

'*Aeromonas intestinalis*' and '*Aeromonas enterica*' isolated from human faeces, '*Aeromonas crassostreae*' from oyster and '*Aeromonas aquatilis*' isolated from lake water represent novel species

M. J. Figueras¹, F. Latif-Eugenín¹, F. Ballester², I. Pujol², D. Tena³, K. Berg⁴, M. J. Hossain⁵, R. Beaz-Hidalgo¹ and M. R. Liles⁵

1) Unidad de Microbiología, Facultad de Medicina y Ciencias de la Salud, IISPV, Universidad Rovira i Virgili, 2) Laboratorio de Referencia de Cataluña Sud. Hospital Universitario St. Joan de Reus, Reus, Spain, 3) Section of Microbiology, University Hospital of Guadalajara, Guadalajara, Spain, 4) Department of Food and Environmental Sciences, University of Helsinki, Finland and 5) Department of Biological Sciences, Auburn University, Auburn, Alabama, USA

Abstract

Four *Aeromonas* strains from clinical and environmental samples differed from known species on the basis of *rpoD* gene sequence. Multilocus phylogenetic analysis and *in silico* DNA-DNA hybridization confirmed them as four new species even though their 16S rRNA gene sequence similarity with their closest relatives was >98.7%, as occurred for other *Aeromonas* spp.

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Corresponding author: M. J. Figueras, Unidad de Microbiología, Facultad de Medicina y Ciencias de la Salud, IISPV, Universidad Rovira i Virgili, Sant Llorenç 21, 43201 Reus, Spain
E-mail: mariajose.figueras@urv.cat

The genus *Aeromonas* belongs to the Family *Aeromonadaceae* and includes oxidase-positive, facultatively anaerobic, Gram-negative bacilli [1,2]. To date, 32 species are recognized, and 19 so far have been implicated in human diarrhoea, bacteraemia or wound infections, and are considered human opportunistic pathogens [2–5]. New data have demonstrated that *Aeromonas* spp. are true enteropathogens [5].

Recently a group of four *Aeromonas* strains could not be assigned to any species. Two of them, I178C^T and I13634^T, were recovered from patients with diarrhoea from two Spanish hospitals; stool samples were collected in plastic containers. Strain I178C^T was isolated, in the absence of other microbes, from the diarrhoeic faeces of a 45-year-old man with vomiting and fever over 2 months. Strain I13634^T was recovered from a 32-year-old woman with abdominal pain lasting several days. One (AOSE3-14A^T) of the other two strains was isolated from *Crassostrea gigas* harvested from Alfacs Bay (River Ebro Delta, Spain), and the other (AE207^T) was isolated from Lake Pyhäjärvi water (Finland).

The phylogenetic tree constructed with the *rpoD* gene, which is a gene we have described earlier as a first-line tool for the identification of *Aeromonas* spp. [2,6], indicated that the four strains formed independent branches from the rest of species, and this was confirmed with the tree constructed with the concatenated sequences of six housekeeping genes (*rpoD*, *gyrB*, *gyrA*, *recA*, *dnaj* and *dnaX*, 3558 bp) (Fig. 1), all performed as previously described [3]. The interspecies 16S rRNA gene sequence (1367 bp) similarity of the four strains with the types of their closest *Aeromonas* species was >98.7% (Supplementary Fig. S1). This is in agreement with the high values of >98.7 to 100% observed among several accepted *Aeromonas* species [2,3,6,7]. The closest species to clinical strain I178C^T was *A. jandaei* (98.85% similarity), while for strain I13634^T they were *A. salmonicida* (99.86%), *A. bestiarum* (99.71%) and *A. piscicola* (99.71%). Strain AOSE3-14A^T grouped with *A. encheleia* (99.93% similarity) and AE207^T with *A. tecta* (99.50% similarity) (Supplementary Fig. S1). The genomes of the four new species were sequenced, and the *in silico* DNA-DNA hybridization (*isDDH*) values with the genomes of the type strains of the closest species was calculated as described elsewhere [3]. The four strains showed *isDDH* values of <70%, which confirmed them as four new species (Fig. 1 and Supplementary Fig. S1).

