Aureimonas endophytica sp. nov., a novel endophytic bacterium isolated from Aegiceras corniculatum

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Abstract

A Gram-negative, motile, aerobic and coccolid rod-shaped bacterium, designated strain 2T4P-2-4ᵀ, was isolated from a piece of surface-sterilized bark of Aegiceras corniculatum collected from Cotai Ecological Zones in Macao, China, and tested by a polyphasic approach to clarify its taxonomic position. Strain 2T4P-2-4ᵀ grew optimally without NaCl at 28–30 °C, pH 7.0–8.0. The 16S rRNA gene sequence of strain 2T4P-2-4ᵀ had the highest similarity (96.2 %) to Aureimonas rubiginis CC-CFT034ᵀ. Phylogenetic analysis showed that the strain grouped with species of the genus Aureimonas. The predominant quinone system of strain 2T4P-2-4ᵀ was ubiquinone 10 (Q-10). The polar lipid profile contained diphosphatidylglycerol, phosphatidylglycerol, phosphatidylcholine, phosphatidylethanolamine, sulfoquinovosyldiacylglycerol, phosphatidylylethanolamine, two unidentified amino lipids, an unidentified aminophospholipid and five unidentified lipids. The predominant cellular fatty acid was C₁₈:ω₂C (61.2 %). The DNA G+C content of strain 2T4P-2-4ᵀ was 69.8 mol%. Based on the phylogenetic, phenotypic and chemotaxonomic features, strain 2T4P-2-4ᵀ is a representative of a novel species of the genus Aureimonas, for which the name Aureimonas endophytica sp. nov. is proposed. The type strain of Aureimonas endophytica sp. nov. is 2T4P-2-4ᵀ (=KCTC 52217ᵀ =CGMCC 1.15367ᵀ).

The family Aurantimonadaceae, within the order Rhizobiales [1], of the class Alphaproteobacteria, contains five genera: Aurantimonas [2], Fulvimarina [3], Martelella [4], Aureimonas [5] and Jiella [6]. Based on the presence or absence of the glycolipid sulfoquinovosyldiacylglycerol (SQDG) and 16S rRNA gene sequence analyses, Aurantimonas altamirensis, Aureimonas ureilytica and Aureimonas frigidaquae of the genus Aurantimonas were reclassified under a new genus Aureimonas as A. altamirensis (the type species), A. ureilytica and A. frigidaquae, respectively [5]. At the time of writing, the genus Aureimonas comprised eight validly named species: A. altamirensis, A. ureilytica and A. frigidaquae [5] isolated from the walls of Altamira Cave [7], air [8] and a water-cooling system [9], respectively; A. ferruginea and A. rubiginis from rusty iron plates [10]; A. jatrophae and A. phyllosphaerae from leaf tissues of Jatropha curcas L. [11] and A. glacistagni from a melt pond on Arctic sea ice [12]. In addition, details of two species from the phyllosphere of Galium album, A. gali and A. pseudogali, have been being published [13]. It is notable that A. altamirensis, the type species of the genus Aureimonas, was recently reported to be isolated not only from human clinical samples including blood, pleural effusions and cornea, but also from an oedematous canine testicle [14].

During a study on the cultivable bacterial diversity of mangrove plants in Cotai Ecological Zones (22°08′23″ N, 113°33′07″ E), Macao, China, a light-yellow strain, designated strain 2T4P-2-4ᵀ, was isolated from a piece of surface-sterilized bark of Aegiceras corniculatum. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain 2T4P-2-4ᵀ was affiliated to genus Aureimonas. Polyphasic taxonomic study showed that strain 2T4P-2-4ᵀ differed from previously described species of the genus Aureimonas and represented a new species. The taxonomic position of this strain is herein reported.

