Fontimonas thermophila gen. nov., sp. nov., a moderately thermophilic bacterium isolated from a freshwater hot spring, and proposal of Solimonadaceae fam. nov. to replace Sinobacteraceae Zhou et al. 2008

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A novel bacterial strain designated HA-01T was isolated from a freshwater terrestrial hot spring located at Hot Springs National Park, Arkansas, USA. Cells were Gram-negative-staining, rod-shaped, aerobic, chemo-organotrophic, oxidase- and catalase-positive, non-spore-forming and motile by means of a single polar flagellum. Growth occurred at 37–60 °C, with an optimum between 45 and 50 °C, and at pH 6.5–8.5, with an optimum between pH 6.5 and 7.0. Phylogenetic analysis based on 16S rRNA gene sequences indicated that the closest relatives of strain HA-01T were Solimonas aquatica NAA-16T (93.8 %), Solimonas flava CW-KD 4T (94.1 %), Solimonas soli DCY12T (93.1 %), Solimonas variicoloris MN28T (94.0 %), Nevskia ramosa Soe1T (91.2 %) and Hydrocarboniphaga effusa AP103T (91.1 %). Major fatty acids consisted of C16:0, iso-C16:0, C16:1ω5c and summed feature 8 (C18:1ω9c, C18:1ω7c and C18:1ω6c). Polar lipids consisted of diphosphatidylglycerol, phosphatidylglycerol and phosphatidylethanolamine, and the major isoprenoid quinone was Q-8. The DNA G+C content was 64.4 mol%. Based on phylogenetic, phenotypic and chemotaxonomic evidence, it is proposed that strain HA-01T represents a novel species in a new genus for which the name Fontimonas thermophila gen. nov., sp. nov. is proposed. The type strain of the type species is HA-01T (＝DSM 23609T＝CCUG 59713T). A new family, Solimonadaceae fam. nov., is also proposed to replace Sinobacteraceae Zhou et al. 2008.

During the course of an investigation into the microbial community structure of the Hale House Hot Spring, located at Hot Springs National Park, Arkansas, USA, a novel bacterial strain was isolated. Phylogenetic analyses using 16S rRNA gene sequences demonstrated that the novel organism belonged to the class Gammaproteobacteria and represents a new line within a cluster of organisms that includes the genera Solimonas, Hydrocarboniphaga, Alkanibacter, Steroidobacter and Nevskia. In particular, the novel strain shared an affinity with members of the genus Solimonas (approximately 93–94 % gene sequence similarity). However, phylogenetic, physiological, biochemical and chemotaxonomic analyses clearly showed that the novel strain should be placed as a novel species within a new genus.

Abbreviation: FAME, fatty acid methyl ester.
The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of Fontimonas thermophila gen. nov., sp. nov. HA-01T is JN415769.

Three supplementary figures and two supplementary tables are available with the online version of this paper.
During the characterization of the micro-organisms from a freshwater artesian spring that erupts in the Hale Bathhouse in Hot Springs, Arkansas, USA (longitude: −93° 3’ 13”, latitude: 34° 30’ 47”), strain HA-01T was isolated and selected for detailed analysis. The physical conditions of the Hale House Spring include a NaCl concentration of less than 10 mg l⁻¹, a temperature of 63.7 °C and a pH of 6.7 (Bell & Hays, 2007). Strain HA-01T was isolated from spring water inoculated directly onto Castenholz plates (Castenholz medium, DSMZ medium no. 86, www.dsmz.de/microorganisms/media_list.php) which were incubated at 60 °C (Brock & Freeze, 1969).

