* and show that a phylogenetic approach including several genes is suitable for improving the complicated taxonomy of the genus.

INTRODUCTION

Bacteria belonging to the genus *Aeromonas* (family *Aeromonadaceae*) are widespread in the environment, especially freshwater, and have been implicated as pathogens in human and animal diseases (Joseph & Carnahan, 2000; Crivelli *et al.*, 2001). The taxonomy of the genus is complex and has been submitted to ongoing change due to newly described species (Pidiyar *et al.*, 2002; Harf-Monteil *et al.*, 2004; Miñana-Galbis *et al.*, 2004) as well as reclassification and emended or extended descriptions of existing taxa (Pavan *et al.*, 2000; Huys *et al.*, 2002, 2003, 2005; Esteve *et al.*, 2003; Demarta *et al.*, 2004b). At present, the genus *Aeromonas* comprises at least 18 species validated on the basis of DNA–DNA hybridizations (Martin-Carnahan & Joseph, 2005).

Phylogenetic analysis based on the 16S rRNA gene is considered an appropriate tool for the reconstruction of evolutionary history and phylogenetic relationships of bacterial genera and it is universally used (Woese *et al.*, 1990; Stackebrandt & Goebel, 1994; Amann *et al.*, 1995). In the case of *Aeromonas*, 16S rRNA gene sequences indicated that the genus is composed of a very tight group of species, some of them differing by only a few nucleotides (Martinez-Murcia *et al.*, 1992). Moreover, some discrepancies have been observed between 16S rRNA gene sequencing and DNA–DNA hybridization results (Sneath, 1993; Martínez-Murcia, 1999) and the existence of 16S rRNA gene polymorphism has been reported recently, particularly in *Aeromonas media* and *Aeromonas veronii* strains (Morandi *et al.*, 2005). Other genes have therefore been evaluated as tools for the phylogenetic and taxonomic analysis of this genus.

Housekeeping genes are considered to be better molecular markers than the 16S rRNA gene for the study of phylogenetic and taxonomic relationships at the species level. In several species of bacteria, nucleotide sequence analysis at multiple protein-encoding loci has led to reliable phylogenies that have improved our understanding of population structure as well as epidemiology (Stackebrandt *et al.*, 2002; Urwin & Maiden, 2003).

Yamamoto & Harayama (1995) designed a set of primers that allowed both the amplification and the nucleotide sequencing of portions of the *gyrB* gene (which encodes the B-subunit of DNA gyrase) from various bacteria and demonstrated that phylogenetic analyses of *gyrB* nucleotide sequences reflected the evolutionary relationships of closely related species (Yamamoto & Harayama, 1996; Yamamoto *et al.*, 1999). *gyrB* sequence analysis has already been used

Abbreviation: HG, hybridization group.

The GenBank/EMBL/DDBJ accession numbers for the new *gyrB* and *rpoB* sequences obtained in this paper are given in Fig. 2.

Table 1. *Aeromonas* strains used in this study

ATCC, American Type Culture Collection (Manassas, VA, USA); CDC, Centers for Disease Control (Atlanta, GA, USA); CECT, Colección Española de Cultivos Tipo (Valencia, Spain); CIP, Collection bactérienne de l'Institut Pasteur (Paris, France); DSM, Deutsche Sammlung von Mikroorganismen und Zellkulturen (Braunschweig, Germany); IBS, Institute of Bacteriology, Louis Pasteur University (Strasbourg, France); LMG, Culture Collection of the Laboratorium voor Microbiologie Gent (Gent, Belgium); NCIMB, National Collection of Industrial, Food and Marine Bacteria (Aberdeen, UK).

