Aeromonas sharmana sp. nov., isolated from a warm spring

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A Gram-negative, facultatively anaerobic bacterial strain designated GPTSA-6T was isolated from a water sample collected from a warm spring in Assam, India. Preliminary analysis of the 16S rRNA gene sequence of this isolate revealed its affiliation to the family Aeromonadaceae. Detailed characterization using a polyphasic approach indicated that strain GPTSA-6T is most closely related to Aeromonas sobria but differs significantly from existing members of the genus Aeromonas. Analysis of the almost-complete (1430 nt) 16S rRNA gene sequence of this strain revealed that its closest relative (99.23% similarity) is an uncultured bacterial clone, A-8, isolated from an algal bloom. Of the taxa with validly published names, Aeromonas sobria ATCC 43979T showed the highest level of sequence similarity (95.13%) with respect to strain GPTSA-6T, followed by Aeromonas molluscorum 848TT and Aeromonas popoffii LMG 17541T (95.04% similarity in both cases). On the basis of the phenotypic, chemotaxonomic and phylogenetic data, it can be concluded that strain GPTSA-6T represents a novel species of the genus Aeromonas, for which the name Aeromonas sharmana sp. nov. is proposed. The type strain is GPTSA-6T (=MTCC 7090T=DSM 17445T).

The family Aeromonadaceae was proposed on the basis of a collection of molecular genetic data, and its phylogenetic locus was suggested as being intermediate between the families Vibrionaceae and Enterobacteriaceae (Colwell et al., 1986). Aeromonads are autochthonous to aquatic environments worldwide and are the usual microbiota of fish, amphibians and other animals (Min˜ana-Galbis et al., 2004). At the time of writing, the family Aeromonadaceae is represented by four genera, Aeromonas, Tolumonas, Oceanimonas and Oceanisphaera (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Root). Of these genera, Aeromonas is the largest, containing 20 species plus 12 subspecies. Tolumonas, Oceanimonas and Oceanisphaera contain one, three and one species, respectively (http://www.bacterio.cict.fr/index.html). In the present communication, we report the taxonomic characterization of strain GPTSA-6T, and, on the basis of the polyphasic analysis, propose that it represents a novel species of the genus Aeromonas.

Strain GPTSA-6T was isolated from water sampled from a warm spring, using dilution plating on TSBA (tryptic soy broth plus 1.5% agar; HiMedia) medium. All phenotypic characterizations were carried out according to standard methods (Cowan & Steel, 1965; Smibert & Krieg, 1994; Murray et al., 1994; Powers, 1995). Growth at various temperatures, pH values and NaCl concentrations was checked on basal TSBA medium. The cells of strain GPTSA-6T were found to be Gram-negative, motile, short rods. Transmission electron microscopy, performed as described previously (Pandey et al., 2002), demonstrated the presence of a single polar flagellum on each cell (see Supplementary Fig. S1 available in IJSEM Online). Detailed characteristics are given in the species description.

Antibiotic sensitivity was checked on Mueller–Hinton agar, using antibiotic-susceptibility discs (HiMedia) at the following antibiotic concentrations: ampicillin (10 μg), bacitracin (8 U), cephalothin (30 μg), chloramphenicol (30 μg), colistin (10 μg), erythromycin (15 μg), gentamicin (10 μg), kanamycin (30 μg), lincomycin (2 μg), meticillin (5 μg) neomycin (30 μg), nitrofurantoin (300 μg), norfloxacin (10 μg), novobiocin (30 μg), penicillin G (10 U), polymyxin B (300 U), rifampicin (2 μg), streptomycin (10 μg), sulfasomidine (300 μg) and tetracycline (3 μg). Susceptibility to the vibriostatic compound O/129 (150 μg) was checked on TSBA medium.

For cellular fatty acid analyses, the strain was grown on TSBA medium at 30 °C for 24 h. Extraction and analysis of the cellular fatty acids were performed according to the procedures for the SHERLOCK Microbial Identification system (MIDI) as described previously (Pandey et al., 2002). The fatty acid profile of strain GPTSA-6T showed a
predominance of saturated and unsaturated unbranched fatty acids and included C_{16:0} (29-0 %), summed feature 3 (C_{16:1}ω7c and/or C_{15:0} iso 2-OH; 29-3 %), C_{18:1}ω7c (24-0 %), C_{12:0} (6-1 %), summed feature 2 (C_{14:0} 3-OH and/or C_{16:1} iso 1; 5-6 %) and C_{14:0} (4-7 %). An increased in the incubation time (to 48 h at 30 °C) did not seem to alter the overall fatty acid profile of the strain.

The genomic G+C content of the strain was determined spectrophotometrically as described previously (Saha et al., 2005). The G+C content of the strain was found to be 60-7 mol%.

