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

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Abstract
A Gram-negative, motile, aerobic and coccus rod-shaped bacterium, designated strain 2T4P-2-4†, 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-2-4† grew optimally without NaCl at 28–30 °C, pH 7.0–8.0. The 16S rRNA gene sequence of strain 2T4P-2-4† had the highest similarity (96.2 %) to Aureimonas rubiginis CC-CT034T. Phylogenetic analysis showed that the strain grouped with species of the genus Aureimonas. The predominant quinone system of strain 2T4P-2-4† was ubiquinone 10 (Q-10). The polar lipid profile contained diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, sulfoquinovosyldiacylglycerol, phosphatidylmethylthanolamine, two unidentified amino lipids, an unidentified aminophospholipid and five unidentified lipids. The predominant cellular fatty acid was C18:ω7C (61.2 %). The DNA G+C content of strain 2T4P-2-4† was 69.8 mol%. Based on the phylogenetic, phenotypic and chemotaxonomic features, strain 2T4P-2-4† 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-2-4† (=KCTC 52217T =CGMCC 1.15367T).

The family Aurantimonadaceae, within the order Rhizobiales [1] of the class Alphaproteobacteria, contains five genera: Aurantimonas [2], Fulvimonas [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, Aurantimonas urelytica and Aureimonas frigidaeae of the genus Aurantimonas were reclassified under a new genus Aureimonas as A. altamirensis (the type species), A. urelytica and A. frigidaeae, respectively [5]. At the time of writing, the genus Aureimonas comprised eight validly named species: A. altamirensis, A. urelytica and A. frigidaeae [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. phyllophaeae from leaf tissues of Jatropha curcas L. [11] and A. glacisagri from a melt pond on Arctic sea ice [12]. In addition, details of two species from the phyllosphere of Galium album, A. galii and A. pseudogalii, 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-2-4†, was isolated from a piece of surface-sterilized bark of Aegiceras corniculatum. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain 2T4P-2-4† was affiliated to genus Aureimonas. Polyphasic taxonomic study showed that strain 2T4P-2-4† 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,

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Keywords: Aureimonas endophytica; Aegiceras corniculatum; endophytic bacterium; Strain 2T4P-2-4†.

Abbreviations: DMDS, dimethyldisulfide; FAME, fatty acid methyl esters; SQDG, sulfoquinovosyldiacylglycerol; Q-10, ubiquinone 10.

The GenBank accession number for the 16S rRNA gene sequence of strain 2T4P-2-4† is KX895883.

Two supplementary figures and one table are available with the online Supplementary Material.
of strain 2T4P-2-4 pure isolates. A light-yellow coloured isolate designated picked up and streaked on ISP 2 agar [16] to obtain the opaque and light yellow in colour. Strain 2T4P-2-4 agar were circular with entire wavy edges, rough, convex, and 10 % (w/v) NaCl. Growth was monitored after 14 days at 28 °C and 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 molybdatophosphoric acid for total lipids, ninhydrin for amino lipids, molybdenum blue for phospholipids and anisaldehyde for glycolipids. The solvent systems of the first and second dimension were chloroform-methanol-water (64:27.5:4, 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 [from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ)] 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-2-4T and closely related species within the genus Aureimonas

Strains: 1, 2T4P-2-4T; 2, A. rubiginis CC-CFT034T; 3, A. altamirensis DSM 21988T. All data shown were obtained in this study unless indicated otherwise. All strains were Gram-staining-negative and positive for oxidase, catalase and urease, but negative for nitrate reduction and hydrolysis of cellulose and Tween 80. In API 20NE Kits, all strains were positive for assimilation of D-mannose and D-mannitol, and negative for assimilation of caprate, trisodium citrate, adipate and phenylacetate. In API ZYM Kits, all strains were positive for acid phosphatase, alkaline phosphatase, leucine arylamidase, trypsin and napthol-AS-BI-phosphohydrolase, but negative for α-chymotrypsin, α-fucosidase, α-galactosidase, β-glucosidase, β-glucuronidase, β-galactosidase, lipase and α-mannosidase. +, positive; −, negative; w, weakly positive; na, no data available. Data on temperature, NaCl and pH range tests given in brackets were taken from original species proposals.