Strain	Hybridization group/origin
Type or reference strains	
A. hydrophila	HG1
A. hydrophila subsp. *hydrophila* ATCC 7966^T	
A. hydrophila subsp. *anaerogenes* CIP 76.15^T	
A. hydrophila subsp. *dhakensis* CIP 107500^T	
A. hydrophila subsp. *ranae* CIP 107985^T	
A. bestiarum CDC 9533-76^T	HG2
A. salmonicida	HG3
A. salmonicida subsp. *salmonicida* ATCC 33658^T	
A. salmonicida subsp. *achromogenes* NCIMB 1110^T	
A. salmonicida subsp. *masoucida* ATCC 27013^T	
A. salmonicida subsp. *pectinolytica* DSM 12609^T	
A. salmonicida subsp. *smithia* NCIMB 13210^T	
A. caviae ATCC 15468^T	HG4
A. media	
A. media CDC 0862-83	HG5A
A. media ATCC 33907^T	HG5B
A. eucrenophila NCIMB 74^T	HG6
A. sobria CIP 7433^T	HG7
A. veronii biogroup Sobria	HG8
CDC 0437-84	
ATCC 51106	
A. jandaei ATCC 49568^T	HG9
A. veronii biogroup Veronii ATCC 35624^T	HG10
Aeromonas sp. ATCC 35941	HG11
A. schubertii ATCC 43700^T	HG12
Aeromonas sp. group 501	HG13
LMG 17321	
CECT 4254	
A. trota ATCC 49657^T	HG14
A. allosaccharophila CECT 4199^T	HG15
A. encheleia DSM 11577^T	HG16
A. popoffii LMG 17541^T	HG17
A. culicicola CIP 107763^T	–
A. molluscorum LMG 22214^T	–
A. simiae	
CIP 107798^T	–
IBS S6874^T	–
Field strains	
F458	Stool of symptomatic child
F589, F474	Stools of patients
F674P	Stool of asymptomatic person
F530D, F666I, F713E, F729F, F544A, F553E, F600C, F533E, F548B	Tap water
F507C	Pipe swab
F567A	Surface swab
V1, V32, V39, V47, V6, V183	Droppings from healthy goats

Table 1. cont.

Strain	Hybridization group/origin
V83	Droppings from a healthy tortoise
V155	Droppings from a healthy hen
V69, V23	Droppings from healthy pigs
V130	Droppings from an ill pig
V15	Droppings from a healthy horse
V30	Droppings from an ill horse
V97, V168	Droppings from an ill calf
JF2638, JF2689	Koi
JF2853	Human wound

to clarify interspecies phylogenetic relationships within *Aeromonas* (Yáñez *et al.*, 2003; Soler *et al.*, 2004) and to characterize novel species (Pidiyar *et al.*, 2003).

DNA-dependent RNA polymerase is a multisubunit enzyme that consists of two α subunits (encoded by the *rpoA* gene), one β subunit (*rpoB*) and one β' subunit (*rpoC*). Comparison of *rpoB* sequences has been used as a basis for phylogenetic analyses among some archaea and bacteria (Klenk & Zillig, 1994; Mollet *et al.*, 1997, Korczak *et al.*, 2004, 2006), but it has never been applied, to our knowledge, to study relationships among *Aeromonas* strains.

The use of *rpoB* sequences for phylogenetic and taxonomic studies of *Aeromonas* strains was therefore evaluated in comparison to *gyrB* using the 16S rRNA gene as a reference. The sequences obtained from these genes were used to characterize 33 *Aeromonas* strains isolated from human and animal specimens (carriers or patients) and environmental samples (freshwater, tap water, surface swabs) in order to investigate their taxonomic position and their genetic relatedness within the genus.

METHODS

Bacterial strains and culture conditions. The *Aeromonas* strains analysed in this study are listed in Table 1. The strains were subcultured overnight at 30 °C under aerobic conditions on Columbia agar base (Oxoid) supplemented with 5 % sheep erythrocytes.

DNA extraction. Genomic DNA was prepared according to a method described previously (Demarta *et al.*, 2000) from cells harvested from blood agar and then resuspended in TE buffer (10 mM Tris/HCl, 1 mM EDTA, pH 8·0). Alternatively, genomic DNA was prepared using an EZNA Bacterial DNA kit (PeqLab) and finally resuspended in water.

