

Aquimonas voraii gen. nov., sp. nov., a novel gammaproteobacterium isolated from a warm spring of Assam, India

P. Saha,¹ S. Krishnamurthi,¹ S. Mayilraj,¹ G. S. Prasad,¹ T. C. Bora² and T. Chakrabarti¹

Correspondence

T. Chakrabarti

tapan@imtech.res.in

¹Microbial Type Culture Collection & Gene Bank (MTCC), Institute of Microbial Technology, Sector 39A, Chandigarh – 160 036, India

²Biotechnology Division, Regional Research Laboratory, Jorhat – 785 006, Assam, India

A bacterial strain designated GPTSA 20^T, which was isolated from a warm spring in Assam, India, was characterized by using a polyphasic approach. The cells were Gram-negative, aerobic rods, which could not utilize or produce acid from most of the carbohydrates tested. The predominant fatty acids were C_{15:0} iso (25.04%), C_{17:1} iso ω9c (19.28%), C_{16:0} iso (17.73%) and C_{11:0} iso 3-OH (9.34%). The G + C content was 75 mol%. From 16S rRNA gene sequence analysis (1433 nucleotides, continuous stretch), it was confirmed that strain GPTSA 20^T belonged to the class 'Gammaproteobacteria'. The closest 16S rRNA gene sequence similarity found (98.2%) was with an uncultured bacterium clone, NB-03 (accession no. AB117707), from an autotrophic nitrifying biofilm. Among culturable bacteria, the closest sequence similarities were with *Fulvimonas soli* (93.0%), *Silanimonas lenta* (92.8%), *Thermomonas hydrothermalis* (92.4%), *Frateruria aurantia* (91.9%), *Rhodanobacter lindaniclasticus* (91.9%), *Thermomonas haemolytica* (91.9%) and *Pseudoxanthomonas taiwanensis* (91.8%); similarities of less than 91.8% were obtained with other members of the class 'Gammaproteobacteria'. From the biochemical, physiological, chemotaxonomic and phylogenetic analysis, it was clear that strain GPTSA 20^T was quite different from members of known genera of the class 'Gammaproteobacteria'. Therefore, it is proposed that strain GPTSA 20^T represents a novel species within a new genus, with the name *Aquimonas voraii* gen. nov., sp. nov. The type strain is GPTSA 20^T (=MTCC 6713^T=JCM 12896^T).

The *Proteobacteria* constitute one of the major cosmopolitan phyla represented by cultivated bacteria (Hugenholtz *et al.*, 1998), and represent the largest phylogenetically coherent group, encompassing over 1300 species with validly published names (Garrity & Holt, 2001). Studies indicate that about 35% of 16S rRNA gene sequences from representatives of the phylum *Proteobacteria* in the ARB database are from uncultured bacteria (Hugenholtz *et al.*, 1998). In this study, we report the taxonomic characterization of a strain, designated GPTSA 20^T, which was isolated from a warm spring water sample collected from Assam, India.

The temperature of the warm spring was 38 °C and the pH was 7.2. The strain was isolated as a slow grower on tryptic

soy broth agar [TSBA; 3% Tryptic soy broth (Difco), 1.5% extra pure agar (Hi-Media)]. The colony morphology, motility and Gram reaction of the strain were determined by using standard methods (Smibert & Krieg, 1994; Murray *et al.*, 1994; Powers, 1995). Cells were 1.5–4.5 μm long and 0.3–0.4 μm wide (Fig. 1), as determined by scanning electron microscopy of a 3 day-old culture grown on TSBA. Growth at various temperatures, pH and NaCl concentrations was determined by using a basal TSBA medium with various pH values and NaCl concentrations. After 3 days of growth on TSBA, the colonies of strain GPTSA 20^T appeared circular, yellowish-brown in colour, glistening and convex, with undulated margins. Cells were Gram-negative, strictly aerobic motile rods. Growth occurred at 25–42 °C and pH 6.0–11.0. Concentrations of NaCl of up to 2% were tolerated. Various biochemical tests, such as hydrolytic and enzymic activities and utilization of various substrates, were performed at 30 °C, as described by Smibert & Krieg (1994). The biochemical and physiological characteristics of strain GPTSA 20^T are given in the genus and species descriptions. Based on the biochemical and physiological

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of *Aquimonas voraii* GPTSA 20^T is AY544768.