The bark of Aegiceras corniculatum was surface-sterilized as described by Qin et al. [15]. After drying in the laminar airflow hood at 25 °C for 2 days, the surface-sterilized bark was ground into a powder using a micromill and distributed on modified Gauze No.2 agar medium [containing per litre distilled water: 1.0 g glucose, 0.5 g peptone, 0.3 g tryptone, 10 g agar, and 1 ml of L-arginine, L-lysine, L-proline and L-ornithine] before being illuminated with artificial light at different wavelengths for 48 h. After picking colonies, they were re-isolated and identified by the same polyphasic approach and the type strain was isolated from a piece of surface-sterilized bark of Aegiceras corniculatum.
0.5 g NaCl, vitamin mixture (0.001 g Vitamin B1, 0.001 g Vitamin B2, 0.001 g Vitamin B3, 0.001 g Vitamin B6, 0.001 g phenylalanine, 0.001 g alanine, 0.0005 g biotin), 20.0 g agar, pH 7.2). After incubation at 28 °C for 4 weeks, colonies were picked up and streaked on ISP 2 agar [16] to obtain the pure isolates. A light-yellow coloured isolate designated 2T4P-2-4 was obtained. After sub-culture on ISP 2 agar at 28 °C, the strain was maintained on ISP 2 agar slant at 4 °C for several weeks and preserved in ISP 2 broth supplemented with 20 % (v/v) autoclaved glycerol at −80 °C.

The cultural, physiological and biochemical characteristics of strain 2T4P-2-4T and the reference strain A. rubiginis CC-CFT034T (=JCM 18445T) [from the Japan Collection of Microorganisms (JCM)] were tested under the same conditions. Cultural characteristics were determined by observing growth of the strain at 28 °C for 3–4 weeks on ISP 2, ISP 3, ISP 4, ISP 5 and ISP 7 agars [16], nutrient agar [17], R2A agar (BD), and tryptic soy agar (TSA; BD). The ISCC-NBS colour charts [18] were used to assess the colony colours. Cell morphology was observed and recorded by transmission electron microscopy (JEM-1400; JEOL) after incubation on ISP 2 agar at 28 °C for 2 days. The pH range (pH 4.0–13.0, at intervals of 1 pH unit) for growth was measured in R2A broth for 4 weeks at 28 °C using the buffer system described by Xu et al. [19]. Salt tolerance was tested on R2A agar supplemented with concentrations of 0, 1, 2, 3, 4, 5, 7 and 10 % (w/v) NaCl. Growth was monitored after 14 days of incubation at 28 °C. The growth temperature of strain 2T4P-2-4T on ISP 2 agar was determined at 4, 8, 20, 25, 28, 30, 37 and 45 °C. Growth was monitored after 14 days. The Gram-staining test was performed as described by Magee et al. [20]. Catalase activity was determined by bubble production in 3 % (v/v) H2O2. Oxidase activity was assessed by using 1 % (w/v) tetramethyl-p-phenylenediamine [21]. Hydrolysis of casein, starch, gelatin, cellulose, Tweens 20, 40 and 80 and production of H2S were examined as described by Gonzalez et al. [22]. The enzyme activities were tested using API ZYM Kits (bioMérieux) according to the manufacturer’s instructions. Acid production from carbon sources was examined using the API 50CH (bioMérieux) system. Oxidation of the carbon sources and sensitivity to antimicrobial compounds were tested using Biolog GEN III MicroPlates. Other biochemical characteristics were tested using API 20NE (bioMérieux) according to the manufacturer’s recommendations.

Strain 2T4P-2-4T was Gram-staining-negative, coccoïd rod-shaped (approximately 1.7–1.9 × 1.2–2.5 μm in size), motile by means of monopolar flagella, non-spore-forming and aerobic (Fig. S1, available in the online Supplementary Material). The colonies after 3 days of incubation on ISP 2 agar were circular with entire wavy edges, rough, convex, opaque and light yellow in colour. Strain 2T4P-2-4T grew well on TSA, ISP 2 agar and R2A agar. Poor growth occurred on ISP 3 agar, ISP 5 agar and ISP 7 agar. No growth occurred on ISP 4 agar and nutrient agar. Strain 2T4P-2-4T was able to grow at 20–37 °C (optimum 28–30 °C), at pH 5.0–9.0 (optimum pH 7.0–8.0) and in the presence of 0–2 % (w/v) NaCl (optimum without NaCl).

The detailed physiological and biochemical characteristics of strain 2T4P-2-4T are given in Table 1 and the species description.