PCR amplification and sequencing. The sets of primers used for amplification and sequencing of 16S rRNA, *gyrB* and *rpoB* genes are listed in Table 2. Nearly complete 16S rRNA gene sequences of the

Table 2. Primers used for PCR amplification and sequencing of 16S rRNA, *gyrB* and *rpoB* genes

Primer positions are given according to *E. coli* numbering. N, Any nucleotide; R, A or G; S, C or G; Y, C or T; M, A or C.

Primer	Position	Sequence (5′→3′)	Reference
16S rRNA			
26f	8–26	AGAGTTTGATCATGGCTCA	Demarta *et al.* (1999)
1391r	1411–1391	GTGTGACGGGCGGTGTGTA	Demarta *et al.* (1999)
308f	289–308	GCTGGTCTGAGAGGATGATC	Demarta *et al.* (1999)
556r	575–556	CTTTACGCCCAGTAATTCCG	Demarta *et al.* (1999)
gyrB			
UP-1	273–312	GAAGTCATCATGACCGTTCTGCAYGCNGGNGGNAARTTYGA	Yamamoto & Harayama (1995)
UP-2r	1527–1485	AGCAGGGTACGGATGTGCGAGCCRTCNACRTCNGCRTCNGTCAT	Yamamoto & Harayama (1995)
UP-1S		GAAGTCATCATGACCGTTCTGCA	Yamamoto & Harayama (1995)
UP-2Sr		AGCAGGGTACGGATGTGCGAGCC	Yamamoto & Harayama (1995)
UP3	548–567	ACTACGAGATCCTGGCCAAG	This paper
UP4	997–1014	TCCTCCCAGACCAAGGAC	This paper
UP5r	917–897	GCCTTCTTGCTGTAGTCCTCT	This paper
UP6r	1283–1259	GCAGAGTCCCCTTCCACTATGTA	This paper
rpoB			
Pasrpob-L	1501–1523	GCAGTGAAAGARTTCTTTGGTTC	Korczak *et al.* (2004)
Rpob-R	2059–2041	GTTGCATGTTNGNACCCAT	Korczak *et al.* (2004)

strains were determined by PCR-based DNA sequencing following a protocol described previously (Demarta *et al.*, 1999). Amplification of *gyrB* was performed as described by Yamamoto & Harayama (1995) using primers UP-1 and UP-2r. Primers UP-1S, UP-2Sr as well as newly designed ones (Table 2) were used to obtain *gyrB* sequences of approximately 1100 bp according to a method described previously (Demarta *et al.*, 2004a). A fragment of about 560 bp from the *rpoB* gene was amplified and sequenced as reported by Korczak *et al.* (2004). The accession numbers of 16S rRNA, *gyrB* and *rpoB* gene sequences are given on the trees.

Phylogenetic analyses. Nucleotide sequences of 16S rRNA, *gyrB* and *rpoB* genes were aligned independently and phylogenetically analysed using MEGA version 3.1 (Kumar *et al.*, 2004). Phylogenetic trees were constructed using the neighbour-joining method with genetic distances computed by employing Kimura's 2-parameter method. A phylogenetic tree was also constructed from combined sequences of *rpoB* and *gyrB* genes.

RESULTS AND DISCUSSION

Isolates of our collection were first identified by 16S rRNA gene amplification and sequencing using the primers indicated in Table 2. Analyses of a fragment of approximately 1330 bp (positions 45–1394 in *Escherichia coli*), comprising the two main hypervariable regions of *Aeromonas*, allowed the construction of the phylogenetic tree in Fig. 1. The taxonomic identification of isolates F544A, F713E, V30 and V168 could not be properly assessed because their sequences were too divergent from those of known hybridization groups (HGs) or genospecies. Moreover, nine strains shared an identical fragment sequence with *Aeromonas salmonicida* and *Aeromonas bestiarum*, species which are hardly differentiated on the basis of 16S rRNA genes (Martinez-Murcia *et al.*, 1992).

