Erythrobacter arachoides sp. nov., isolated from ice core

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Abstract

A Gram-stain-negative, rod-shaped bacterial strain, designated RC4-10-4T, belonging to the genus Erythrobacter, was isolated from the East Rongbuk Glacier on the Tibetan Plateau. Strain RC4-10-4T grew optimally at pH 7.0, at 25°C and in the presence of 2% (w/v) NaCl. Summed feature 3 (C16:1ω6c and/or iso-C15:0 2-OH), summed feature 8 (C18:1ω7c and/or C18:1ω6c) and C16:0 were the major fatty acids. The major polar lipids were phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol, sphingoglycolipid and phosphatidylcholine. Carotenoid was detected in the cells. The DNA G+C content of the novel strain was 66.4 mol%. Phylogenetic analysis based on 16S rRNA gene sequences revealed that strain RC4-10-4T formed a distinct phylogenetic lineage within the cluster comprising Erythrobacter strains. Similarities between the 16S rRNA gene sequences of strain RC4-10-4T and the closely related strains Erythrobacter luteus KCTC 42179T, Erythrobacter gangjinensis KCTC 22330T, Erythrobacter odishensis KCTC 23981T and Erythrobacter atlanticus KCTC 42697T were 98.0, 97.6, 97.5 and 97.2%, respectively. Based on the phenotypic and phylogenetic characteristics, strain RC4-10-4T represents a novel species of the genus Erythrobacter, for which the name Erythrobacter arachoides sp. nov. is proposed, with the type strain RC4-10-4T (=CGMCC 1.15507T=JCM 31277T).

The genus Erythrobacter was first proposed by Shiba and Simidu [1] with the description of a single species, Erythrobacter longus OCh101T. At the time of writing, this genus contains 18 species with validly published names (www.bacterio.net/-allnamesdl.html) which were isolated from different resources, such as seaweed [1, 2], seawater [3–11], soil [12] and sediment [13–16]. In this study, we describe a novel strain, designated RC4-10-4T, which was isolated from an ice core in East Rongbuk Glacier (86.96°E, 28.02°N) on the Tibetan Plateau. The section of ice core was melted in sterile Nalgene bottles at 4°C. Subsequently, 200 µl melt water from separate sections of the ice core was used for cultivation. After incubation at 4°C for 30 days on R2A agar [17], some orange bacterial colonies were recovered. Isolates were routinely cultured on R2A agar at 25°C and maintained as a glycerol suspension (15%, w/v) at −80°C.

Bacterial genomic DNA was extracted according to the method of Marmur [18] from cells grown on R2A agar for 3 days at 25°C. Purity was measured by using a Nanodrop spectrophotometer (2000c; Thermo). The 16S rRNA gene sequence was amplified by using the universal bacterial primers 27F (5′-AGAGTTTGATCCTTGTCAG-3′) and 1492R (5′-CGGTACCTTGGTGACTCTTGA-3′). To determine the approximate phylogenetic affiliation, the 16S rRNA gene sequence was compared with those available in the GenBank database by using the BLAST program (NCBI) and Ezbiocloud [19]. Phylogenetic trees were reconstructed on the basis of 16S rRNA gene sequence analysis using the neighbour-joining, maximum-likelihood and maximum-parsimony algorithms in the MEGA 7 software package [20] with 1000 bootstrap replications (Fig. 1). It was found that strain RC4-10-4T was most related to Erythrobacter luteus KCTC 42179T, Erythrobacter gangjinensis KCTC 22330T, Erythrobacter odishensis KCTC 23981T and Erythrobacter atlanticus KCTC 42697T at levels of 98.0, 97.6, 97.5 and 97.2%, respectively. So they were considered as reference strains for subsequent comparisons under the same laboratory conditions.

The genomic DNA G+C content was estimated from the midpoint value (Tm) of the thermal denaturation profile

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Abbreviations: Q-10, Ubiquinone-10; Tm, melting temperature.