Amplification of the 16S rRNA gene from strain GPTSA-6ᵀ was done with primers 27f (5’-AGAGTTTGATCCTG-GCTCAG-3’) and 1492r (5’-TACGGYTACCTTGGTATCAG-3’). The amplification reaction and purification of amplicons were performed as described previously (Pandey et al., 2002). The amplified product was sequenced by the dideoxy chain terminator method, using the BigDye Terminator kit (Perkin-Elmer), followed by capillary electrophoresis on an ABI 310 Genetic Analyzer (Applied Biosystems). An almost-complete (1430 nt) 16S rRNA gene was obtained using the dideoxy chain terminator method, followed by capillary electrophoresis on an ABI 310 Genetic Analyzer (Applied Biosystems). An almost-complete (1430 nt) 16S rRNA gene was used as the query to search for homologous sequences in the GenBank database. Sequence analysis revealed that its closest relative (99-04 %) was an uncultured bacterial clone, A-8, reported during analysis of dissolved organic matter and a bacterial community involved in the degradation of an algal bloom (Kasuga et al., 2003). With regard to cultured bacteria, strain GPTSA-6ᵀ showed most similarity with Aeromonas sobria ATCC 43979ᵀ (95-13 %) followed by Aeromonas molluscum 8487ᵀ (95-04 %), Aeromonas popoffii LMG 17541ᵀ (95-04 %), Aeromonas eucrenophila ATCC 2309ᵀ (94-92 %), Aeromonas encheleia CECT 4342ᵀ (94-90 %), Aeromonas salmonicida ATCC 33658ᵀ (94-85 %), Aeromonas veronii ATCC 35624ᵀ (94-85 %), Aeromonas caviae ATCC 15468ᵀ (94-84 %), Aeromonas bestiarum CIP 7430ᵀ (94-77 %), Aeromonas allosaccharophila CECT 4199ᵀ (94-77 %) and Aeromonas hydrophilia ATCC 7966ᵀ (94-77 %). Sequence similarity with other species of the genus Aeromonas was less than 94-77 %. The strain showed much less sequence similarity with Tolunomas auensis DSM 9187ᵀ (91-95 %), Oceanimonas doudoroffii ATCC 27123ᵀ (90-70 %) and Oceanisphaera litoralis DSM 15406ᵀ (90-57 %), the type strains of the type species of the three other genera within the family Aeromonadaceae. Sequences from its closest uncultured relative and 22 type strains representing different species of the genera Aeromonas, Tolunomas, Oceanimonas and Oceanisphaera of the family Aeromonadaceae and of the genus Vibrio within the family Vibrionaceae were used for phylogenetic analysis. All these sequences were aligned by the CLUSTAL_X program (Thompson et al., 1997) and edited manually. Aligned sequences were analysed by the PHYLIP software package version 3.5c (Felsenstein, 1993). Pairwise evolutionary distances for the aligned sequences were computed using the DNADIST program with the Kimura two-parameter model (Kimura, 1980). To obtain a confidence value for the aligned sequence dataset, bootstrap analysis of 100 replications was done using SEQBOOT. A phylogenetic tree showing the relationship between GPTSA-6ᵀ and other

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*DWPP, Drinking-water production plant; F, fish; MF, monkey faeces; W, water; WES, wedge shells; WSW, water from warm spring.

Table 1. Differential characteristics of strain GPTSA-6ᵀ and related Aeromonas species

Taxa: 1, strain GPTSA-6ᵀ; 2, A. sobria (data from Popoff, 1984; Holt et al., 1994); 3, A. simiae (Harf-Montell et al., 2004); 4, A. hydrophila (Popoff, 1984; Holt et al., 1994); 5, A. molluscum (Minaña-Galbis et al., 2004); 6, A. popoffii (Huys et al., 1997). Symbols: +, 85–100 % of strains positive; −, >85 % of strains negative; V, around 50 % of strains positive; V+, 50–84 % of strains positive; d, 11–89 % of strains positive; ND, data not available.
reference strains was constructed using the neighbour-
joining method (Saitou & Nei, 1987) and the UPGMA
algorithm. Distance matrix data obtained from DNASIT
were also used to construct a phylogenetic tree, by using
KITSCH. Consensus trees for each of these methods
were generated using CONSENSE from the PHYLIP package.
Distance-based phylogenetic analysis was also performed
with the TREECON software package (Van de Peer & De
Wachter, 1997), using both Kimura (Kimura, 1980) and
Jukes–Cantor (Jukes & Cantor, 1969) correction. Irrespec-
tive of the tree-generation software packages used, the
overall tree topologies were similar in all cases. Phylogenetic
analyses revealed that strain GPTSA-6T falls within the
radiation of the family Aeromonadaceae (neighbour-joining
analysis shown in Fig. 1). However, together with its
closest uncultured bacterial relative, it formed a cluster
that was well separated from the Aeromonas cluster and the
Tolumonas–Oceanimonas–Oceanisphaera cluster with a very
high bootstrap value.

