**Fabivirga thermotolerans** gen. nov., sp. nov., a novel marine bacterium isolated from culture broth of a marine cyanobacterium


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A Gram-stain-negative, red, non-spore-forming, strictly aerobic bacterium, designated strain A4T, was isolated from culture broth of a marine cyanobacterium. Cells were flexible rods with gliding motility. Phylogenetic analysis, based on 16S rRNA gene sequences, revealed that strain A4T formed a coherent cluster with members of the genera *Roseivirga* and *Fabibacter*, and represents a distinct lineage in the family *Flammeovirgaceae*. Thermotolerance and a distinctive cellular fatty acid profile could readily distinguish this isolate from any bacteria of the genera *Roseivirga* and *Fabibacter* with a validly published name. On the basis of the phenotypic, chemotaxonomic and phylogenetic characteristics, strain A4T is suggested to represent a novel species in a novel genus, for which the name *Fabivirga thermotolerans* gen. nov., sp. nov. is proposed. The type strain is A4T (=KCTC 42507T =CGMCC 1.15111T).

The genus *Roseivirga* was firstly established by Nedashkovskaya *et al.* (2005a), whereafter the description of the genus *Roseivirga* was emended twice (Nedashkovskaya *et al.*, 2005b; Nedashkovskaya *et al.*, 2008). One year later, the genus *Fabibacter*, close phylogenetically to the genus *Roseivirga*, was proposed by Lau *et al.* (2006). Additionally, bacteria from the genera *Roseivirga* and *Fabibacter* share many phenotypic features and, perhaps, will be joined to form a single genus (Nedashkovskaya & Ludwig, 2010). However, from the point of view of the heterogeneity of the family *Flammeovirgaceae*, it should be noted that *Roseivirga* can be considered to be a member of a novel family in the future (Nedashkovskaia & Ludwig, 2010). Thus, considering the possibility of a novel family represented by *Roseivirga* in the future, the genus *Fabibacter* should be preserved at present. In this study, a marine bacterium, isolated from the culture broth of a seawater-dependent cyanobacterium, was subjected to polyphasic taxonomic characterization. Phylogenetic analysis demonstrated that it has a close relationship with members of the genera *Roseivirga* and *Fabibacter*. Phylogenetic, morphological, phenotypic and chemotaxonomic profiles from this study and from the literature support strain A4T representing a novel species of a novel genus, for which the name *Fabivirga thermotolerans* gen. nov., sp. nov. is proposed.

Aquatic samples were collected from a culture pond of a marine cyanobacterium, *Plectonema* sp., on 25th December, 2014. The algae broth was diluted to 10⁻³ fold with sterilized seawater and 100 µl of the diluted solution was spread on marine agar 2216 (MA, Difco) supplemented with 30% (v/v) glycerol at 25 °C and in marine broth 2216 (MB, Difco) supplemented with 30% (v/v) glycerol at -80 °C. The type strains *Roseivirga ehrenbergii* KMM 6017T, *Roseivirga spongicola* UST030701-084T, *Roseivirga marina* PSR1T, *Fabibacter halotolerans* UST030701-097T, *Fabibacter pacificus* DY-53T and *Fabibacter misakiensis* SK-8T were obtained from the Zhejiang University, the Second Institute of Oceanology-State Oceanic Administration People’s Republic of China (SIO-SOA) and the Korean Collection for type Cultures (KCTC) to be used as reference strains in the following studies.

Genomic DNA extraction of strain A4T was performed by following the protocols described by Barbeyrion *et al.* (1984). An almost-complete 16S rRNA gene sequence was obtained using PCR with the universal primers 27F and 1492R (Lane, 1991). The 16S rRNA gene sequence of strain A4T was aligned with that of closely related...
members from the EzBiocloud database (Kim et al., 2012) using the SINA software package in the SILVA rRNA database (Pruesse et al., 2012). Phylogenetic trees were reconstructed using the neighbour-joining (Saitou & Nei, 1987), minimum-evolution (Rzhetsky & Nei, 1992) and maximum-likelihood (Felsenstein, 1981) algorithms in the software package MEGA version 6.0 (Tamura et al., 2013). The topology of the phylogenetic tree was assessed by using the bootstrap resampling method of Felsenstein (1985) with 1000 replicates. The evolutionary distance matrices were calculated by the Kimura two-parameter model (Kimura, 1980).

