

Taxonomic Note

The reference strain *Aeromonas hydrophila* CIP 57.50 should be reclassified as *Aeromonas salmonicida* CIP 57.50

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The use of reference strains is a critical element for the quality control of different assays, from the development of molecular methods to the evaluation of antimicrobial activities. Most of the strains used in these assays are not type strains and some of them are cited erroneously because of subsequent reclassifications and descriptions of novel species. In this study, we propose that the reference strain *Aeromonas hydrophila* CIP 57.50 be reclassified as *Aeromonas salmonicida* CIP 57.50 based on phenotypic characterization and sequence analyses of the *cpn60*, *dnaJ*, *gyrB* and *rpoD* genes.

The selection and use of reference strains that are genetically stable is a critical element for achieving quality control goals in antimicrobial susceptibility testing. The reclassification of recognized species and the description of novel species can make it difficult to keep track of the most up to date taxonomic opinion and can lead to confusing citations. As an example, the quality control strain ATCC 43055 can be cited either as *Eggerthella lenta* or *Eubacterium lentum* and both names have been validly published. However, the most recent taxonomic opinion places this strain as a member of the genus *Eggerthella* (Wade *et al.*, 1999). This problem can also occur at an interspecific level if the strain used for quality control is not the type strain, for example, *Aeromonas hydrophila* ATCC 7965 (=NCTC 7812=CIP 57.50) is a reference strain frequently used to assess antimicrobial susceptibilities, the sensitivity and specificity of PCR protocols and stress responses, among other assays (Isonhood *et al.*, 2002; Huddleston *et al.*, 2006; Skočibušić *et al.*, 2006; Al Amri *et al.*, 2007; Giddens & Bean, 2007; Gordon *et al.*, 2008).

Over the past few years, the taxonomy of the genus *Aeromonas* has become increasingly complex due to the description of several novel species and the rearrangement of species described thus far (Miñana-Galbis *et al.*, 2007; Demarta *et al.*, 2008; Beaz-Hidalgo *et al.*, 2009). An

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA, *cpn60*, *dnaJ*, *gyrB* and *rpoD* gene sequences of *A. hydrophila* CIP 57.50 are FJ936134, FJ936135, FJ936136, FJ936137 and FJ936138, respectively.

The alignments and the distance matrix for the *cpn60*, *dnaJ*, *gyrB* and *rpoD* gene sequences of *A. hydrophila* strain CIP 57.50 with the type strains of *A. bestiarum*, *A. hydrophila* and *A. salmonicida* are available with the online version of this paper.

example of this complexity is the difficulty in discriminating between the phenotypically and genetically closely related *A. hydrophila*, *Aeromonas bestiarum* and the mesophilic strains of *Aeromonas salmonicida* (Miñana-Galbis *et al.*, 2002; Martínez-Murcia *et al.*, 2005). Nevertheless, alternative methods for species delineation, e.g. multilocus enzyme electrophoresis (MLEE) and housekeeping gene sequencing, have clarified most of the controversial taxa found in the genus *Aeromonas* (Miñana-Galbis *et al.*, 2004, 2009; Soler *et al.*, 2004; Nhung *et al.*, 2007).

In our laboratory, we have performed an extensive phenotypic characterization, PCR amplification and sequencing of the 16S rRNA, *cpn60*, *dnaJ*, *gyrB* and *rpoD* genes for *A. hydrophila* strain CIP 57.50 (=ATCC 7965=NCTC 7812) using previously described methods (Soler *et al.*, 2004; Miñana-Galbis *et al.*, 2007, 2009; Nhung *et al.*, 2007). *A. hydrophila* strain CIP 57.50, a mesophilic, motile and non-pigmented strain, was phenotypically identified as *A. salmonicida* after testing positive for sorbitol fermentation and negative for lactate assimilation. These results contradicted those expected for a strain belonging to *A. hydrophila* (Miñana-Galbis *et al.*, 2002, 2007). Nevertheless, the strain could not be assigned to any of the subspecies of *A. salmonicida* since most of these are psychrophilic and non-motile (*A. salmonicida* subsp. *achromogenes*, *A. salmonicida* subsp. *masoucida*, *A. salmonicida* subsp. *salmonicida* and *A. salmonicida* subsp. *smithia*). *A. salmonicida* subsp. *pectinolytica*, the only mesophilic subspecies described to date, is non-motile, pigmented and negative for the lysine decarboxylase test and for aesculin hydrolysis, opposite characteristics to those found for *A. hydrophila* strain CIP 57.50 (Pavan *et al.*, 2000; Martin-Carnahan & Joseph, 2005).