For the chemotaxonomic analyses of quinones and polar lipids, strain 2T4P-2-4T together with the reference strain A. rubiginis CC-CFT034T were grown in ISP 2 broth at 28 °C with 180 r.p.m. for 4 days. Quinones were isolated and purified as described by Collins et al. [23], and then analysed and identified by HPLC-MS according to the method of Guo et al. [24]. The polar lipids were extracted and analysed by two-dimensional TLC on silica gel 60 F254 plates (Merck) according to the method of Minnikin et al. [25]. Polar lipid profiles of the strain 2T4P-2-4T and the reference strain A. rubiginis CC-CFT034T were visualized with molybdophosphoric acid for total lipids, ninhydrin for aminolipids, molybdenum blue for phospholipids and anisaldehyde for glycolipids. The solvent systems of the first and second dimension were chloroform–methanol–water (64:27:5, v/v) and chloroform–methanol–acetic acid–water (80:18:12:5, v/v), respectively. For the analysis of whole-cell fatty acids, strain 2T4P-2-4T, A. rubiginis CC-CFT034T and A. altamirensis DSM 21988T were cultured simultaneously on ISP 2 agar at 28 °C for 3 days. The whole-cell fatty acids were saponified, methylated and extracted according to the standard protocol described by Sasser [26], and analysed by gas chromatography (Agilent 7890B) according to the instructions of the Sherlock Microbial Identification System (version 6.2; MIDI) with the RTSBA 6 database. A gas chromatograph (Agilent 7890B) coupled with an Agilent 5977B single quadrupole mass spectrometer was used to further confirm the types of fatty acids by the Nist14 Library software database according to the method described by Tuo et al. [27]. The dimethyl disulfide (DMDS) derivatization of the fatty acid methyl esters (FAME) extracts was performed as described by Nichols et al. [28], and the double-bond position was determined by the same GC-MS described above. A capillary column HP-5MS (30 m × 0.25 mm i.d. × 0.25 μm film thickness; Agilent Technologies) was used for separation of the DMDS adducts of monounsaturated FAME. The initial temperature of 80 °C was maintained for 1 min, then raised to 280 °C at the rate of 8 °C min−1 and held for 15 min. Helium was used as the carrier gas with a flow rate of 1.0 ml min−1. Injection (2 μl) was made in splitless mode at an injector temperature of 250 °C. Mass spectra were obtained using electron impact (EI; 70 eV).

For calculation of the G+C content, the genomic DNA of strain 2T4P-2-4T was prepared as described by Marmur [29] and was determined by reversed-phase HPLC as described by Mesbah et al. [30].

