

The recently proposed species *Aeromonas sharmana* sp. nov., isolate GPTSA-6^T, is not a member of the genus *Aeromonas*

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Summary. A new species of the genus *Aeromonas*, *Aeromonas sharmana* sp. nov., was recently described on the basis of a single isolate, strain GPTSA-6^T, obtained from a warm spring in India. The description of this new species included biochemical characterization, antibiotic susceptibility, cellular fatty-acid profiles, and 16S rRNA gene sequencing, but not DNA-DNA hybridization data. In the present article, phylogenetic analysis (branch distances in the tree and nucleotide signatures) of the 16S rRNA of isolate GPTSA-6^T, together with certain phenotypic characteristics of the isolate reported in the earlier description, clearly indicate that this microorganism does not belong to the genus *Aeromonas* as known to date, although it falls within the radiation of the family *Aeromonadaceae*. Emendation from the List of Prokaryotic Names with Standing in Nomenclature is consequently proposed. [*Int Microbiol* 2007; 10(1):61-64]

Key words: *Aeromonas sharmana* · *Aeromonas* · phylogenetic analysis · phenotypic characterization

Introduction

Species of *Aeromonas* are common inhabitants of aquatic environments and have been described in connection with fish and human diseases [1,5,19]. This genus belongs to the family *Aeromonadaceae* [4,14,28] and, according to the last edition of *Bergey's Manual of Systematic Bacteriology* [12], the genus comprises the following species: *Aeromonas hydrophila*, *Aeromonas bestiarum*, *Aeromonas salmonicida*,

Aeromonas caviae, *Aeromonas media*, *Aeromonas eucrenophila*, *Aeromonas sobria*, *Aeromonas veronii* (biovars *sobria* and *veronii*), *Aeromonas jandaei*, *Aeromonas schubertii*, *Aeromonas trota*, *Aeromonas allosaccharophila*, *Aeromonas encheleia*, *Aeromonas popoffii*, and the two DNA homology groups, *Aeromonas* sp. (HG11), *Aeromonas* sp. (HG13; formerly Enteric Group 501), that remain without a species name. Three new species, *Aeromonas culicicola* [17], *Aeromonas simiae* [6], and *Aeromonas molluscorum* [16] have recently been described. Furthermore, *Aeromonas enteropelogenes* [23], *Aeromonas ichthiosmia* [24], and *Aeromonas culicicola* [17] are now considered synonyms of *A. veronii*, *A. trota*, and *A. veronii*, respectively [2,3,7–9,12,18].

In a recent issue of the *International Journal of Systematic and Evolutionary Microbiology*, Saha and Chakrabarti

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[21] proposed the new taxa "*Aeromonas sharmana* sp. nov." for the single strain GPTSA-6^T isolated from a warm spring in India. The description of this new species included biochemical characterization, antibiotic susceptibility, cellular fatty-acid profiles, and 16S rRNA gene sequencing. DNA-DNA hybridization data were not provided. The name has already been added to the List of Prokaryotic Names with Standing in Nomenclature [<http://www.bacterio.cict.fr/>] and the NCBI taxonomy database [<http://www.ncbi.nlm.nih.gov/Taxonomy/Browser>]. Strain duplicates are available as MTCC7090^T and DSMZ1744^T, and the 16S rRNA sequence accession number is DQ013306. In the present study, strain DSMZ1744^T was subjected to 16S rRNA sequencing to confirm whether it belonged or not to the genus *Aeromonas*.

Materials and methods

Bacterial strains and culture conditions. Isolate GPTSA-6^T was obtained from the German Collection of Microorganisms as strain DSMZ1744^T. The strain was grown on TSA (Trypticase Soy Agar, Difco, Barcelona, Spain) at 30°C.

16S rRNA gene sequencing. Genomic DNA extraction and PCR amplification were carried out as previously described [15]. PCR products were purified with the Concert Rapid PCR Purification System (Life Technologies; Barcelona, Spain) following the manufacturer's instructions. Sequencing primers were those described by Martínez-Murcia et al. [15] and sequences were determined by using the BigDye Terminator V3.1 Cycle Sequencing Kit in the ABI 3100-Avant Genetic Analyzer (Applied Biosystems), according to the manufacturer's instructions and services supplied by the Molecular Diagnostics Center (MDC), Orihuela, Spain.

Phylogenetic data analysis. The nucleotide sequences were aligned according to the Clustal X program, version 1.8 [27]. For alignments, previously published reference sequences of *Aeromonas* spp. [13,14,18] and these of other genera retrieved from the EMBL database were used. Genetic distances were obtained by Kimura's 2-parameter model [10] and evolutionary trees were constructed by the neighbor-joining method [22] with the MEGA program [11].