Table 1. Sequence distances (%) between *A. hydrophila* strain CIP 57.50 and the type strains of *A. bestiarum*, *A. hydrophila* and *A. salmonicida*

Sequence alignments and distances (Jukes–Cantor model and pairwise deletion option) were calculated with MEGA4 software.

Type strains	16S rRNA gene		<i>cpn60</i>		<i>dnaJ</i>		<i>gyrB</i>		<i>rpoD</i>	
	%	GenBank nos	%	GenBank nos	%	GenBank nos	%	GenBank nos	%	GenBank nos
<i>A. bestiarum</i>	0.0	X60406	6.0	EU306796	6.3	AB280554	3.0	AY101774	7.5	AY169326
<i>A. hydrophila</i> subsp. <i>hydrophila</i>	1.1	X60404	7.0	EU306804	10.2	AB280561	7.7	AY101778	11.8	AY169325
<i>A. hydrophila</i> subsp. <i>ranae</i>	1.2	AJ508766	8.0	EU306805	11.0	AB280562	8.6	AM262162	10.2	EF465509
<i>A. salmonicida</i> subsp. <i>achromogenes</i>	0.1	X60407	1.1	EU306824	1.8	AB280568	1.3	AY101785	2.3	AY169329
<i>A. salmonicida</i> subsp. <i>masoucida</i>	0.1	X74680	1.1	EU306825	1.7	AB280569	1.2	AY101786	2.4	AY169330
<i>A. salmonicida</i> subsp. <i>pectinolytica</i>	0.1	AF134065	1.3	EU306827	2.4	AB280570	1.9	AY101810	2.3	AY169324
<i>A. salmonicida</i> subsp. <i>salmonicida</i>	0.0	X60405	1.1	EU306828	1.9	AB280571	1.5	AY101773	2.5	AY169327
<i>A. salmonicida</i> subsp. <i>smithia</i>	0.1	AB027544	1.1	EU306829	1.8	AB280572	1.4	AM262159	2.4	AY169331

The 16S rRNA gene sequence of *A. hydrophila* strain CIP 57.50 (GenBank accession no. FJ936134) was identical to those of *A. bestiarum* CIP 74.30^T and *A. salmonicida* subsp. *salmonicida* NCIMB 1102^T and showed a single nucleotide difference with respect to *A. salmonicida* subsp. *pectinolytica* 34mel^T. Differences of two nucleotides were found when strain CIP 57.50 was compared with the type strains of the other subspecies of *A. salmonicida*. Distance values >1% (16–17 nucleotide differences) were obtained between the 16S rRNA gene sequence of *A. hydrophila* CIP 57.50 and those of the type strains of the two subspecies of *A. hydrophila* (Table 1).

In contrast, the sequencing of housekeeping genes allowed us to identify *A. hydrophila* strain CIP 57.50 to the species level. Previous studies (Soler *et al.*, 2004; Nhung *et al.*, 2007; Miñana-Galbis *et al.*, 2009) have established the following intraspecific threshold values based on divergences of gene sequences: 3.5% for *cpn60*, 3.3% for *dnaJ*, 2.3% for *gyrB* and 2.6% for *rpoD*. The sequences of the *cpn60*, *dnaJ*, *gyrB* and *rpoD* genes in *A. hydrophila* strain CIP 57.50 (GenBank accession nos FJ936135, FJ936136, FJ936137 and FJ936138, respectively) showed sequence distances below the interspecific threshold values with respect to those of the type strain of *A. salmonicida*, while the values were clearly higher than those of the type strains of *A. hydrophila* and *A. bestiarum* (Table 1). Moreover, *A. hydrophila* strain CIP 57.50 could be distinguished from all of the subspecies of *A. salmonicida*, showing a distance range of 1.1–2.5% depending on the gene or subspecies analysed (Table 1). The alignments and the distance matrix for the *cpn60*, *dnaJ*, *gyrB* and *rpoD* gene sequences are available as supplementary material in IJSEM Online.

Although a low discriminatory power between highly related species of the genus *Aeromonas* is obtained from 16S rRNA gene sequence analysis, it has been demonstrated that sequencing of housekeeping genes is an effective method for discriminating between species of the genus *Aeromonas* (Küpfer *et al.*, 2006; Saavedra *et al.*, 2006;

Miñana-Galbis *et al.*, 2009). The results obtained in this study strongly suggest that the reference strain *A. hydrophila* CIP 57.50 (=ATCC 7965=NCTC 7812) belongs to the species *A. salmonicida* rather than *A. hydrophila*.

In order to avoid other possible misclassifications, it would be useful if the reference strains deposited in culture collections were updated and checked using the latest identification tools since 16S rRNA gene sequencing is often insufficient for the identification of species. Additionally, considering the numbers of descriptions of novel species published each year, the number of key phenotypic tests used to identify isolates at the species level should be increased.

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