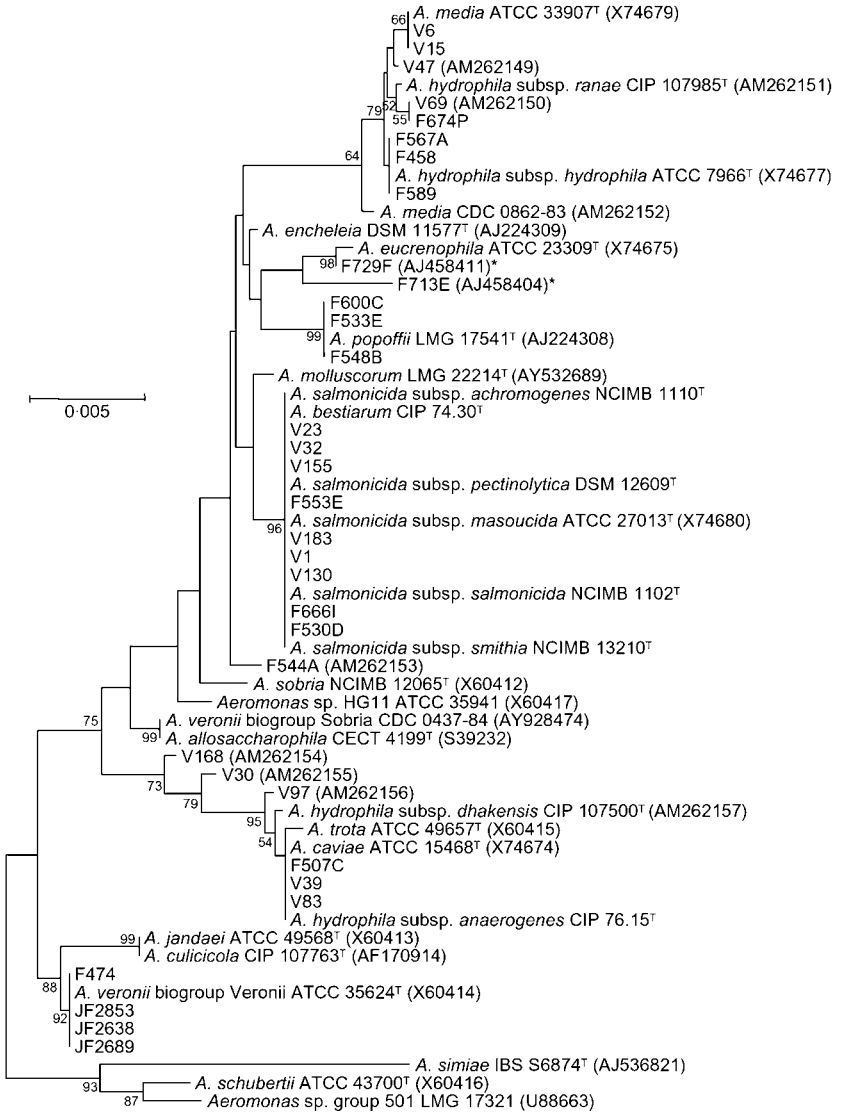

Fig. 1. Unrooted neighbour-joining phylogenetic tree based on a fragment of 1321 bp of the 16S rRNA genes of *Aeromonas* strains. Numbers at nodes indicate bootstrap values (percentages of 1000 replicates). Sequence accession numbers are in parentheses. Strains without accession numbers gave sequences that were found to be identical to another deposited sequence included on the tree; sequences marked by an asterisk were found to be identical to the deposited sequence indicated, although the original source strain is not included on the tree. Bar, 5 changes per 1000 positions

Using universal primers (Table 2), we could amplify and sequence *gyrB* fragments of approximately 1100 nucleotides (positions 346–1467 in *E. coli* K-12). Maximal interspecies divergence of *gyrB* sequences was found to be 15·2 %, whereas the mean divergence was 7·76 %, values close to those reported by Yáñez *et al.* (2003).

Primers used to amplify and sequence the *rpoB* genes of bacteria belonging to the family *Pasteurellaceae* (Table 2) proved also to be suitable for *Aeromonas*, allowing the amplification and the sequencing of a 558 nucleotide fragment, which was used to draw the phylogenetic tree in Fig. 2(a). The *rpoB* region amplified corresponded to codons 500–686 of the 1342 amino acid β subunit in *E. coli* (Ovchinnikov *et al.*, 1981). This segment represents the most variable part of the gene in many bacterial species (Mollet *et al.*, 1997). The maximal interspecies divergence was 13 %, with a mean interspecies divergence of 6·07 %. *rpoB* sequences were therefore found to be more conserved than *gyrB* sequences among *Aeromonas* strains.