Results and Discussion

The 16S rRNA gene sequence of "*Aeromonas sharmana* sp. nov.", strain DSMZ1744^T, was determined and compared with the sequence of isolate GPTSA-6^T deposited in the public databases under accession number DQ013306 [<http://www.ncbi.nlm.nih.gov>]. Nucleotide differences were not found. The sequence was aligned with 16S rRNA from all currently described *Aeromonas* species [18] and with those of representative species of several related genera belonging to the families *Aeromonadaceae*, *Enterobacteriaceae*, and *Vibrionaceae*, which were retrieved from the NCBI database. The resulting phylogenetic tree (Fig. 1), consistent with the

one reported in the species description [21], showed that this strain clustered at a relatively long distance from the genus *Aeromonas*.

A preliminary assessment of the data published by Saha and Chakrabarti [21] indicated that isolate GPTSA-6^T does not belong to the genus *Aeromonas*, as currently defined. The 16S rRNA sequence of this isolate was newly determined to confirm the published data. Neighbor-joining 16S rRNA phylogenetic analysis indicated that strain GPTSA-6^T falls within the radiation of the family *Aeromonadaceae*, having the genus *Aeromonas* as its closest phylogenetic neighbor (Fig. 1), in agreement with the tree shown by Saha and Chakrabarti [21]. However, this strain is clearly well-separated from the *Aeromonas* cluster, as revealed by the relatively long distance of branches between the new isolate and all *Aeromonas* species. The highest 16S rRNA sequence similarity values were reported to range between 94.8 and 95.1% for the 1430 nucleotides of isolate GPTSA-6^T and the homologous sequences of *Aeromonas* species. The authors concluded that "sequence similarity found between GPTSA-6^T and various species of the genus *Aeromonas* ($\leq 95.13\%$) strongly indicates that this strain represents a novel species". However, we would argue that this low similarity indicates just the opposite, i.e., that the strain does not belong to *Aeromonas*. Our conclusion is based on the shallow phylogenetic depth of the 16S rRNA tree of the genus *Aeromonas*, as defined 15 years ago, which shows that interspecies sequence similarity values range between 96.7 and 100% [14,20]. Recent updates of the phylogeny, including the addition of new species [12,13,18], has not changed this picture. Despite the fact that there are no generally approved threshold values for 16S gene similarities with respect to genus delineation, it is obvious that the phylogenetic distance, or sequence similarity, found between the proposed new species and the aeromonads better fits a genus-to-genus relationship.

As an example, inter-genus sequence similarity for *Plesiomonas shigelloides* and *Serratia marcescens* is ca. 94.6%, for *Escherichia coli* and *Salmonella* sp. ca. 96.6%, both within the radiation of the family *Enterobacteriaceae*. Note that these values are quite different from those found between *Aeromonas* and the other genera provisionally included in the *Aeromonadaceae* (91.95% with *Tolumonas auensis*, 90.70% with *Oceanimonas doudoroffii*, and 90.57% with *Oceanisphaera litoralis*), reinforcing the questioned validity of their placement as stated in *Bergey's Manual* [12]. These low percentages are similar to those found between genera belonging to different families of the Gamma-proteobacteria, for instance *E. coli*-*Aeromonas* spp. (90.3%), *Aeromonas* spp.-*Vibrio anguillarum* (90-91%), and the vibrios versus some enterobacteria, with values around 90% [14].