Isolation was achieved by repeated transfers onto fresh medium at 60 °C. Once isolated into pure culture, cells were routinely cultured on plates containing yeast extract and tryptone at a concentration of 2 g l⁻¹, at 60 °C. Long-term storage was performed by adjusting cultures to a 20 % glycerol concentration (v/v) and storage at −80 °C. Growth was assessed on LB (Difco), TSA (Difco) and R2A (Difco) plate media. Cell morphology and motility were examined via phase-contrast microscopy using an Olympus CX41 microscope. Flagella staining was performed using Ryu flagella stain according to the methods of Heimbrook et al. (1989). Growth characteristics of the strain were investigated under a variety of conditions using a simple yeast extract and tryptone broth at a concentration of 2 g l⁻¹ to 3 % (w/v, tested at intervals of 0.5 %). Growth on substrate concentrations of 2 g l⁻¹ (Chang et al., 2004). All growth experiments were performed with shaking at 200 r.p.m. and monitored via a Spectronic 20D+ (Thermo) at 600 nm for a period of up to 10 days. Catalase production was tested for using a 3 % H₂O₂ solution and the presence of oxidase was tested for using a Ryu flagella stain (Fig. S2). Growth occurred on TSA and R2A, but not on LB plates. After 4 days of growth on R2A at 45 °C, colonies were approximately 1.0–1.5 mm in diameter, transparent, pale yellow, smooth, convex and circular. Catalase and oxidase activities were positive. Growth was observed at temperatures between 37 and 60 °C with an optimum between 45 and 50 °C. In addition, growth also occurred between pH 6.5 and 8.5 with an optimum between pH 6.5 and 7.0. No growth was observed under anaerobic conditions. The results for the Biolog and API test systems are given in the species description.

Only complex substrates containing amino acids such as yeast extract, tryptone, Casamino acids or peptone produced growth. No growth was observed in the presence of the following substrates: acetate, citrate, lactate, L-proline, L-glutamate, L-arginine, L- aspartic acid, pyruvate, glucose, fructose, benzoate, ethanol, succinate, arabinose or xylose. Starch was not hydrolysed, nitrate reduction was not observed, and no DNase activity was observed.

DNA was isolated using a standard phenol:chloroform extraction using a modification of the method described by Lawson et al. (1989). DNA G+C mol% content of DNA was determined by HPLC (Mesbah et al., 1989) using a Prevail C18 reversed-phase column (Alltech) at room temperature with a mobile phase of 25 mM KH₂PO₄:CH₃CN (96 : 4), pH 2.5. The G+C content of the genomic DNA was 64.4 mol%. The universal bacterial primers 27F (5'-AGAGTTTGATCCTGTCAG) and 1492R (5'-GGCTACCTTGTTACGACTT) (numbers of primers refer to 5'-positions relative to Escherichia coli 16S rRNA) were used to amplify the 16S rRNA genes as previously described (Rios-Hernandez et al., 2003). The amplified products were treated with ExoSAP-IT (USB Corporation) to remove excess primers and nucleotides and directly sequenced with primers directed towards conserved positions of the rRNA gene (Hutson et al., 1993). Sequencing reactions were performed using the BigDye Terminator, version 3.1, cycle sequencing kit (Applied Biosystems) as described by the manufacturer and were analysed using an Applied Biosystems 3730 DNA Analyser. The closest known relatives of the new isolate was identified by performing database searches using the FASTA program (Lipman & Pearson, 1985). These sequences and those of other related strains were retrieved from GenBank and aligned with the newly determined sequence using CLUSTAL via the MEGA5 program (Tamura et al., 2011). Phylogenetic analyses were conducted in MEGA5 using the neighbour-joining method (Saitou & Nei, 1987). The stability of the groupings was estimated by bootstrap analysis (1000 replications). All major branching nodes were confirmed by maximum-parsimony and maximum-likelihood analyses (data not shown) and confirmed the robust nature of this group of organisms, the branching order and high bootstrap values. The results of the phylogenetic analyses indicated that the novel organism

available in IJSEM online). A single polar flagellum was observed with a Ryu flagella stain (Fig. S2). Growth occurred on TSA and R2A, but not on LB plates. After 4 days of growth on R2A at 45 °C, colonies were approximately 1.0–1.5 mm in diameter, transparent, pale yellow, smooth, convex and circular. Catalase and oxidase activities were positive. Growth was observed at temperatures between 37 and 60 °C with an optimum between 45 and 50 °C. In addition, growth also occurred between pH 6.5 and 8.5 with an optimum between pH 6.5 and 7.0. No growth was observed under anaerobic conditions. The results for the Biolog and API test systems are given in the species description.

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belonged to the class *Gammaproteobacteria*. The novel bacterium was most closely related to *Solimonas aquatica* NAA-16<sup>T</sup>, *Solimonas flava* CW-KD 4<sup>T</sup>, *Solimonas soli* DCY12<sup>T</sup> and *Solimonas variicoloris* MN28<sup>T</sup>, with pairwise similarity values of 93.8%, 94.1%, 93.1% and 94.0%, respectively (Fig. 1). More distantly related organisms included Alkanibacter difficilis DSM 14084<sup>T</sup> (91.2%), Hydrocarboniphaga effusa AP103<sup>T</sup> (91.1%), Nevskia ramosa Soe<sup>T</sup> (91.2%) and Steroidobacter denitrificans DSM 18526<sup>T</sup> (87.0%). This cluster was supported by a significant bootstrap value of 100%. There is no precise correlation between 16S rRNA gene sequence divergence and species delineation, but it is generally recognized that divergence values of 3% or more are significant (Stackebrandt & Goebel, 1994). The sequence divergence values of 6% or greater, displayed between the novel isolate and species within the genus *Solimonas*, combined with physiological and chemotaxonomic criteria strongly suggested that the new isolate represented a novel genus.