The *A. veronii*/*A. culicicola*/*A. allosaccharophila* group

Trees derived from *rpoB* and *gyrB* sequences grouped these species on the same branch, but the relative positions of single strains differed. In the case of *rpoB* sequences (Fig. 2a), the reference strains *A. allosaccharophila* CECT 4199^T (HG15), *A. culicicola* CIP 107763^T and *A. veronii* ATCC 35624^T (HG10) showed a mean interspecies divergence of 1 %. The same low interspecies resolution among these species was reported previously from *rpoD* sequence analysis (Soler *et al.*, 2004). The *rpoB* sequences of *A. culicicola* CIP 107763^T and *A. veronii* ATCC 35624^T (HG10) were hard to differentiate, presenting a divergence of only 0·58 %. Recent investigations (Huys *et al.*, 2005) concluded that members of the species *A. culicicola* in fact belong to the species *A. veronii*. Analysis of *rpoB* sequences, as well as *gyrB* and *rpoD* (Soler *et al.*, 2004), confirmed that *A. culicicola* could be considered as a synonym of *A. veronii*. Strains JF2638, JF2689, JF2853 and F474 were consequently allocated to the latter species (Fig. 2a). The strain *A. veronii* biogroup Sobria CDC 0437-84 (HG8) was found to be very similar to *A. veronii* biogroup Veronii ATCC 35624^T (HG10) when *rpoB* sequences were analysed.

The partial *gyrB* sequences for this group of species showed an interspecies divergence of about 4 %. The mean intraspecies divergence for the subgroup formed by strains JF2638, JF2689, JF2853, F474, *A. veronii* ATCC 35624^T (HG10) and *A. culicicola* CIP 107763^T was 2·3 %. The partial *gyrB* sequence of the strain *A. veronii* biogroup Sobria CDC 0437-84 was more closely related to that of *A. allosaccharophila* CECT 4199^T (HG15) than to that of *A. veronii* biogroup Veronii ATCC 35624^T, as found by Pidiyar *et al.* (2003), but an almost identical *rpoB* sequence to *A. veronii* biogroup Veronii ATCC 35624^T. This finding therefore represents a case of inconsistency similar to that reported by Yáñez *et al.* (2003) regarding strain CECT 4911, classified as *A. veronii* by Huys *et al.* (1996) but possessing *gyrB* and 16S rRNA gene sequences similar or identical to those of *A. allosaccharophila* CECT 4199^T. It should be mentioned that the strain *A. veronii* biogroup Sobria CDC 0437-84 was found to carry at least two divergent copies of the 16S rRNA gene (Morandi *et al.*, 2005)

The *A. bestiarum*/*A. salmonicida*/*A. popoffii* group

Six strains of animal origin (V1, V130, V183, V32, V155 and V23) as well as three strains isolated from tap water (F666I, F553E and F530D) showed identical 16S rRNA gene sequences to the *A. salmonicida* and *A. bestiarum* type strains when the stretch of 1321 bp was considered (Fig. 1). These two species are difficult to separate on this basis because they are reported to differ in only two nucleotide positions over the entire 16S rRNA gene sequence (Martin-Carnahan & Joseph, 2005).

These species could be separated using *gyrB* (Yáñez *et al.*, 2003; this study) or *rpoD* (Soler *et al.*, 2004), which showed a resolving power to distinguish *A. salmonicida* from *A. bestiarum* ranging from 6·8 to 8·7 %. *rpoB* sequences could also separate these species, although with a lower resolving power (interspecies divergence of 2·6 %). Our collection strains were placed in *A. salmonicida* genospecies HG3 (strains V1, V130, V183, V32, V155, V23 and F553E) and in *A. bestiarum* genospecies HG2 (F530D and F666I) by both *rpoB* and *gyrB* sequence analysis. Strains belonging to *A. salmonicida*, comprising the subspecies we analysed, formed a very uniform group, with respective intraspecies substitution rates of 1·3 and 0·8 % for *gyrB* and *rpoB*.