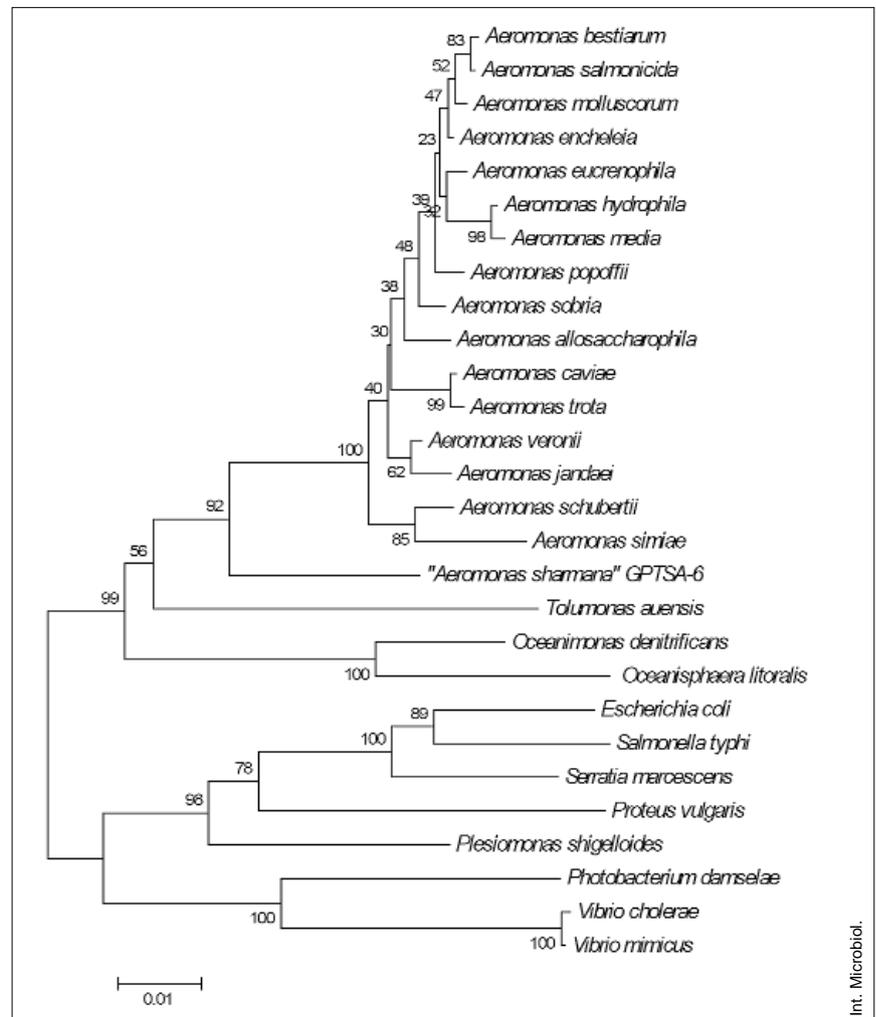


Fig. 1. Phylogenetic tree showing the 16S rRNA sequence relationships of “*Aeromonas sharmana*” sp. nov., strain GPTSA-6^T, to all currently described *Aeromonas* species and representative species of some related genera belonging to families *Aeromonadaceae*, *Enterobacteriaceae*, and *Vibrionaceae*.

In addition, Saha and Chakrabarti [21] indicated that when 16S rRNA gene sequence similarities are lower than 97%, overall genomic relatedness is less than 70% [25]. Thus, only a basal relationship is to be expected from the hybridization of total chromosomal DNA belonging to strains from different genera, as is the case with strain GPTSA-6^T and *Aeromonas*. So far, all species of *Aeromonas* have been defined on the basis of DNA-DNA hybridization studies because their interspecies 16S rRNA relationships are ca. 98% or higher (in most cases > 99%), which corresponds to the 16S rRNA gene similarity threshold value found for the majority of bacterial genera.

Also, a detailed analysis of the deposited 16S rRNA sequence of isolate GPTSA-6^T after alignment with these of the genus *Aeromonas* showed significant nucleotide differences in positions that are conserved in all *Aeromonas* spp. As only two examples, the two 16S rRNA signature regions located at positions 86–106 and 584–604 are absolutely conserved for many hundreds of strains belonging to all *Aeromonas* spp.,

characterized in our laboratory at the Molecular Diagnostics Center (MDC). These are genus-specific targets for PCR detection applied directly to pure cultures or for preliminary screening of colonies. However, the sequence of strain GPTSA-6^T showed, respectively, three and four mismatches (plus a deletion). These genus-specific signatures and many others, lend additional support for the finding that this strain is outside the phylogenetic frame of the genus *Aeromonas*.

Phenotype continues to play an important role for species delineation [26]. Strain GPTSA-6^T does not fulfil several of the genus-specific characters of *Aeromonas* because it does not reduce nitrate to nitrite and a catalase reaction was not clearly evident. While catalase is a key for *Aeromonadaceae*, GPTSA-6^T showed a “very weakly positive” catalase reaction; the interpretation of this test seems subjective, as the response to a catalase test is usually clear-cut. In other words, a “very weak” result may be considered negative. In addition, Saha and Chakrabarti [21] indicated that certain fatty acids present in many members of the genus *Aeromonas* are not

detected in strain GPTSA-6^T. In our opinion, all these phenotypic characteristics are consistent with the genetic data, indicating that strain GPTSA-6^T is not a member of the genus *Aeromonas*.

Note that the 16S rRNA sequence of strain GPTSA-6^T [accession number DQ013306, <http://www.ncbi.nlm.nih.gov>], was first submitted under “*Manjusharmella aquatica*”, gen. nov., sp. nov., after Dr Manju Sharma, as a novel genus of the family *Aeromonadaceae*” (unpublished, as of May 10, 2005). By clicking on the organism name, more details were obtained about this taxon deposited in the NCBI taxonomic database. For example, the name “*Halofoba aquatica*” was also given. These names were not validly published at the time of submission of the corresponding sequence entry; however, it is evident that, initially, the rank of genus was proposed for this bacterium by the same authors, as all the above-discussed data indeed seem to indicate.

In our view, it also does not seem reasonable to amend the genus description (phylogenetic depth based on the 16S rRNA gene and genus phenotypic characters) to accommodate the single strain GPTSA-6^T. Therefore, for the time being and awaiting further research, the genus status, and perhaps the name *Manjusharmella aquatica* tentatively suggested by Saha and Chakrabarti [21], must be reconsidered for strain GPTSA-6^T. Consequently, the name “*Aeromonas sharmana* sp.nov.” should be amended in the List of Prokaryotic Names with Standing in Nomenclature.

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