Fatty acid methyl ester (FAME) analysis was performed on cells grown for 3 days on R2A agar at 45 °C. Biomass was recovered from the third quadrants representing growth in the late-exponential phase as recommended by the manufacturer. The fatty acids were determined using the MIDI Sherlock Microbial Identification System (Version 6.1, QI-TSA database), as described previously (Kämpfer & Kroppenstedt, 1996; Miller, 1982; Sasser, 1990). A complex FAME profile was generated for strain HA-01<sup>T</sup>, with the major fatty acids consisting of C<sub>16:0</sub>, iso-C<sub>16:0</sub>, C<sub>16:1ω5c</sub> and summed feature 8 (probably C<sub>18:1ω7c</sub>). These results are similar to those for the related genera *Solimonas*, *Hydrocarboniphaga* and *Nevskia* which contain C<sub>16:0</sub> and C<sub>18:1ω7c</sub> as the predominant fatty acids, however differences are present in the differentiation of these taxa (Table 1). It is possible that minor perturbations are a result of different incubation temperatures, an unavoidable aspect given the different temperature optima of the various organisms. A detailed FAME profile comparison between strain HA-01<sup>T</sup> and the closest phylogenetic relatives is provided in Table S1.

Lipid and quinone analyses were carried out by the Identification Service of the DSMZ and Dr B. J. Tindall, DSMZ, Braunschweig, Germany (Tindall et al., 2007). Several polar lipids were detected including diphasphatidylglycerol (DPG), phosphatidylglycerol (PG), phosphatidylethanolamine (PE), an unknown phospholipid (PL1) as well as two unidentified aminolipids (AL1, AL2) (Fig. S3). Phylogenetic analysis demonstrated that the novel organism's closest relatives were members of the genus *Solimonas*. Although the novel bacterium shared some similarities with these organisms, the patterns for members of the genus *Solimonas* generally appear to be more complex with a greater number of unidentified compounds (Shue et al., 2011). The novel strain contained a ubiquinone with eight isoprene units (Q-8) that is commonly found in species belonging to the class *Gammaproteobacteria*.

In addition to its unique 16S rRNA gene sequence, there are a number of features that may be used to clearly distinguish the novel organism from members of other genera; most notable is that the novel species represents, to the authors’ knowledge, the first reported thermophilic isolate within the related genera of *Solimonas* (Shue et al., 2011), *Hydrocarboniphaga* (Liu et al., 2011), *Nevskia* (Kim et al., 2011), *Alkanibacter* (Friedrich & Lipski, 2008) and *Steroidobacter* (Fahrbach et al., 2008). The novel bacterium possesses an optimal growth temperature of between 45 and 50 °C and readily grows at 60 °C, a value considerably higher than those reportedly used for cultivation of members of related genera. The use of molecular methods, and in particular 16S rRNA gene sequencing, allows almost unequivocal identification of micro-organisms and is now advocated for the initial screening of isolates (Rainey, 2011). Therefore the isolation of similar strains or closely related taxa will be facilitated by the characteristics given in Tables 1 and S2. Based on phylogenetic, biochemical and chemotaxonomic properties, we propose that the unknown rod-shaped strain should be classified as representing a new genus, *Fontimonas* gen. nov., with the type species *Fontimonas thermophila*. Sheu et al. (2011) recently reclassified *Sinobacter flavus*, the sole member of the genus *Sinobacter* and the type genus for the family *Sinobacteraceae*, as *Solimonas flava* (Zhou et al., 2008). With the genus name *Sinobacter* now defunct there is now
### Table 1. Characteristics that are useful in the differentiation of strain HA-01T from related taxa