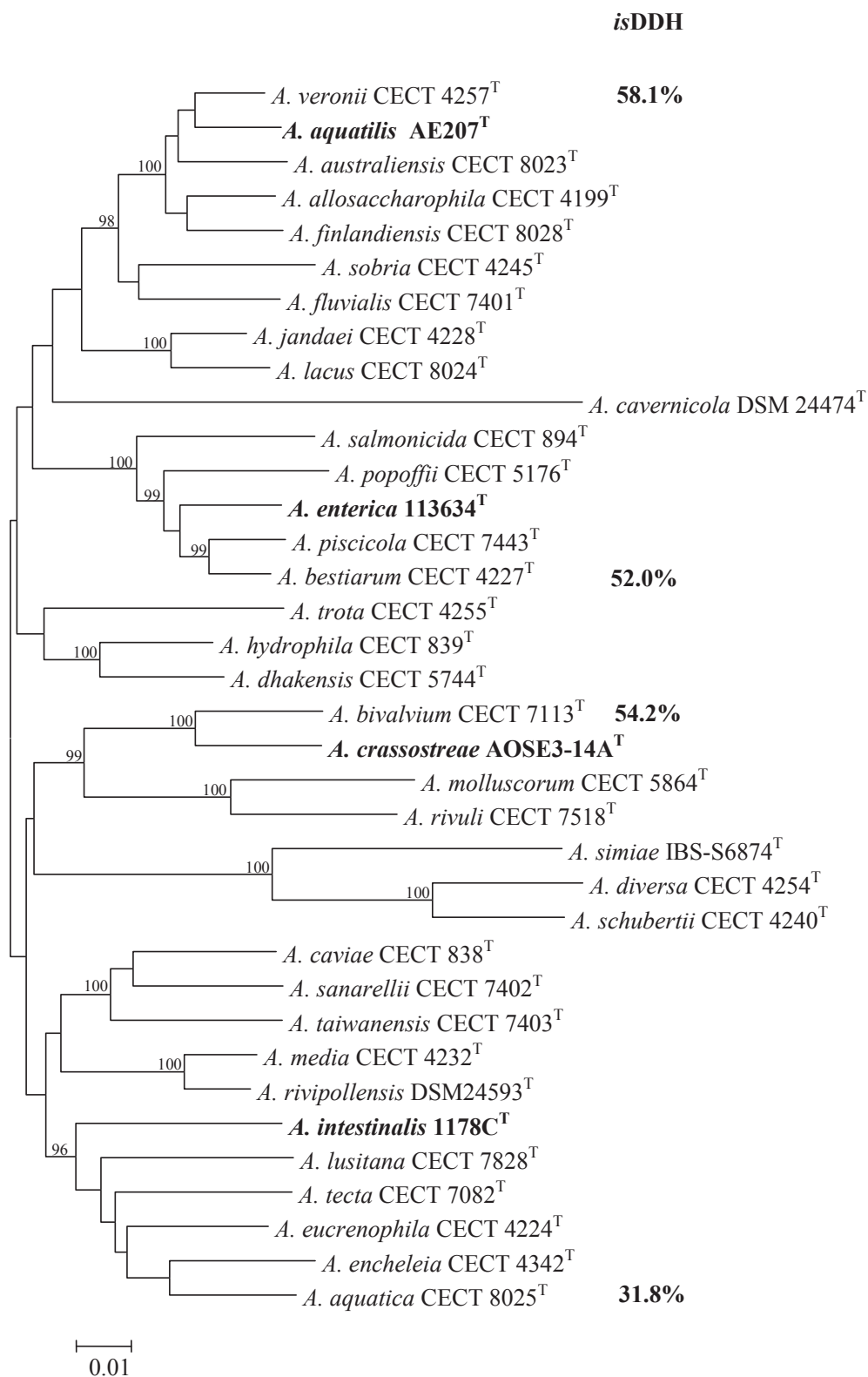


FIG. 1. Neighbour-joining phylogenetic tree obtained with concatenated sequences of six housekeeping genes (*rpoD*, *gyrB*, *gyrA*, *recA*, *dnaJ* and *dnaX*, 3558 bp) showing position of four new *Aeromonas* spp. (bold) relative to 32 *Aeromonas* spp. Numbers at nodes represent bootstrap percentages obtained by repeating analysis 1000 times. Scale bar = 0.01 estimated substitution per site. *In silico* DNA-DNA hybridization (isDDH) represents genetic similarity obtained between genome of each of four new species and genomes of their closest neighbour species.

The clinical strains 1178C^T and 113634^T were initially identified with MicroScan W/A and Vitek II, respectively. In both cases, the identifications obtained were erroneous and masked these new species under the species *A. hydrophila* and *A. sobria*, respectively.

More than one phenotypical characteristic distinguished the four new species from their closest relatives. The most important were the nonacid production from D-sucrose but production from lactose by strain 1178C^T, the production of acid from L-fucose by 113634^T, the use DL-lactate by AOSE3-14A^T and the mannitol-negative reaction of strain AE207^T that also showed to be susceptible to the vibriostatic agent O/129.

The names proposed are as follows: '*Aeromonas intestinalis*' (in.tes.ti.na'lis, N.L. fem. adj. *intestinalis*, 'pertaining to the intestine'), '*Aeromonas enterica*' (en.te'ri.ca, Gr. n. *enteron*, 'gut, bowel, intestine'; L. fem. suff. *-ica*, suffix used with the sense 'pertaining to'; N.L. fem. adj. *enterica*, 'pertaining to intestine'), '*Aeromonas crassostreae*' (crass.os'tre.ae, N.L. gen. n. *crassostreae*, 'of the oyster genus *Crassostrea*') and '*Aeromonas aquatilis*' (a.qua'ti.lis, L. fem. adj. *aquatilis* 'aquatic, growing in water').

Nucleotide sequence accession number

The GenBank/European Molecular Biology Laboratory/DNA Data Bank of Japan accession numbers of the 16S rRNA gene sequences of strains 1178C^T (= CECT 8980^T = LMG 29048^T), 113634^T (= CECT 8981^T = LMG 29049^T), AOSE3-14A^T (= CECT 8982^T = LMG 29050^T) and AE207^T (= CECT 8026^T = LMG 26714^T) are LT630759, LT630760, LT630761 and LT630765, respectively. The *rpoD*, *gyrB*, *gyrA*, *recA*, *dnaj* and *dnaX* of the other strains of the four novel species have also been deposited under the accession numbers LT630710–LT630712 and LT630716, LT630717–LT630719 and LY630723, LT630724–LT630726 and LT630730, LT630731–LT630733 and LT630737, LT630738–LT630740 and LT630744, LT630745–LT630747 and LT630751, respectively.

Deposit in a culture collection

All the type strains have been deposited in the Culture Collections of Spain (CECT) and Belgium (LMG): '*A. intestinalis*' 1178C^T (= CECT 8980^T = LMG 29048^T), '*A. enterica*' 113634^T

(= CECT 8981^T = LMG 29048^T), '*A. aquatilis*' AE207^T (= CECT 8026^T = LMG 26714^T) and '*A. crassostreae*' AOSE3-14A^T (= CECT 8982^T = LMG 29050^T).

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.nmni.2016.11.019>.

Conflict of Interest

None declared.

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