The predominant quinone of strain 2T4P-2-4T was ubiquinone-10 (Q-10). The polar lipid profiles of strain 2T4P-2-4T and the reference strain A. rubiginis CC-CFT034T are
Table 1. Differential characteristics between strain 2T4P-2-4<sup>T</sup> and closely related species within the genus *Aureimonas*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1†</th>
<th>2*</th>
<th>3</th>
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<tr>
<td>Cell size (µm)</td>
<td>1.7–1.9×1.9–2.5</td>
<td>1.3×2.3&lt;sup&gt;†&lt;/sup&gt;</td>
<td>0.9×1.1&lt;sup&gt;†&lt;/sup&gt;</td>
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<tr>
<td>Colony colour</td>
<td>Light yellow</td>
<td>Pale yellow&lt;sup&gt;†&lt;/sup&gt;</td>
<td>Yellow&lt;sup&gt;†&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cell morphology</td>
<td>Coccolid rod</td>
<td>Short rod&lt;sup&gt;†&lt;/sup&gt;</td>
<td>Short rod&lt;sup&gt;†&lt;/sup&gt;</td>
</tr>
<tr>
<td>Flagellation</td>
<td>&gt;1, polar</td>
<td>NA</td>
<td>-†</td>
</tr>
<tr>
<td>Colony morphology</td>
<td>Circular with entire wavy edges, convex, rough</td>
<td>Circular, entire, smooth&lt;sup&gt;†&lt;/sup&gt;</td>
<td>Circular, convex, smooth&lt;sup&gt;†&lt;/sup&gt;</td>
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<tr>
<td>Growth on TSA</td>
<td>+</td>
<td>–</td>
<td>+†</td>
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<tr>
<td>pH range</td>
<td>5–9</td>
<td>5–9 (5–81&lt;sup&gt;†&lt;/sup&gt;)</td>
<td>NA</td>
</tr>
<tr>
<td>Temperature range for growth (°C)</td>
<td>20–37</td>
<td>8–30 (20–301&lt;sup&gt;†&lt;/sup&gt;)</td>
<td>10–40&lt;sup&gt;†&lt;/sup&gt;</td>
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<tr>
<td>NaCl tolerance range (% w/v)</td>
<td>0–2</td>
<td>0–2 (0–11&lt;sup&gt;†&lt;/sup&gt;)</td>
<td>0–5&lt;sup&gt;†&lt;/sup&gt;</td>
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<tr>
<td>Hydrolysis of:</td>
<td></td>
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<tr>
<td>Tween 40</td>
<td>–</td>
<td>+</td>
<td>–†</td>
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<tr>
<td>Tween 20</td>
<td>w</td>
<td>+</td>
<td>–†</td>
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<tr>
<td>Casein</td>
<td>–</td>
<td>w</td>
<td>–†</td>
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<tr>
<td>Starch</td>
<td>–</td>
<td>–</td>
<td>+†</td>
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<tr>
<td>Carbon source utilization:</td>
<td></td>
<td></td>
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<tr>
<td>d-Glucose</td>
<td>–</td>
<td>+</td>
<td>+†</td>
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<tr>
<td>l-Arabinose</td>
<td>–</td>
<td>+</td>
<td>+†</td>
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<tr>
<td>Malose</td>
<td>–</td>
<td>w</td>
<td>+†</td>
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<tr>
<td>N-acetyl-d-glucosamine</td>
<td>–</td>
<td>w</td>
<td>–†</td>
</tr>
<tr>
<td>Potassium gluconate</td>
<td>–</td>
<td>–</td>
<td>+†</td>
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<tr>
<td>Malic acid</td>
<td>–</td>
<td>–</td>
<td>+†</td>
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<tr>
<td>API ZYM:</td>
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<td></td>
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<tr>
<td>Valine arylamidase</td>
<td>w</td>
<td>–</td>
<td>–†</td>
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<tr>
<td>α-Glucosidase</td>
<td>+</td>
<td>–</td>
<td>–†</td>
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<tr>
<td>Cystine arylamidase</td>
<td>w</td>
<td>–</td>
<td>–†</td>
</tr>
<tr>
<td>N-acetyl-β-glucosaminidase</td>
<td>+</td>
<td>+</td>
<td>–†</td>
</tr>
<tr>
<td>Esterase (C4)</td>
<td>–</td>
<td>–</td>
<td>+†</td>
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<tr>
<td>Esterase lipase (C8)</td>
<td>–</td>
<td>–</td>
<td>+†</td>
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<tr>
<td>G+C content (mol%)</td>
<td>69.8</td>
<td>67.7&lt;sup&gt;†&lt;/sup&gt;</td>
<td>71.8&lt;sup&gt;†&lt;/sup&gt;</td>
</tr>
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</table>

<sup>†</sup>Data performed in this study.

<sup> Data from other studies indicated as: a, Lin et al. [10]; b, Jurado et al. [7]; c, Cho et al. [12]; d, Madhaiyan et al. [11]; e, Rathsack et al. [5]; f, Weon et al. [8].</sup>

shown in Fig. S2. The polar lipids of strain 2T4P-2-4<sup>T</sup> comprised phosphatidylglycerol, diphosphatidylglycerol, phosphatidycholine, phosphatidylethanolamine, sulfoquinovosyldiacylglycerol, phosphatidylmethylethanolamine, two unidentified amino lipids, an unidentified aminophospholipid, and five unidentified lipids. The cellular fatty acid contents of strain 2T4P-2-4<sup>T</sup> and the related species of genus *Aureimonas* are given in Table S1. The whole-cell fatty acids of strain 2T4P-2-4<sup>T</sup> contained large amounts of C<sub>18:1ω7c</sub> (61.2 %), moderate amounts of C<sub>16:0</sub> (11.2 %) and C<sub>18:1ω9c</sub> (11.3 %), small amounts of C<sub>19:0 cyclo</sub> ω8c (5.6 %), C<sub>16:1ω7c</sub> (5.0 %) and C<sub>18:0</sub> (2.6 %). The DNA G+C content of strain 2T4P-2-4<sup>T</sup> was 69.8 mol%, which is in the range of the G+C content (63.9 to 71.8 mol%) reported for species within the genus *Aureimonas* [5, 7, 9, 13]. The major components of quinones, polar lipids of the reference strain *A. rubiginis* CC-CFT034<sup>T</sup> in our study were largely consistent with previous reports [10].