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Sterotaxa</th>
<th>Solimonas</th>
<th>Nokia</th>
<th>Hydroaromaphaga</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Flagellar arrangement</strong></td>
<td>Single polar</td>
<td>Single polar</td>
<td>Single polar</td>
<td>Single polar</td>
</tr>
<tr>
<td><strong>Oxidase</strong></td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td><strong>Nitrate reduction</strong></td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td><strong>Temperature range for growth (°C)</strong></td>
<td>37–60</td>
<td>23–37</td>
<td>10–42</td>
<td>10–42</td>
</tr>
<tr>
<td><strong>DNA G+C content (mol%)</strong></td>
<td>C₆₅₋₇₅</td>
<td>C₆₅₋₇₅</td>
<td>C₆₅₋₇₅</td>
<td>C₆₅₋₇₅</td>
</tr>
<tr>
<td><strong>Major cellular fatty acids</strong></td>
<td>i-C₁₆₋₇₅, C₁₈₋₁₀₉₆, summed feature 8</td>
<td>i-C₁₆₋₇₅, C₁₈₋₁₀₉₆, summed feature 8</td>
<td>i-C₁₆₋₇₅, C₁₈₋₁₀₉₆, summed feature 8</td>
<td>i-C₁₆₋₇₅, C₁₈₋₁₀₉₆, summed feature 8</td>
</tr>
<tr>
<td><strong>Hydrocarbons</strong></td>
<td>DPG, PG, PE, AL</td>
<td>DPG, PG, PE, AL</td>
<td>DPG, PG, PE, AL</td>
<td>DPG, PG, PE, AL</td>
</tr>
<tr>
<td><strong>Polar lipids</strong></td>
<td>DPG, PG, PE, AL</td>
<td>DPG, PG, PE, AL</td>
<td>DPG, PG, PE, AL</td>
<td>DPG, PG, PE, AL</td>
</tr>
</tbody>
</table>

Data from Sheu et al. (2011), Fahrbach et al. (2008), Liu et al. (2011), Kim et al. (2011). DPG, diphosphatidylglycerol; PE, phosphatidylethanolamine; PL, unknown phospholipids; APL, unknown aminophospholipids; AL, unknown aminolipids; L, unknown polar lipids. Summed feature 3 is composed of iso-C₁₅₋₀, C₁₆₋₀, C₁₈₋₀ and summed feature 8 is composed of C₁₈₋₀, i-C₁₆₋₀ and C₁₈₋₁₀₉₆. Positive, negative, ND, not determined or no data available. All strains were reported to have been rod-shaped, Gram-negative-staining, catalase-positive and contain Q-8 as the major isoprenoid quinone.

### Description of Fontimonas thermophila gen. nov., sp. nov.

**Fontimonas** [Fonti.m.o.nas. L. adj. fontis a spring, fountain; L. fem. n. monas a unit, monad; N.L. fem. n. Fontimonas a monad (bacterium) isolated from a spring).

Cells are Gram-negative-staining, aerobic, non-spore-forming rods. Motile by a single polar flagellum. Aerobic and chemo-organotrophic. Oxidase and catalase-positive. Capable of growth at 60 °C. Major fatty acids (>10%) are C₁₆₋₀, iso-C₁₆₋₀, C₁₆₋₁₀₉₆ and summed feature 8 (C₁₈₋₁₀₉₆, C₁₈₋₁₀₉₆ and C₁₈₋₁₀₉₆). Polar lipids are diphosphatidylglycerol, phosphatidylglycerol and phosphatidylethanolamine. The isoprenoid quinone is Q-8. The type strain is *Fontimonas thermophila* sp. nov.

### Description of Fontimonas thermophila sp. nov.

**Fontimonas thermophila** [ther.mo’phi.la. Gr. adj. thermos hot; N.L. adj. philus -a -um (from Gr. adj. philos -é -on) friend, loving; N.L. fem. adj. thermophila heat-loving).