Three supplementary figures are available with the online Supplementary Material.
The genomic DNA G+C content of strain RC4-10-4<sup>T</sup> was 66.4 mol%. DNA–DNA hybridization between strain RC4-10-4<sup>T</sup> and the reference strains was carried out with by using the optical renaturation method [21–23]. The temperature used in the optical renaturation method was 80°C. Strain RC4-10-4<sup>T</sup> shared 37.6, 29.8, 15.4 and 35.8% DNA relatedness with <i>E. luteus</i> KCTC 42179<sup>T</sup> (98.0% similarity), <i>E. gangjinensis</i> KCTC 22330<sup>T</sup> (97.6%), <i>E. odishensis</i> KCTC 23981<sup>T</sup> (97.5%) and <i>E. atlanticus</i> KCTC 42697<sup>T</sup> (97.2%), respectively.

To further determine the taxonomic position of the novel isolate, a series of phenotypic and genotypic approaches were used. Cell morphology was examined by transmission electron microscope (JEM-1230; JEOL). Cells of the novel strain were rod-shaped, 1.1–1.4 µm long and 0.3–0.6 µm wide (Fig. S1, available with the online Supplementary Material). Gram reaction and catalase activity tests were conducted according to the method described by Smibert and Krieg [24]. Oxidase activity was analysed with oxidase reagent. Hydrolysis of starch, gelatin, aesculin and Tweens 20, 40, 60 and 80 were determined as described by Cowan and Steel [25]. Motility was assessed by the hanging-drop method. Growth at various temperatures (0–35°C) was tested in R2A broth in 5°C increments. The pH range (5.0–11.0) for growth was determined in R2A broth at 25°C. The pH values of <6, 6–9 and >9 were obtained by using sodium acetate/acetic acid, Tris/HCl and Na<sub>2</sub>CO<sub>3</sub> buffers, respectively. Growth in the absence of NaCl and in the presence of 0.2, 0.4, 0.6, 0.8, 1, 2, 3, 4, 5 and 6% NaCl was also investigated in the same medium at 25°C. Physiological and biochemical characteristics and other enzyme activities were determined by using API 20E, API 20NE and API ZYM strips (bioMérieux) following the manufacturer’s instructions at 25°C. Differences in the physiological characteristics between RC4-10-4<sup>T</sup> and all the reference strains are given in Table 1.

Fig. 1. Neighbour-Joining phylogenetic tree for strain RC4-10-4<sup>T</sup>, based on 16S rRNA gene sequence analysis. Number at nodes indicated bootstrap percentages (based on 1000 replications). Bar, 0.005 accumulated changes per nucleotide. Circles indicate that the corresponding nodes were also obtained by using the maximum-likelihood and maximum-parsimony algorithms.
System (MIDI, version 6.0). The predominant fatty acids of strain RC4-10-4 T and the reference strains were summed feature 8 (C18:1 ω7c and/or C19:1 ω6c) and summed feature 3 (C16:1 ω7c and/or iso-C15:0 2-OH) (Table 2). There were no significant differences in the dominant components between strain RC4-10-4 T and reference strains, except that strain RC4-10-4 T had a relatively higher proportion of summed feature 3 (C16:1 ω7c and/or iso-C15:0 2-OH) (Table 2).