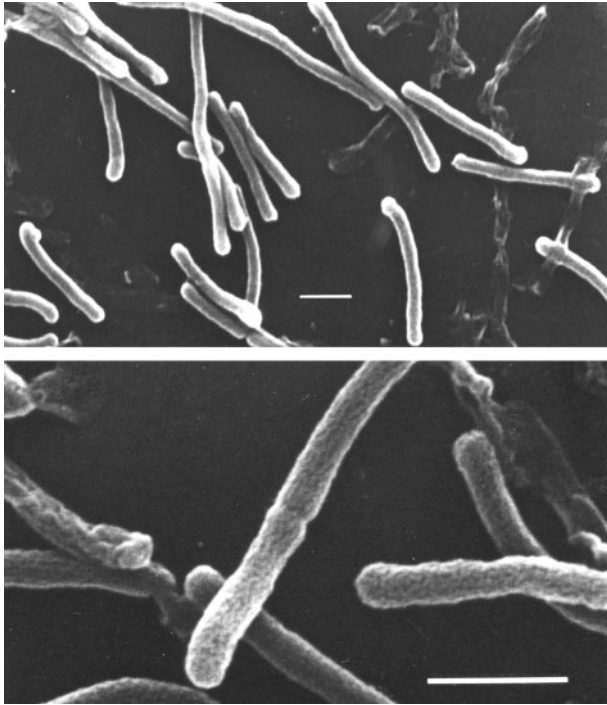


Fig. 1. Scanning electron micrographs of strain GPTSA 20^T. Bars, 1 μ m.

characteristics, it was not possible to place the strain into any known genus.

For cellular fatty acid analysis, the strain was grown on TSBA medium at 30 °C for 3 days. 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 20^T had a predominance of saturated, mono-unsaturated and saturated hydroxy branched fatty acids. The predominant fatty acids were C_{15:0} iso, C_{17:1} iso ω 9c, C_{16:0} iso and C_{11:0} iso 3-OH. The fatty acid profile is summarized in Table 1.

The genomic DNA of strain GPTSA 20^T was isolated by using Marmur's protocol (Johnson, 1994). The G + C content of genomic DNA was determined spectrophotometrically (Lambda 35 spectrophotometer; Perkin Elmer) by using the thermal denaturation method (Mandel & Marmur, 1968). The G + C content of strain GPTSA 20^T was 75 mol%. The full-length 16S rRNA gene of GPTSA 20^T was amplified by PCR using the primers 8-27f (5'-AGAGTTTGATCCTGGCTCAG-3') and 1500r (5'-AGAAA-GGAGGTGATCCAGGC-3'). PCR was performed as described previously (Pandey *et al.*, 2002). The product was purified by using a Qiaquick gel extraction kit (Qiagen), and sequenced by using the dideoxy chain-termination method with the BigDye terminator kit (Applied Biosystems). The primers used for sequencing were 8-27f, 357f (5'-CTCCTACGGGAGGCAGCAG-3'),

Table 1. Fatty acid profile of strain GPTSA 20^T grown on TSBA at 30 °C

Values represent the mean of five analyses. Each of the following (not shown) constitute less than 1% of the total: C_{16:1} iso H, C_{12:0} iso, C_{15:0} anteiso, C_{16:1} ω 7c alcohol, C_{16:1} ω 5c, C_{11:0} 2-OH, C_{13:0} iso, C_{10:0} iso and C_{10:0}.

Fatty acid	Content (%)
C _{11:0} iso	7.44
C _{11:0} iso 3-OH	9.34
C _{14:0} iso	1.41
C _{14:0}	1.11
C _{15:1} iso F	2.29
C _{15:0} iso	25.04
C _{16:0} iso	17.73
C _{16:0}	4.52
C _{17:1} iso ω 9c	19.28
C _{17:0} iso	2.82
Summed feature 3*	4.16

*Summed feature 3 comprises C_{15:0} iso 2-OH and/or C_{16:1} ω 7c.

1110r (5'-GGGTTGCGCTCGTTG-3') and 1500r. The reaction products were analysed by capillary electrophoresis on an ABI 310 Genetic Analyzer (Applied Biosystems).

An almost complete (1433 nucleotides, continuous stretch) 16S rRNA gene sequence of strain GPTSA 20^T was used to search for similar sequences in the GenBank database. The closest similarity (98.2%) found was with an uncultured bacterial clone from an autotrophic nitrifying biofilm (Kindaichi *et al.*, 2004). The sequences of this uncultured bacterium and 15 other type strains with greater than 90.4% similarity to that of GPTSA 20^T were used for phylogenetic analysis. The sequences were aligned using the CLUSTAL_X program (Thompson *et al.*, 1997) and edited manually. Aligned sequences were analysed by using the PHYLIP software package, version 3.5c (Felsenstein, 1993). Pairwise evolutionary distances for the aligned sequences were computed using the DNADIST program with Kimura's two-parameter model (Kimura, 1980). To obtain a confidence value for the aligned sequence dataset, bootstrap analysis of 100 replications was performed using SEQBOOT. A phylogenetic tree showing the relationships between GPTSA 20^T and other reference strains was constructed using the neighbour-joining method (Saitou & Nei, 1987) and the unweighted pair group arithmetic average (UPGMA) linkage algorithm. Distance matrix data obtained from DNADIST 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. Analysis of the almost complete (1433 nucleotides) 16S rRNA gene sequence of GPTSA 20^T revealed that the strain belonged to the class '*Gammaproteobacteria*', as indicated by the signature oligonucleotides CTAATA-CCG, YCACAYYG (Y=pyrimidine), CTAACYYG and