The 16S rRNA gene fragment of strain A4T was a continuous stretch of 1511 bp. Sequence similarity calculations demonstrated that strain A4T was most closely related to R. marina PSRT and R. spongicola UST030701-084T, sharing equal sequence similarities of 94.8 %. Other phylogenetically close relatives were F. halotolerans UST030701-097T (94.2 %), F. misakiensis SK-8T (93.9 %), R. ehrenbergii KMM 6017T (93.3 %), F. pacificus DY53T (93.3 %) and R. echinicomitans KMM 6058T (93.3 %). Phylogenetic analysis, based on 16S rRNA gene sequences using the neighbour-joining algorithm, illustrated that strain A4T forms a distinct monolineage out of the robust clade consisting of members of the genera Fabibacter and Roseivirga in the family Flammoevirgaceae (Fig. 1). This tree topology was also supported by the minimum-evolution and maximum-likelihood algorithms.

The morphology of cells in the exponential growth phase was observed by transmission electron microscopy (Hitachi; TEM System-H7650). The cells on MA plates were pre-processed by negative staining with 1 % (w/v) phosphotungstic acid. Cell motility was examined by the hanging drop method (Bernardet et al., 2002). Endospore formation and other morphological characteristics were examined by the procedures described by Dong & Cai (2001). The Gram reaction was performed according to the method described by Gerhardt et al. (1994) with cells grown on MA plates at 30 °C for 72 h. Anaerobic growth was tested in accordance with the protocols of Barbeyron et al. (2001). Production of flexirubin-type pigments was detected according to the method of Reichenbach et al. (1974). Production of H₂S was assayed using the procedure described by Bruns et al. (2001). Catalase activity was detected by oxidase test strips (Huankai, China). Catalase activity was assessed by bubble production in a 3 % (v/v) hydrogen peroxide solution (Smibert & Krieg, 1994). The temperature range for growth was assayed by observing colony formation after inoculation of strain A4T on GYP plates [Wang et al., 2013b; amended with (all w/v) 0.5 % sodium pyruvate, 0.5 % glucose and 0.5 % sucrose (new GYP)] and incubation at 4, 10, 15, 20, 25, 30, 33, 37, 41, 45, 48, 50, 53 and 55 °C in an aerobic condition for 2–5 days. The pH range for growth was determined by following the method described by Wang et al. (2013a). Growth at different NaCl concentrations was detected by

![Fig. 1. Neighbour-joining phylogenetic tree of strain A4T, based on nearly complete 16S rRNA gene sequences, showing the position of strain A4T and related taxa. Sphingobacterium spiritivorum ATCC 33861T was used as the outgroup. The same topology was observed in the minimum-evolution tree. Lines in bold indicate branches also found in the maximum-likelihood tree. Bootstrap values (expressed as percentages of 1000 replications, >70 %) are shown at branching points. Bar, 0.02 substitutions per nucleotide position.](image-url)
measuring the turbidity of the broth after incubating the bacterium at 25/33 °C in MB [amended with (all w/v) 0.5 % sodium pyruvate, 0.5 % glucose and 0.5 % sucrose (new MB)] with the addition of an NaCl gradient: 0, 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 14, 16 and 20 % (w/v) NaCl (note: NaCl in MB itself was not calculated). Hydrolysis of casein, starch, Tween 20, Tween 40, Tween 80, gelatin and chitin were assayed by inoculation of strain A4T on MA plates supplemented with corresponding substrates. Growth on sole carbon sources and enzymic activities were examined by using Biolog GEN III MicroPlates and bioMe´ rieux API 20NE after 24 h pre-incubation of strain A4T in sterile seawater at 27 °C.