Strain RC4-10-4 T was cultivated for 5 days at 25 °C to obtain the cell mass required for respiratory quinone and polar lipid analysis. Respiratory quinone was extracted and purified according to the methods of Collins [26], and analysed by high-performance liquid chromatography according to Wu et al. [27]. The predominant respiratory quinone of strain RC4-10-4 T was Q-10. Polar lipids were extracted from 150 mg of freeze-dried cells of strain RC4-10-4 T and examined by two-dimensional thin-layer chromatography as described by Minnikin [28]. Ethanolic ninhydrin, molybdenum blue (Sigma), α-naphthol and molybdophosphoric acid were used to detect amino lipids, phospholipids, glycolipids and total polar lipids, respectively. The major polar lipids of strain RC4-10-4 T were phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol, phosphatidylglycerol, phosphatidylcholine, sphingoglycolipid and four unidentified lipids (Fig. S2).
Pigment analysis was performed as described by Saga [29] using cells of strain RC4-10-4\(^{-T}\) in the middle-late logarithmic phase. Pigments were extracted in acetone–methanol–solution (7:2:1). The absorption spectrum of the cell extracts was analyzed by using SpectraMax M5 equipment. The spectra of pigments extracted from cells showed only one peak, which indicated the presence of carotenoids. No peak was above 600 nm. There were no photosynthetic light-harvesting complexes in the cells (Fig. S3).

In conclusion, on the basis of genotypic and phenotypic characteristics, strain RC4-10-4\(^{-T}\) is clearly a member of the genus *Erythrobacter*. Analyses of phylogenetic affiliation, 16S rRNA gene sequences, the major fatty acids (summed feature 8, summed feature 3 and C\(_{16:0}\)), the predominant respiratory quinone (Q-10) and the major polar lipids (phosphatidyethanolamine, phosphatidylethanolamine, diphosphatidylglycerol and phospha-tidylglycerol, sphingoglycolipid and phospha-tidylylglycerol) support the affiliation of strain RC4-10-4\(^{-T}\) to the genus *Erythrobacter*.

### DESCRIPTION OF *ERYTHROBACTER ARACHOIDES* SP.NOV.

*Erythrobacter arachoides* sp. nov. (a.r.a.cho’i.des. N.L. n. arachis, peanut; L. suff. –oides, looking like; N.L. masc. adj. arachoides, looking like a peanut).

Cells are aerobic, Gram-stain-negative. Colonies are orange, convex and opaque after incubation on R2A agar at 25°C for 5 days. Growth occurs at 0–35°C (optimally at 25°C), pH 6–9 (optimally at 7) with 0–3% NaCl (optimally at 2%). Indole and H\(_2\)S are not produced. Nitrate and nitrite reduction does not occur. The cells are positive for hydrolysis of catalase, Tween 20, 40, 60, 80 and negative for hydrolysis of oxidase and starch. The fatty acid profile is mostly composed of summed feature 8 (C\(_{18:1}\)ω7c and/or C\(_{18:1}\)ω6c), Summed feature 3 (C\(_{16:1}\)ω7c and/or iso-C\(_{15:0}\)2-OH) and C\(_{16:0}\). The major polar lipids are phosphatidylethanolamine, phosphatidylglycerol, diphasphatidylglycerol and sphingoglycolipid. Q-10 is the dominant respiratory quinone. In the API ZYM test, cells are positive for alkaline phosphatase and trypsin, weak for esterase (C4), esterase lipase (C8), leucine, cysteine arylamidase and naphthol-AS-B1-phosphohydrolase, and negative for lipase (C14), valine arylamidase, α-chymotrypsin, acid phosphatase, α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase, α-mannosidase and α-fucosidase. In API 20NE tests, cells are positive for aesculin hydrolysis, weakly positive for β-galactosidase, and negative for adipic acid, capric acid, phenylacetic acid, malic acid and assimilation of D-glucose, L-arabinose, D-mannose, D-mannitol, N-acetylglucosamine and maltose. In API 20E tests, cells are negative for citrate utilization, H\(_2\)S, indole and acetoin production, activities of arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, urease, tryptophan deaminase and gelatinase, and acid production from D-glucose, mannanol, inositol, sorbitol, rhannosucrose, melibiose, amygdalin and arabinose.

The type strain, RC4-10-4\(^{-T}\) (＝CGMCC 1.15507\(^{T}\)=JCM 31277\(^{T}\)), was isolated from an ice core in the East Rongbuk Glacier, Tibetan Plateau. The DNA G+C content of the type strain is 66.4 mol% (Tm).

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

### References
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