Strains of *A. popoffii* (LMG 17541^T, F533E, F548B and F600C) appeared to be closely related to the two above-mentioned species by both *rpoB* and *gyrB*, in agreement with previous reports (Pidiyar *et al.*, 2003; Yáñez *et al.*, 2003) as well as DNA–DNA hybridization results (Huys *et al.*; 1997b). Probably the best resolution to separate these species can be obtained by using *rpoD* sequences (Soler *et al.*, 2004). The finding of perfect matches between the two markers *rpoB* and *gyrB* in strains F533E, F548B and F600C suggests that they could be clonally related.

The *A. encheleia*/*Aeromonas* sp. HG11/ *A. eucrenophila* group

A lasting controversy within the genus *Aeromonas* is represented by the species *A. encheleia* HG16 and the unnamed *Aeromonas* sp. HG11. DNA–DNA relatedness levels reported by Huys *et al.* (1997a) regarding the strains used in our study clearly allocated *Aeromonas* sp. HG11 strain ATCC 35941 to the species *A. encheleia*, despite some atypical reactions in phenotypic tests. As already found for *rpoD* (Soler *et al.*, 2004), *rpoB* sequences, showing an interspecies divergence of only 0·78 %, did not highlight any phylogenetic divergence between these strains and were therefore congruent with the suggestion that these two taxa