Cells of strain A4T were Gram-stain-negative, non-spore-forming, curved rods, 0.4–1.0 μm in length (Fig. S1, available in the online Supplementary Material). Cells were motile by gliding. Colonies on new GYP plates were pink or red, shiny and convex with smooth surfaces and entire edges. Neither flexirubin-type pigments nor H2S production were detected. Strain A4T was oxidase- and catalase-positive. Growth occurred at 25/33 °C, pH 6.0–9.0, and with 0–14 % (w/v) NaCl, while optimal growth was observed at 33–41 °C, pH 7.5–8.0 and with no NaCl addition to new MB. Strain A4T is more thermotolerant (25–50 vs 4–45 °C) than all members of the genera Roseivirga and Fabibacter with validly published names. Tween 20, casein, gelatin and starch could be hydrolysed by the bacterium, but Tweens 40, 80 and chitin could not. Utilization of sole carbon sources, enzymic activities and other phenotypic properties of strain A4T have been listed in Table 1 and in the species description.

Biomass prepared for the analysis of respiratory quinones, polar lipids and DNA base composition was obtained from MB after 72 h incubation at 27 °C. Respiratory quinones were extracted by following the method described by Collins (1985) and determined by the reversed phase HPLC method (Komagata & Suzuki, 1987). Polar lipids of strain A4T and of the reference strains were extracted using the method of Kamekura (1993), and identified by two-dimensional thin-layer chromatography, followed by spraying with detection reagents (Tindall, 1990). Total DNA extraction was performed according to Barbeuron et al. (1984), and the G+C content was assayed by using the HPLC method (Mesbah et al., 1989). Cell biomass for fatty acid composition analysis was acquired from new GYP plates after incubation at 30 °C for 12 h, 33 °C for 12 h, and 25 °C for 48 h. Cellular fatty acids were extracted according to the Sherlock Microbial Identification System (MIID) instructions. The cellular fatty acid profile was analysed by GC (Agilent G6890N) and identified by using the Microbial Identification software package (Sherlock Version 6.0).

The only respiratory quinone of strain A4T was MK-7, which was consistent with other members of the family Flammeovirgaceae (Nedashkovskaya & Ludwig, 2010; Yoon et al., 2011). The genomic DNA G+C content of strain A4T was 40.3 mol%, similar to that of members from the genera Roseivirga (40.2–43.7 mol%) and Fabibacter (39.1–42.5 mol%). The predominant fatty acids (>5 %) of strain A4T included iso-C15 : 1G, iso-C17 : 03-OH, iso-C15 : 0 and iso-C16 : 03-OH. This profile was similar to those of the genera Roseivirga and Fabibacter (Table S1). However, the distinctive fatty acid profile could not be hydrolysed by the bacterium, but Tweens 40, 80 and chitin could not. Utilization of sole carbon sources, enzymic activities and other phenotypic properties of strain A4T have been listed in Table 1 and in the species description.

**Table 1.** Characteristics of strain A4T and the type strains of related taxa in the family Flammeovirgaceae

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3*</th>
<th>4</th>
<th>5</th>
<th>6</th>
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<tr>
<td>Cell shape</td>
<td>CR</td>
<td>SR</td>
<td>R</td>
<td>CR</td>
<td>CR</td>
<td>CR</td>
<td>CR</td>
<td>CR</td>
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<tr>
<td>Colony colour</td>
<td>Red(v)</td>
<td>Red(v)</td>
<td>Pink</td>
<td>Pink(v)</td>
<td>Pink(v)</td>
<td>Red(v)</td>
<td>Pink(v)</td>
<td>Red(v)</td>
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<tr>
<td>Temperature range for growth (°C)</td>
<td>25–50</td>
<td>4–41</td>
<td>4–31</td>
<td>(12)</td>
<td>15–45</td>
<td>15–45</td>
<td>(12)</td>
<td>15–45</td>
</tr>
<tr>
<td>Salinity range for growth (% w/v) (optimal)</td>
<td>0–14(0)</td>
<td>0–16(0–1)</td>
<td>1–8</td>
<td>0–12(1)</td>
<td>0–14(1)</td>
<td>0–16(0–1)</td>
<td>0–12(1)</td>
<td>0–8(1)</td>
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<td>Nitrate reduction§</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
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<tr>
<td>Hydrolyse of§</td>
<td>Casein</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
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<td>Gelatin</td>
<td>+</td>
<td>/</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
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<tr>
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<td>+</td>
<td>−</td>
<td>−</td>
<td>W</td>
<td>+</td>
<td>W</td>
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<td>Tween 40</td>
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<td>+</td>
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<td>+</td>
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<td>+</td>
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<td>Urea</td>
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<td>+</td>
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<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>(G+C) (mol%)§</td>
<td>40.3</td>
<td>40.2</td>
<td>41.3</td>
<td>43.7</td>
<td>41.9</td>
<td>42.5</td>
<td>40.8</td>
<td>39.1</td>
</tr>
</tbody>
</table>