TCACACCATG, which are present at approximate positions 170, 315, 510 and 1410 (*Escherichia coli* numbering system; Brosius *et al.*, 1978), respectively, in all members of the class ‘*Gammaproteobacteria*’ (Woese, 1987; Stackebrandt *et al.*, 1988; Hauben *et al.*, 1997). From sequence analysis at the RDP site (Ribosomal Database Project-II release 9; <http://rdp.cme.msu.edu/index.jsp>), it was apparent that GPTSA 20^T was a member of the *Rhodanobacter lindaniclasticus* subgroup, within the *Xanthomonas* group of the class ‘*Gammaproteobacteria*’. Among the cultured bacteria, the maximum similarity of the 16S rRNA gene sequence found was with *Fulvimonas soli* (93.0%), followed by *Silanimonas lenta* (92.8%), *Thermomonas hydrothermalis* (92.4%), *R. lindaniclasticus* (91.9%), *Frateuria aurantia* (91.9%), *Thermomonas haemolytica* (91.9%), *Pseudoxanthomonas taiwanensis* (91.8%), *Thermomonas fusca* (91.5%), *Thermomonas brevis* (91.5%), *Lysobacter enzymogenes* (91.4%), *Luteimonas mephitis* (91.2%), *Pseudoxanthomonas broegbernensis* (91.0%), *Stenotrophomonas maltophilia* (90.8%), *Xanthomonas campestris* (90.6%) and *Xanthomonas axonopodis* (90.4%). At the phylogenetic level, strain GPTSA 20^T formed a distinct cluster with an uncultured ‘*Gammaproteobacteria*’ phylotype, separate from the *Fulvimonas*–*Rhodanobacter*–*Frateuria* cluster (Fig. 2). The genomic relatedness of GPTSA 20^T with these taxa may not be high, because it has been observed that, when the 16S rRNA gene sequence similarity between bacterial strains is less than 97%, the whole genomic DNA–DNA hybridization level is less than 70% (Stackebrandt & Goebel, 1994).

The biochemical characteristics, fatty acid profile, mol% G+C content (Table 2) and 16S rRNA gene sequence of strain GPTSA 20^T were quite different from those of its phylogenetically close relatives. Moreover, the closest similarity (93.0%) of the 16S rRNA gene sequence of GPTSA 20^T with a culturable bacterium (*Fulvimonas soli*) was much lower than 95%, a value that was proposed as a ‘practicable

border zone for genus definition’ (Ludwig *et al.*, 1998). We therefore propose that strain GPTSA 20^T represents a novel species within a new genus, for which the name *Aquimonas voraii* gen. nov., sp. nov., is proposed.

Description of *Aquimonas* gen. nov.

Aquimonas (A.qui.mo’nas. L. n. *aqua* water; L. fem. n. *monas* a unit, monad; N.L. fem. n. *Aquimonas* a water monad, referring to the isolation of the type species from a warm spring water sample).

Gram-negative, strictly aerobic, mesophilic, motile rods, occurring singly or in pairs. Oxidase- and catalase-positive, but negative for H₂S production, acid production from D-cellobiose and growth on maltose. Major whole-cell fatty acids are C_{15:0} iso, C_{17:1} iso ω9c, C_{16:0} iso and C_{11:0} iso 3-OH. The proposed genus is affiliated to the class ‘*Gammaproteobacteria*’, with only one proposed species.

The type species is *Aquimonas voraii*.

Description of *Aquimonas voraii* sp. nov.

Aquimonas voraii (vo’ra.i.i. N.L. masc. gen. n. *voraii* named after V.C. Vora, a distinguished biotechnologist and founder director of the Institute of Microbial Technology, Chandigarh, India).