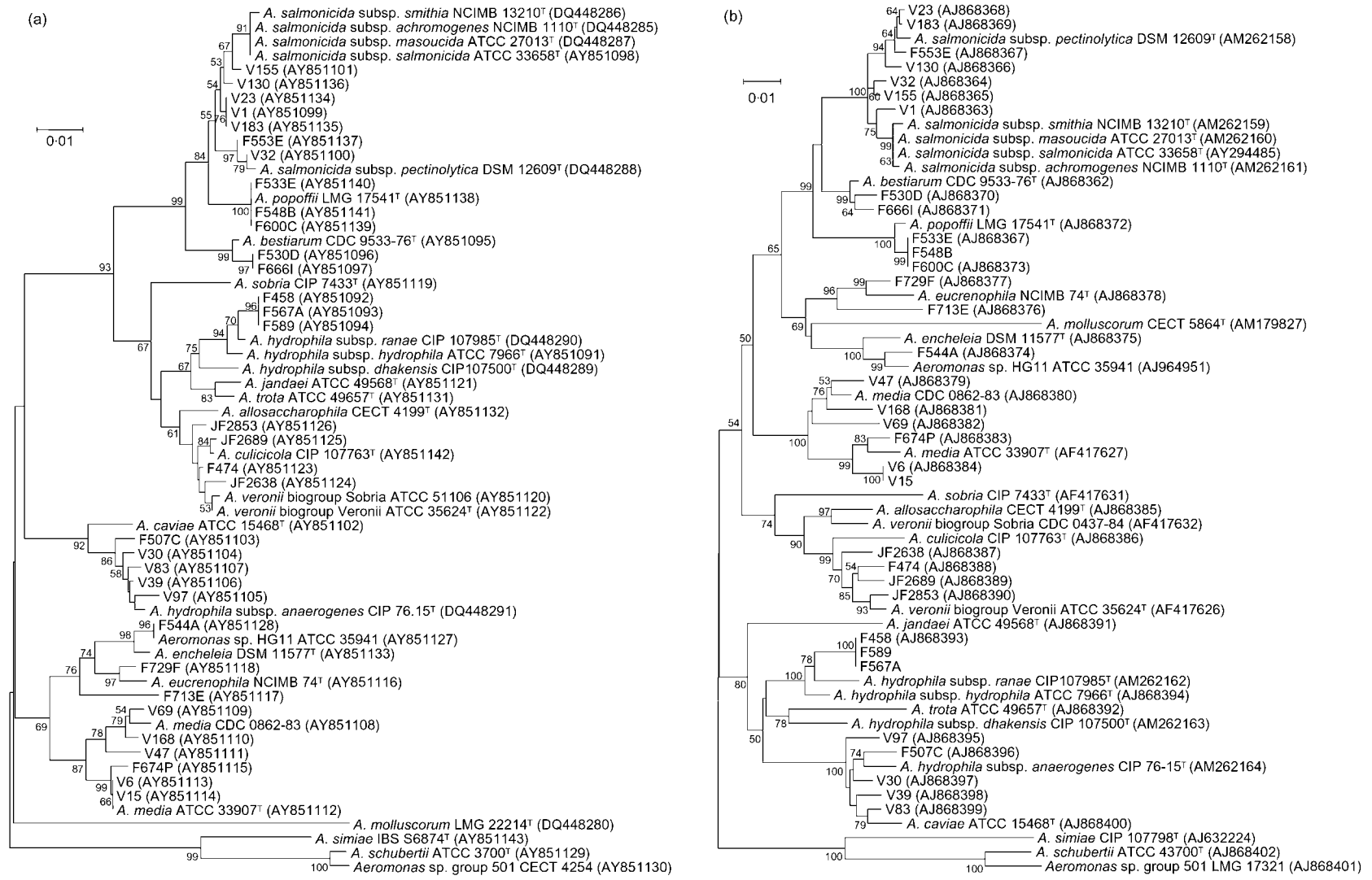

Fig. 2. Unrooted neighbour-joining phylogenetic trees based on *rpoB* (a) and *gyrB* (b) gene sequences of *Aeromonas* strains. Numbers at nodes indicate bootstrap values (percentages of 1000 replicates). Sequence accession numbers are in parentheses; strains without accession numbers gave sequences that were found to be identical to another deposited sequence included on the tree. Bars, 1 change per 100 positions.

should be considered as a single species. The interspecies divergence based on our *gyrB* sequences was 2·3 %, in agreement with results reported by Yáñez *et al.* (2003); this value was also close to the intraspecies nucleotide substitution threshold of 2 % found by Yáñez *et al.* (2003) for most *Aeromonas* species. Since only one field isolate (strain F544A) clustered within these strains by both methods, further insight in this group was not possible.

Two strains (F713E and F729F) clustered in the *A. eucrenophila* branch, but strain F713E showed an *rpoB* sequence divergence from *A. eucrenophila* NCIMB 74^T of 3 % (4·36 % for *gyrB*). 16S rRNA gene analysis showed that this strain possessed the same nucleotide sequence as a group of *Aeromonas* strains described by Demarta *et al.* (2004b). *rpoB* and *gyrB* sequencing results strengthened the hypothesis of the existence of a novel species in the genus *Aeromonas*, closely related to *A. eucrenophila*.

The *A. media* HG5A/*A. media* HG5B group

rpoB, *gyrB* and 16S rRNA gene sequences were established for *A. media* ATCC 33907^T (HG5B) and *A. media* CDC 862-83 (HG5A) and for strains V168, V47, V69, F674P, V6 and V15. Apart from strain V168, which clustered with *Aeromonas caviae* HG4, these strains were grouped in a heterogeneous cluster with *Aeromonas hydrophila* ATCC 7966^T in the 16S rRNA gene tree (Fig. 1). *rpoB* sequence analysis (Fig. 2a) divided these strains into two related subgroups, diverging by 1·9 %. The same topology was found with the *gyrB* tree (Fig. 2b), with a divergence rate for this marker of 3·74 %. The *gyrB* sequences of strains V47, V69, V168 and *A. media* CDC 0862-83 showed a mean similarity of 97·8 % with isolates 57, 480 and 610, strains analysed by Soler *et al.* (2004) which formed a phylogenetically distinct cluster in their *gyrB* tree.

These results confirmed previous outcomes on the differentiation of the genospecies *A. media* HG5 into two distinct lines matching the subgroups 5A and 5B, which might constitute two subspecies (Martin-Carnahan & Joseph, 2005).