*Data from Nedashkovskaya et al. (2005b).
†This NaCl content did not include that of marine broth 2216 media.
§Data are in parentheses are from Lau et al. (2006) and Wong et al. (2015).
distinguish strain A4<sup>T</sup> from members of the genera Roseivirga and Fabibacter (Table S1). The major polar lipids of strain A4<sup>T</sup> included phosphatidylethanolamine, two unidentified lipids, one unidentified aminolipid and two unidentified phospholipids (Fig. S2). Minor amounts of three unidentified lipids and three unidentified aminophospholipids were also detected in this bacterium. Bacteria from this study could be distinguished from each other by the polar lipid profiles, although they shared phosphatidylethanolamine and some aminolipids (Fig. S2; Pan et al., 2015).

In summary, phenotypic, physiological and chemotaxonomic properties support strain A4<sup>T</sup> forming a coherent phylogenetic cluster with bacteria from the genera Fabibacter and Roseivirga, although the thermostolerance, distinctive fatty acid profile and other phenotypic properties could well distinguish strain A4<sup>T</sup> from those type strains. Features proposed to differentiate the genus Fabibacter from Roseivirga, such as starch hydrolysis, cell shape, NaCl tolerance, temperature range for growth, genomic G+C contents and distinctive cellular fatty acid profiles of each strain (Lau et al., 2006; Nedashkovskaya & Kim, 2010), could not solidly support the original proposal due to novel type species assignment and the uniformity of data acquisition methods (Table 1). It is difficult to determine if these differences are inter-genus or intra-genus and inter-species although they can readily determine if these differences are inter-genus or intra-genus. Considering the possibility of Roseivirga representing a novel family in the future (Nedashkovskaya & Ludwig, 2010), it is necessary for the genus Fabibacter to remain, and to build a novel genus represented by strain A4<sup>T</sup> to display the heterogeneity of the novel family candidate. Thus, strain A4<sup>T</sup> is suggested to represent a novel species in a novel genus, for which, the name Fabivirga thermotolerans gen. nov., sp. nov. is proposed.

**Description of Fabivirga thermotolerans sp. nov.**

Fabivirga thermotolerans (ther.mo.to’le.rans. Gr. adj. thermos hot; L. part. adj. tolerans tolerating; N.L. part. adj. thermotolerans tolerating heat).

Characteristics are as given for the genus. In addition, cells are non-spore-forming, usually 0.4–1.0 μm in width and 2.0–9.0 μm in length; they are motile by gliding. Colonies on MA are pink or red, circular and convex with entire margins. Flexirubin-type pigments are not produced. Growth occurs at 25–50 °C, pH 6.0–9.0 and with 0–14 % (w/v) NaCl addition to marine broth 2216. Growth occurs optimally at 30 °C, pH 7.5–8.0, and with no NaCl added to marine broth 2216. Tween 20, gelatin, starch and casein are hydrolysed, but Tween 40, Tween 80 and chitin are not. Indole and H<sub>2</sub>S are not produced. Assimilates D-glucose, L-arabinose, D-mannose, D-mannitol, N-acetylglucosamine, maltose, L-sodium glutamate, L-proline, L-tryptophan, potassium gluconate, adipic acid, L-arginine, myo-inositol, malic acid, sucrose, trehalose and phenylacetic acid, but not capric acid, glycine, pyruvic acid, L-tyrosine, lactose, L-histidine, D-xylose, maleic acid and trisodium citrate. β-Glucosidase and β-galactosidase are positive, while L-arginine dehydrodrolase and urease are negative.

The type strain A4<sup>T</sup> (=KCTC 42507<sup>T</sup>=CGMCC 1.15111<sup>T</sup>), was isolated from the culture broth of a cyanobacterium Plectonema sp., in Guangzhou, PR China. The genomic DNA G+C content of the type strain is 40.3 mol%.

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**References**