The genomic DNA of strain 2T4P-2-4<sup>T</sup> was extracted using the method described by Li et al. [31], and the prepared DNA was used as the template to amplify the 16S rRNA gene by PCR with the primers 27F (5'-AGAGTTTGATCC TGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTAC WCC-

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GACTT-3') [32]. The purified PCR products were cloned using the pEASY-T1 Cloning Kit (TransGen Biotech) to obtain the almost complete 16S rRNA gene sequence according to the manufacturer’s instructions. Gene sequencing was performed using primers M13F (5'-GTGTTCCTCCG TACGAC-3'), M13R (5'-CAGGAAAACAGCTATGAC-3') and 51.W1F (5'-CCGCTAACTCTTCTTGA-3') by an ABI PRISM 3730XL DNA Analyzer (Foster City, CA), and then three sequence fragments were assembled using Seq-Man [33] to obtain the nearly full-length 16S rRNA gene sequence. The almost complete 16S rRNA gene sequence of the strain 2T4P-2-4T was submitted to the EzTaxon-e server (http://www.ezbiocloud.net/) [34] and NCBI for BLAST search, and then the 16S rRNA gene sequence similarity values between strain 2T4P-2-4T and the related species were obtained. Multiple alignments were made using the CLUSTAL_X tool in MEGA version 5.0. Phylogenetic trees were constructed using neighbour-joining [35], maximum-likelihood [36] and maximum-parsimony [37] methods with MEGA version 5.0 [38]. Evolutionary distances were calculated using Kimura’s two-parameter model [39]. The topologies of the phylogenetic trees were evaluated by a bootstrap method with 1000 replications [40].

An almost full-length 16S rRNA gene sequence (1465 bp) of strain 2T4P-2-4T was obtained. The results of the BLAST search in EzTaxon-e showed that the 16S rRNA gene sequence of strain 2T4P-2-4T had the highest sequence similarity (96.2 %) with that of strain A. rubiginis CC-CFT034T. The 16S rRNA gene sequence similarities between strain 2T4P-2-4T and all other related species of the genus Aureimonas were less than 95.7 %. The phylogenetic trees based on 16S rRNA gene sequences, generated by using maximum-likelihood algorithms, showed that strain 2T4P-2-4T fell within the lineage of the genus Aureimonas and formed a distinct subclade with A. rubiginis CC-CFT034T. The proportion of fatty acid 18:1ω7c in fatty acids of strain 2T4P-2-4T was phylogenetically affiliated to the genus Aureimonas, but the relatively lower sequence similarities (<97.0%) with recognized species of the genus Aureimonas and the phylogenetic position showed that strain 2T4P-2-4T should represent a novel species of the genus Aureimonas. The conclusion is also supported by chemotaxonomic characteristics of strain 2T4P-2-4T. Compositions of the major polar lipids, the predominant respiratory quinone, and large amounts of C18:1ω7c in fatty acids of strain 2T4P-2-4T are all in line with the description of the genus Aureimonas. Moreover, the presence of a distinct sulfoquinovosyldiacylglycerol (SQDG) was clearly seen in strain 2T4P-2-4T and is the special chemotaxonomic feature of genus Aureimonas [5]. However, distinct differences have been observed within strain 2T4P-2-4T, A. rubiginis CC-CFT034T and A. altamirensis DSM 21988T. It is notable that the proportion of fatty acid C18:1ω7c detected in 2T4P-2-4T (61.2 %) is similar to that in A. rubiginis CC-CFT034T (61.0 %), which is obviously lower than that in A. altamirensis DSM 21988T (79.2 %), the proportion of fatty acid C18:1 2-OH detected in strain 2T4P-2-4T (11.3 %) is slightly lower than that in A. rubiginis CC-CFT034T (14.0 %), which is obviously higher than that in A. altamirensis DSM 21988T (2.2 %), the proportions of fatty acids C19:0 cyclo ω8c and C16:1ω7c detected in strain 2T4P-2-4T (5.6 and 5.0 %, respectively) are higher than those in A. rubiginis CC-CFT034T (1.7 and 2.1 %, respectively) and A. altamirensis DSM 21988T (1.7 and 1.3 %, respectively), the proportion of fatty acid C18:0 detected in 2T4P-2-4T (2.6 %) is close to that in A. altamirensis DSM 21988T (1.9 %), which is obviously lower than that in strain A. rubiginis CC-CFT034T (6.0 %), the fatty acid C14:0 2-OH absent in strain 2T4P-2-4T and A. altamirensis DSM 21988T is obvious in A. rubiginis CC-CFT034T (5.0 %). The details of fatty acid profiles of strain 2T4P-2-4T, A. rubiginis CC-CFT034T and A. altamirensis DSM 21988T are given in Table S1. In the polar lipid profiles, an unidentified lipid (L5) detected in strain 2T4P-2-4T was absent in A. rubiginis CC-CFT034T (Fig. S2). Furthermore, the differences in morphological, cultural and physiological characteristics between strain 2T4P-2-4T and related species of genus Aureimonas can be found in Table 1.