The remaining groups

The *rpoB* sequence of *A. caviae* ATCC 15468^T (HG4) clustered with five sequences derived from our environmental strains (four of animal origin and one from a pipe swab), as well as with *A. hydrophila* subsp. *anaerogenes* CIP 76.15^T, which is considered to belong to the species *A. caviae*. The mean sequence diversity of this species was calculated to be 1·23 % for *rpoB* and 1·83 % for *gyrB*.

Strains F458, F589 (isolated from stools of patients) and F567A (isolated from a surface swab) showed identical 16S rRNA, *rpoB* and *gyrB* gene sequences, classifying them as *A. hydrophila* HG1. The identical genetic profiles in three independent markers showed the possible clonal origin of these strains, which were epidemiologically unrelated. The strain *A. hydrophila* subsp. *dhakensis* CIP 107500^T was found to be the more divergent, regardless of the gene used to draw the phylogenetic tree, among the strains belonging to the species *A. hydrophila*. The 16S rRNA gene sequence placed this strain close to the type strains of *A. caviae* and *Aeromonas trota*.

A. sobria (HG7), *Aeromonas jandaei* (HG9), *Aeromonas schubertii* (HG12), *Aeromonas* sp. group 501 (HG13), *A. trota* (HG14) and *Aeromonas simiae* were represented in our trees by their type (or reference) strains only. The *gyrB*-derived tree for these species (Fig. 2b) did not differ from those obtained by other authors (Pidiyar *et al.*, 2003; Yáñez *et al.*, 2003; Soler *et al.*, 2004). In the *rpoB* tree, only the relative branching positions were subject to change (Fig. 2a).

Since the resolving power of *gyrB* and *rpoB* differed in separating some species, a tree (not shown) was derived using the combined sequences joined end to end ($\sim$1600 nucleotides). Overall, the combined tree confirmed the positions of species and strains already found in the individual trees. Its reliability in differentiating closely related taxa was improved, as attested by bootstrap values greater than those obtained in single gene analysis.

The majority of the strains isolated from tap water were identified as *A. bestiarum* or *A. popoffii* or they were found to belong to the group *A. eucrenophila*/*A. encheleia*/*Aeromonas* sp. HG11. No further distribution of the strains in the phylogenetic tree was noticed related to their origin. Strains isolated from animal droppings were scattered among different genospecies, regardless of the kind of the animal or its health status. It is therefore conceivable that the pathogenic potential of aeromonads is related both to certain host determinants and to strain properties such as the presence of virulence factors.

In conclusion, the use of *rpoB* and *gyrB* allowed us to clarify the taxonomy and the phylogenetic relationships of *Aeromonas* strains isolated from humans, animals and the environment. 16S rRNA gene sequencing was useful to define isolates at the genus level only. In contrast, *gyrB* sequences were a powerful tool in differentiating *Aeromonas* genospecies, confirming previous work on *Aeromonas* (Yáñez *et al.*, 2003; Soler *et al.*, 2004) and similar findings in other bacterial genera (Fukushima *et al.*, 2002; Radice *et al.*, 2006). Moreover, due to the sequence diversity that we could observe in our strains at the intraspecies level, we can agree completely with the suggestion of using *gyrB* sequences not only for the identification of species in a phylogenetic framework but also for strain differentiation, as proposed by Yáñez *et al.* (2003). Although *rpoB* sequences are more conserved than *gyrB* sequences in the genus *Aeromonas*, the present work demonstrated that the resolution of *rpoB* was sufficient to infer phylogenetic relationships and taxonomic identifications within *Aeromonas*. Moreover, a similar reliability to *gyrB* for differentiating related *Aeromonas* species could be obtained from analysis of an *rpoB* fragment of only 560 bp. In fact,

these sequences allowed differentiation between *A. salmonicida* and *A. bestiarum*, as well as *A. media* HG5A and *A. media* HG5B. Furthermore, strain groupings obtained with *rpoB* were in agreement with the taxonomic classification of all species and subspecies currently recognized in the genus *Aeromonas*. Finally, *rpoB* sequences contributed to a clearer understanding of some still controversial taxa of the genus *Aeromonas*.

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