The low levels of sequence similarity found between
GPTSA-6T and various species of the genus Aeromonas
(≤95.13 %) strongly indicate that this strain represents a
novel species. It is generally accepted that, when 16S rRNA
gene sequence similarity is lower than 97 %, overall genomic
relatedness is less than 70 % (Stackebrandt & Goebel, 1994),
and these two criteria are considered crucial for prokaryotic
classification purposes. On the basis of phenotypic properties,
strain GPTSA-6T could be differentiated from its five closest
phylogenetic relatives belonging to the genus Aeromonas
(Table 1). The type strains of Oceanimonas doudoroffii and
Oceanisphaera litoralis are both strictly aerobic, negative
for asesculin hydrolysis, can reduce nitrate to nitrite, can
grow at 10 °C, tolerate 7 % NaCl, cannot grow in medium
lacking NaCl, all of which contrast with the properties of
strain GPTSA-6T, and they have G + C contents of 54 and
56.4 mol%, respectively (Baumann et al., 1983; Brown
et al., 2001; Romanenko et al., 2003). Similarly, T. auensis
differs from strain GPTSA-6T in being non-motile, in being
unable to utilize cellulose, δ-lactose, maltose, δ-mannitol,
and sucrose but being able to hydrolyse Tweens 40 and 80,
and in having a comparatively low genomic G + C content
(49 mol%) (Fischer-Romo et al., 1996). In terms of the fatty acid composition, fatty acids C13:0 iso, C13:0 C17:0 iso,
C17:1ω8c, C15:0 iso, C16:0 iso, C17:1ω9c iso, C16:1ω9c alcohol, which are present in many members
of the genus Aeromonas (Huys et al., 1994), were not
detectable in strain GPTSA-6T. On the whole, GPTSA-6T
is phylogenetically related to the members of the genus
Aeromonas. Thus, on the basis of the results of our poly-
phasic approach, we conclude that strain GPTSA-6T repre-
sents a novel species within the genus Aeromonas, for which
we propose the name Aeromonas sharmana sp. nov.

Description of Aeromonas sharmana sp. nov.

Aeromonas sharmana (shar.ma’na. N.L. fem. adj. sharmana
named after Dr Manju Sharma, a biologist and a great
proponent of research on microbial diversity in India).

Gram-negative, facultatively anaerobic, mesophilic, motile,
short rods occurring in singly or in pairs. Single polar
flagellum. Colonies on TSA after 36 h growth are round,
convex and generally irregular margins, opaque and whitish

Fig. 1. Neighbour-joining phylogenetic tree showing the relationships between strain GPTSA-6T and related taxa. The tree
was drawn using TREECON with the Kimura correction. Bootstrap values above 50 are shown at the nodes. Escherichia coli
ATCC 11775T was used as an outgroup. Bar, 0.02 base substitutions per site.
in colour without the production of any diffusible pigment. Cells are 1–2 μm long and 0·4–0·5 μm wide. Oxidase-positive and very weakly positive for catalase. Grows at temperatures between 15 and 42 °C, at pH 5·7–8·0 and can tolerate 2 % NaCl. Does not produce indole, gas from glucose, H2S, gelatinase, phenylalanine deaminase, DNase, arginine dihydrolase, lysine decarboxylase or ornithine decarboxylase. Hydrolyses starch, asculin and ONPG but not casein, urea, fat or Tween 20, 40 and 80. Does not reduce nitrate to nitrite. Produces acid from arbutin, D-melezitose, D-raffinose, L-rhamnose, D-ribose, D-sorbitol, L-sorbose, xylitol, D-xylose and L-xylose. Utilizes L-arabino-, D-cellobiose, D-fructose, D-glucose, D-galactose, nystose, D-arabitol, dulcitol, glycerol, myo-inositol, D-melibiose, D-melezitose, D-raffinose, L-rhamnose, D-ribose, D-sorbitol, L-sorbose, xylitol, D-xylose and L-xylose. Utilizes L-arabinose, D-cellobiose, D-fructose, D-glucose, D-galactose, D-lactose, D-mannose, D-maltose, sucrose and D-xylose (weakly) but not D-arabinose, L-arabitol, arbutin, D-amygdalin, adonitol, dulcitol, glycerol, melezitose, L-rhamnose, D-ribose, D-sorbitol, L-sorbose, acetate, citrate, fumarate, glutarate, malate, propionate or succinate as sole carbon sources. The type strain is resistant to the vibriostatic compound O/129, lincomycin and methicillin but is susceptible to ampicillin, bacitracin, cephalothin, chloramphenicol, colistin, erythromycin, gentamicin, kanamycin, neomycin, nitrofurantoin, novobiocin, polymyxin B, penicillin G, rifampicin, streptomycin, sulfasomidine and tetracycline. The major whole-cell fatty acids are C16:0 (29·0%), summed feature 3 (C16:1ω7c and/or C15:0 iso 2-OH; 29·3%), C18:1ω7c (24·0%), C12:0 (6·1%), summed feature 2 (C14:0 3-OH and/or C16:1 iso I; 5·6%) and C14:0 (4·7%). The genomic DNA G+C content is 60·7 mol%. The type strain, GPTSA-6T (=MTCC 7090T = DSM 17445T), was isolated from a water sample from a warm spring in Assam, India.

Acknowledgements

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