In conclusion, based on the phylogenetic analysis, phenotypic, biochemical and chemotaxonomic data provided, the novel strain 2T4P-2-4T is proposed to represent a novel species within the genus Aureimonas, for which the name Aureimonas endophytica sp. nov. is proposed.

**DESCRIPTION OF AUREIMONAS ENDOPHYTICA SP. NOV.**

Aureimonas endophytica (en.do.phyti.ca. Gr. pref. endo within; Gr. n. phyton plant; L. fem. suff. -ica adjectival suffix used with the sense of belonging to; N.L. fem. adj. endophytica within plant, endophytic, pertaining to the original isolation from plant tissues).

Cells are Gram-stain-negative, non-spore-forming, motile with monopolar flagella, aerobic and coccolid rod-shaped (1.7–1.9 × 1.9–2.5 μm) after incubation for 2 days at 28 °C on ISP 2 agar. Colonies on ISP 2 agar for 3 days are circular with entire wavy edges, rough, convex, opaque and light yellow in colour. Good growth occurs on ISP 2 agar, R2A agar and TSA, poor growth occurs on ISP 3 agar, ISP 5 agar and ISP 7 agar, no growth occurs on ISP 4 agar and nutrient agar. The growth temperature is between 20 and 37 °C. pH range is from 5.0 to 9.0 and the species can tolerate less than 2 % (w/v) NaCl, and the best growth occurs at 28–30 °C, pH 7.0–8.0 and without NaCl. Cells are positive for catalase, oxidase and weakly positive for hydrolysis of Tween 20. Hydrolysis of Tween 40, Tween 80, starch, cellulose, casein and urease, nitrate reduction and H2S production are negative. According to API ZYM strip test results, positive for N-acetyl-β-D-glucosaminidase, acid phosphatase, alkaline phosphatase, α-glucosidase, leucine arylamidase, naphthol-AS-
D-xylopyranoside, raffinose, D-nosamine, D-N-galactosamine, D-glucose, gluconate, glycol, inulin, 2-ketogluconate, 5-ketogluconate, D-fructose, D-arabinose, D-ribose, D-xylose, mannitol, D-mannose, D-glucuronic acid, pyruvic acid, pyridoxal phosphate, D-fucose, D-lucose, D-galactose, galacturonic acid, D-galactonic acid lactone, gelatin, gentiobiose, D-glucuronic acid, α-D-glucose, glucuronamide, D-glucuronic acid, glycerol, α-hydroxybutyric acid, D-keto-glutaric acid, L-lactic acid methyl ester, D-malic acid, D-malic acid, methyl ester, L-malic acid, maltose, D-mannitol, D-mannose, methyl β-D-glucoside, methyl pyruvate, mucleic acid, myo-inositol, pectin, L-pyroglutamic acid, quinic acid, L-rhamnose, D-saccharic acid, D-serine, L-serine, D-sorbitol, sucrose, trehalose and turanose, cells are sensitive to aztreonam, fusidic acid, lithium chloride, potassium tellurite, minocycline, niaproof 4, sodium bromate, 1% sodium lactate, troleandomycin, vancomycin and toluenesulphonamide. The predominant quinone is Q-10. The dominant fatty acid is C₁₉:1ω7c. The G+C content of the genomic DNA is 69.8 mol%.