In addition to the description of the genus, the species has the following characteristics. Colonies on TSBA after 3 days of incubation at 30 °C appear circular, yellowish-brown, glistening and convex, with undulated margins. Cells are 1.5–4.5 μm long and 0.3–0.4 μm wide. Growth occurs at 25–42 °C and pH 6.0–11.0, and NaCl concentrations of up to 2% can be tolerated. Hydrolyses casein, starch, gelatin and Tweens 20, 40 and 80, but not urea, aesculin or *o*-nitrophenyl galactoside. Cannot ferment lactose and cannot utilize L-arabinose, L-arabitol, arbutin, D-amygdalin,

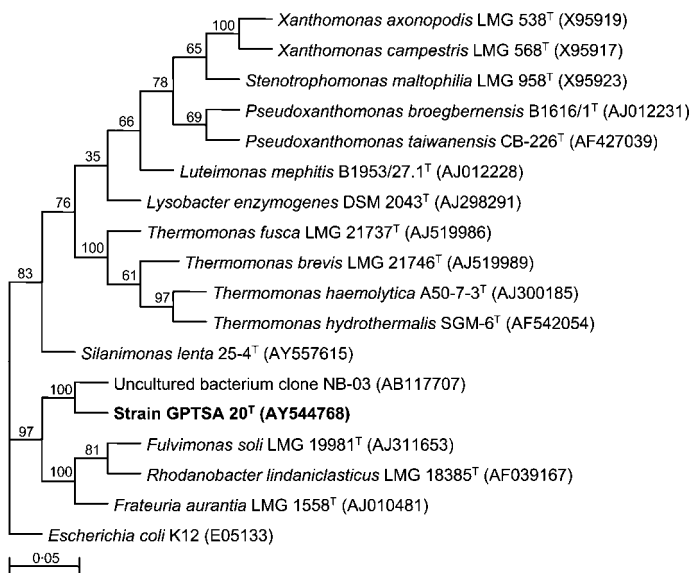


Fig. 2. Phylogenetic tree based on the neighbour-joining method showing the relationships between strain GPTSA 20^T and related taxa. Bootstrap values as percentages of 100 replications are shown at the branch points. The 16S rRNA gene sequence of *E. coli* K12 was used as an outgroup. Bar, 0.05 substitution per site.

Table 2. Comparison of phenotypic characteristics and G+C content of strain GPTSA 20^T with other closely related species

Strain/species: 1, GPTSA 20^T; 2, *Fulvimonas soli* (data from Mergaert *et al.*, 2002); 3, *R. lindaniclasticus* (Nalin *et al.*, 1999); 4, *Frateriuria aurantia* (Swings *et al.*, 1984); 5, *T. hydrothermalis* (Alves *et al.*, 2003); 6, *S. lenta* (Lee *et al.*, 2005). +, Positive; -, negative; ND, not determined.

Characteristic	1	2	3	4	5	6
Motility	+	+	-	+/-	-	+
Growth temperature	Mesophilic	Mesophilic	Mesophilic	Mesophilic	Slightly thermophilic	Slightly thermophilic
Starch degradation	+	+	-	-	ND	+
Hydrolysis of:						
Aesculin	-	+	+	-	+	-
Gelatin	+	-	ND	-	+	+
H ₂ S production	-	ND	ND	+	ND	ND
Acid production from:						
D-Cellobiose	-	ND	+	ND	+	ND
D-Fructose	-	ND	-	ND	ND	+
D-Glucose	-	-	+	+	+	-
D-Xylose	-	-	+	+	ND	ND
D-Mannose	-	-	-	+	ND	-
L-Arabinose	-	-	-	+	ND	ND
Growth on maltose	-	+	-	+	+	ND
Major fatty acids	C _{15:0} iso, C _{17:1} iso ω9c, C _{16:0} iso, C _{11:0} iso 3-OH	C _{15:0} iso, C _{17:1} iso ω9c, C _{17:0} iso	ND	ND	C _{15:0} iso, C _{16:0} iso, C _{11:0} iso, C _{11:0} iso 3-OH	C _{15:0} iso, C _{16:0} iso, C _{17:0} iso, C _{17:0} iso cis9
G + C content (mol%)	75	71.7	63	62-64	64.7	50.7

D-cellobiose, D-fructose, D-glucose, glycerol, D-galactose, *myo*-inositol, D-lactose, D-maltose, D-mannitol, D-mannose, D-melibiose, D-melezitose, D-raffinose, L-rhamnose, D-ribose, D-sorbitol, L-sorbose, D-xylose, acetate, citrate, fumarate, glutarate, malate, propionate or succinate as sole carbon sources, but weakly utilizes dulcitol, adonitol and D-arabinose. Acid is not produced from any of the carbohydrates mentioned above, and also from inulin, salicin, sucrose, trehalose, xylitol or L-xylose. Negative for indole formation, oxidation-fermentation reaction, methyl red and Voges-Proskauer tests, nitrate reduction and utilization of xylan, cellulose and dextrin. Negative for lysine and ornithine decarboxylase, DNase and phenylalanine deaminase activities. Grows on nutritionally poor medium such as TSBA100 (TSB, 100-fold diluted and solidified with agarose) and, therefore, may be considered as a facultative oligophile. The genomic G + C content is 75 mol%.

The type strain is GPTSA 20^T (=MTCC 6713^T=JCM 12896^T), which was isolated from a warm spring water sample collected from Assam, India.

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