Cells are Gram-negative, aerobic, non-spore-forming, rod-shaped and motile by a single polar flagellum. After 4 days of growth on R2A agar at 45 °C, colonies are transparent, pale yellow, smooth, convex, circular and approximately 1.0–1.5 mm in size. Growth occurs at 37–60 °C (optimum 45–50 °C), at pH 6.5–8.5 (optimum pH 6.5–7.0) and up to NaCl concentrations of 1% (w/v) (optimum 0%). Catalase- and oxidase-positive and does not reduce nitrate to nitrite. Does not demonstrate DNase activity or starch hydrolysis. Substrate utilization using standard methods only occurred in the presence of complex substrates containing amino acids including peptone, tryptone, Casamino acids and yeast extract. No growth under standard growth conditions with acetate, citrate, lactate, L-proline, L-glutamate, L-arginine, L-aspartic acid, pyruvate, glucose, fructose, benzoate, ethanol, succinate, arabinose or xylene. According to the GN2 Biolog identification system, the following substrates are utilized: Tween-40 and 80, α-ketovaleric acid, L-alaninamide, L-alanine, L-α-lactyl-glycine, γ-glutamyl-L-glutamic acid and L-proline. According to the GN2 Biolog identification system, the following substrates are weakly utilized, pyruvic acid methyl ester and glycol L-aspartic acid. Using the same system, negative reactions are found for utilization of x-cyclodextrin, dextrin, glycogen, N-acetyl-D-galactosamine, N-acetyl-D-glucosamine, adonitol, L-arabinose, D-arabitol, cellulbiose, i-erythritol, D-fructose, L-fucose, D-galactose, gentiobiose, α-D-glucose, myo-inositol, α-D-lactose, lactulose, maltose, D-mannitol, D-mannose, melibiose, methyl β-D-glucoside, D-psicose, raffinose, L-rhamnose, D-sorbitol, sucrose, trehalose, turanose, xylitol, acetic acid, cis-aconitic.
acid, citric acid, formic acid, D-galactonic acid lactone, D-galacturonic acid, D-glucosaminic acid, D-glucuronic acid, α-hydroxybutyric acid, β-hydroxybutyric acid, γ-hydroxybutyric acid, p-hydroxyphenylacetic acid, itaconic acid, α-ketobutyric acid, α-ketoglutaric acid, DL-lactic acid, malonic acid, propionic acid, quinic acid, D-sacharic acid, sebacic acid, succinic acid, bromosuccinic acid, succinamic acid, glucuronamide, D-alanine, L-asparagine, L-aspartic acid, L-glutamic acid, L-histidine, hydroxy-L-proline, L-leucine, L-ornithine, L-phenylalanine, L-tryptophan, γ-aminobutyric acid, urocanic acid, inosine, uridine, thymidine, phenylethylamine, putrescine, 2-aminoethanol, 2,3-butanediol, glycerol, DL-x-glycerol phosphate, α-D-glucose 1-phosphate and D-glucose 6-phosphate. Using the API ZYM kit, positive reactions were obtained for alkaline phosphatase, acid phosphatase, α-galactosidase, α-glucosaminidase, α-glucosidase, β-glucosidase, β-glucuronidase, γ-glucosidase, N-acetyl-β-glucosaminidase, ε-mannosidase and α-fucosidase are not detected. Major fatty acids (＞10 %) are C₁₆:0, iso-C₁₆:0, C₁₆:03c and summed feature 8 (C₁₈:1ω9c, C₁₈:1ω7c and C₁₈:1ω6c). Polar lipids are diphosphatidylglycerol, phosphatidylglycerol, phosphatidyl-N-palmitoyl-l-rhizochitin, phosphatidylethanolamine and an unknown phospholipid as well as two unidentified aminolipids. Respiratory quinone is Q-8.

The type strain, HA-O1T (=DSM 23609T=CCUG 59713T), was isolated from a freshwater hot spring located at Hot Springs National Park, Hot Springs, Arkansas, USA. The DNA G+C content of the type strain is 64.4 mol%.

**Description of Solimonadaceae fam. nov.**

*Solimonadaceae* (So’li.mo’na.da.ceae. N.L. masc. n. *Solimonas* type genus for the family; suff. -aceae ending to denote a family; N.L. fem. pl. n. *Solimonadaceae* the *Solimonas* family).

Cells are Gram-negative, may be non-motile or motile by means of a single polar flagellum. Non-endospore-forming, aerobic or facultatively anaerobic. Oxidase and catalase-positive. Q-8 is the predominate isoprenoid quinone. Major fatty acids are C₁₆:0, C₁₈:1ω9c, iso-C₁₆:0 and summed feature 8 (C₁₈:1ω9c, C₁₈:1ω7c or C₁₈:1ω6c). The pattern of 16s rRNA gene signatures of members of the family consists of nucleotides at positions 369 (G), 514 (U), 537 (A), 560 (U), 580–761 (U–A), 778–804 (U–U) and 1129–1143 (C–A). The family is a member of the class Gammaproteobacteria, order Xanthomadaceae. The family contains the genera *Solimonas* and *Fontimonas*. The type genus is *Solimonas*.

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**References**