| Characteristic | 1† | 2* | 3
|---------------|----|----|---
| Cell size (µm) | 1.7–1.9×1.9–2.5 | 1.3×2.3a | 0.9×1.1b |
| Colony colour | Light yellow | Pale yellow†a | Yellow†b |
| Cell morphology | Coccolid rod | Short rod†a | Short rod†b |
| Flagellation | >1, polar | NA | -†b |
| Colony morphology | Circular with entire wavy edges, convex, rough | Circular, entire, smooth†a | Circular, convex, smooth†b |
| Growth on TSA | + | – | +†c |
| pH range | 5–9 | 5–9 (5–81ª) | NA |
| Temperature range for growth (°C) | 20–37 | 8–30 (20–30†ª) | 10–40†b |
| NaCl tolerance range (% w/v | 0–2 | 0–2 (0–1†ª) | 0–5†a |
| Hydrolysis of: | | | |
| Tween 40 | – | + | –†d |
| Tween 20 | w | + | –†b |
| Casein | – | w | –†b |
| Starch | – | – | +†c |
| Carbon source utilization: | | | |
| D-Glucose | − | + | +†b |
| L-Arabinose | − | + | +†d |
| Maltose | − | w | +†d |
| N-acetyl-D-glucosamine | − | w | –†b |
| Potassium gluconate | − | – | +†d |
| Malic acid | − | – | +†d |
| API ZYM: | | | |
| Valine arylamidase | w | – | –†f |
| α-Glucosidase | + | – | –†d |
| Cystine arylamidase | w | – | –†b |
| N-acetyl-β-glucosaminidase | + | + | –†b |
| Esterase (C4) | – | – | +†b |
| Esterase lipase (C8) | – | – | +†b |
| DNA G+C content (mol%) | 69.8 | 67.2†a | 71.8†b |

Data performed in this study.
†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].

shown in Fig. S2. The polar lipids of strain 2T4P-2-4T comprised phosphatidylglycerol, diphosphatidylglycerol, phosphatidylcholine, phosphatidylethanolamine, sulfoquinovosyldiacylglycerol, phosphatidylmethylthanolamine, two unidentified amino lipids, an unidentified aminophospholipid and five unidentified lipids. The cellular fatty acid contents of strain 2T4P-2-4T and the related species of genus Aureimonas are given in Table S1. The whole-cell fatty acids of strain 2T4P-2-4T contained large amounts of C18:1ω7c (61.2%), moderate amounts of C16:0 (11.2%) and C18:1 2-ОН (11.3%), small amounts of C19:0 cyclo ω8c (5.6%), C16:1ω7c (5.0%) and C18:0 (2.6%). The DNA G+C content of strain 2T4P-2-4T 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-CFT034T in our study were largely consistent with previous reports [10].

The genomic DNA of strain 2T4P-2-4T 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’-AGAGTTTGATCCTGGGCTCAG-3’) and 1492R (5’-GGTTACCCGTAGGTGAC-3’).
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'-GTTTTCCCAAGTTCAACGGAC-3') and M13R (5'-CAGGAAACAGCTATGAC-3') and 51.W1F (5'-CCCGTCATTGCTTCTGATGG-3') by an ABI PRISM 3730XL DNA Analyzer (Foster City, CA), and then three sequence fragments were assembled using SeqMan [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 maximum-likelihood algorithms, showed that strain 2T4P-2-4T fell within the lineage of the genus Aureimonas and formed a distinct clade with A. rubiginis CC-CFT034T. The position of strain 2T4P-2-4T did not vary with the methods of tree reconstruction used and it was supported by high bootstrap values (Fig. 1). It was clear that 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 sulfoquinovosyl diacylglycerol (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.phy’ti.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 coccoid 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-glucosaminidase, acid phosphatase, alkaline phosphatase, α-glucosidase, leucine arylamidase, naphthol-AS-
BL-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-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-lxoyse, mannitol, D-mannose, D-ribose (weakly), L-rhamnose, L-sorbose (weakly), sorbitol (weakly), xylitol (weakly), L-xylene and D-xylene (weakly), but not from N-acetylglucosamine, D-adonitol, aesculin, D-arabitol, L-arabitol, arbutin, amygdalin, cellobose, erythritol, D-gentiobiose, D-glucose, gluconate, glyco-gen, glycol, inulin, 2-ketoglucuronate, 5-ketoglucuronate, lactose, maltose, melezitose, melibiose, methyl α-D-glucopyranoside, methyl α-D-mannopyranoside, methyl β-D-xlyopyranoside, raffinose, D-sucrose, salicin, starch, D-tagato-se, 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-β-D-mannosamine, D-arabitol, D-aspartic acid, bromosuccinic acid, cellobiose, citric acid, dextrin, D-fructose, D-fructose-6-phosphate, D-fucose, L-fucose, D-galactose, galacturonic acid, D-galactonic acid lactone, gelatin, gentiobiose, D-glucuconic acid, α-D-glucose, glucuronamide, D-glucuronic acid, glycero1, 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-glucose, methyl pyruvate, mucleic acid, myo-inositol, pectin, L-pyrroglutamic 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, novobiocin, 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 11775T were used as outgroup. Bar, 2 nt substitutions per 100 nt.
The type strain, 2T4P-2-4T (=KCTC 52217T=CGMCC 1.15367T) was isolated from a piece of bark of Agieciras corniculatum collected from Cotai Ecological Zones in Macao, China.

Funding information
This research was partly supported by the National Natural Sciences Foundation of China (NSFC, grant no. 81172963 and grant no. 81321004) and the Research Committee of the University of Macau (ref:MYRG2016-00056-FST).

Acknowledgements
We are grateful to the Environmental Protection Bureau, Government of the Macao SAR, for assistance in sampling at Macao’s mangrove wetland and to Dr J.-N. Liang at the Institute of Microbiology, Chinese Academy of Sciences for her assistance in observing the cell morphology by transmission electron microscopy.

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

References

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