**Fig. 1.** Maximum-likelihood phylogenetic tree showing the phylogenetic position of strain 2T4P-2-4 and related species within the family *Aurantimonadaceae* on the basis of 16S rRNA gene sequences termini 67 and 1478. Numbers at nodes refer to bootstrap values (based on 1000 replicates; only values >70% are shown at branch points). Filled circles indicate that the corresponding nods were also obtained in both the neighbour-joining and maximum-parsimony trees. Type strains of *Escherichia coli* ATCC 11775 were used as outgroup. Bar, 2 nt substitutions per 100 nt.

BI-phosphohydrolase and trypsin. Weakly positive for cystine arylamidase and valine arylamidase. Negative for α-chymotrypsin, esterase (C4), esterase lipase (C8), α-fucosidase, α-galactosidase, β-galactosidase, β-glucosidase, β-glucuronidase, lipase (C14) and α-mannosidase. In the API 50CH strips, acid is produced from D-arabinose (weakly), L-arabinose, dulcitol (weakly), D-fructose, D-fucose, L-fucose, D-galactose, inositol (weakly), D-lyxose, mannitol, D-mannose, D-ribose (weakly), L-rhamnose, L-sorbose (weakly), sorbitol (weakly), xylitol (weakly), L-xylene and D-xylose (weakly), but not from N-acetylgalactosamine, D-adenylate, aesculin, D-arabitol, L-arabitol, arbutin, amygdalin, cellulose, erythritol, D-gentiobiose, D-glucose, gluconate, glycogen, glycol, inulin, 2-ketogluconate, 5-ketogluconate, lactose, maltose, melezitose, melibiose, methyl α-D-glucopyranoside, methyl α-D-mannopyranoside, methyl β-D-xlyopyranoside, raffinose, D-sucrose, salicin, starch, D-tagatose, trehalose and turanose. In the BIOLOG system, positive for oxidation of acetic acid, acetoacetic acid, N-acetyl-D-galactosamine, N-acetyl-D-glucosamine, N-acetyl-D-mannosamine, D-arabitol, D-aspartic acid, bromosuccinic acid, cellulbiose, citric acid, dextrin, D-fructose, D-fructose-6-phosphate, D-fucose, L-fucose, D-galactose, galacturonic acid, D-galactonic acid lactone, gelatin, gentiobiose, D-glucuronic acid, α-D-glucose, glucuronamide, D-glucuronic acid, glycerol, α-hydroxybutyric acid, D-keto-glutaric acid, L-lactic acid, D-lactic acid methyl ester, D-malic acid, D-malic acid, maltose, D-mannitol, D-mannose, methyl β-D-glucoside, methyl pyruvate, mucic acid, myo-inositol, pectin, L-pyroglutamic acid, quinic acid, L-rhamnose, D-saccharic acid, D-serine, L-serine, D-sorbitol, sucrose, trehalose and turanose, cells are sensitive to aztreonam, fusidic acid, lithium chloride, potassium tellurite, minocycline, niaproof 4, sodium bromate, 1% sodium lactate, troleandomycin, vancomycin and toluenesulphonamide. The predominant quinone is Q-10. The polar lipids comprise phosphatidylglycerol, diphosphatidylglycerol, phosphatidylincholine, phosphatidylethanolamine, sulfoquinovosyldiacylglycerol, phosphatidymethylethanolamine, two unidentified amino lipids, an unidentified aminophospholipid and five unidentified lipids. The predominant fatty acid is C₁₈:1ω7c. The G+C content of the genomic DNA is 69.8 mol%.
The type strain, 2T4P-2-4^T (=KCTC 52217^T=CGMCC 1.15367^T) was isolated from a piece of bark of Aegiceras corniculatum collected from Cotai Ecological Zones in Macao, China.

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Conflicts of interest
The authors declare that there are no conflicts of interest.

References

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