**Altererythrobacter xinjiangensis** sp. nov., isolated from desert sand, and emended description of the genus *Altererythrobacter*

Xiuqing Xue,1 Kundi Zhang,2 Feng Cai,1 Jun Dai,1 Yang Wang,1 Erkin Rahman,3 Fang Peng1 and Chengxiang Fang1

1China Center for Type Culture Collection (CCTCC), College of Life Sciences, Wuhan University, Wuhan 430072, PR China
2Qingdao Institute of Bioenergy and Bioprocess Technology, Chinese Academy of Sciences, Qingdao, PR China
3College of Life Science and Technology, Xinjiang University, Urumchi 830046, PR China

A Gram-negative, rod-shaped, non-motile, strictly aerobic bacterium, strain S3-63T, was isolated from desert sand of Xinjiang, China. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain S3-63T had highest similarity to type strains of the genus *Altererythrobacter*, i.e. *Altererythrobacter marinus* H32T (97.2 % similarity), *Altererythrobacter marensis* MSW-14T (95.9 %), *Altererythrobacter aestuarii* KCTC 22735T (95.5 %), *Altererythrobacter epoxidivorans* JCS350T (95.1 %), *Altererythrobacter namhicola* KCTC 22736T (95.1 %), *Altererythrobacter luteolus* SW-109T (95.0 %) and *Altererythrobacter indicus* LMG 23789T (93.5 %). Growth occurred at 20–37 °C (optimum 30 °C), at pH 7.0–9.0 (optimum pH 8.0) and in 0–3 % (w/v) NaCl (optimum 1 %). The major respiratory quinone was ubiquinone-10 and the predominant cellular fatty acids were C18:1ω7c (50.8 %), summed feature 3 (C16:1ω7c and/or C16:1ω6c; 12.6 %), C16:0 (12.3 %), C14:0 2-OH (7.3 %) and C17:1ω6c (4.5 %). The DNA G+C content was 64.6 mol%. Therefore, the phylogenetic, physiological and chemotaxonomic data demonstrated that strain S3-63T represents a novel species of the genus *Altererythrobacter*, for which the name *Altererythrobacter xinjiangensis* sp. nov. is proposed; the type strain is S3-63T (=CCTCC AB 207166=KIP 110125). An emended description of the genus *Altererythrobacter* is provided.

The genus *Altererythrobacter* was created by Kwon et al. (2007) and, at the time of writing, comprised seven species with validly published names: *Altererythrobacter epoxidivorans* (Kwon et al., 2007), *A. luteolus* (Yoon et al., 2005; Kwon et al., 2007), *A. indicus* (Kumar et al., 2008), *A. marinus* (Lai et al., 2009), *A. marensis* (Seo & Lee, 2010), and *A. namhicola* and *A. aestuarii* (Park et al., 2011). Members of the genus *Altererythrobacter* share many phenotypic and chemotaxonomic characteristics, such as the absence of motility, formation of yellow-coloured colonies and rod-shaped cells. C18:1ω7c and ubiquinone-10 are the dominant fatty acid and respiratory quinone, respectively. All current members of the genus *Altererythrobacter* have been isolated from seawater environments. Strain S3-63T was isolated from the desert of Xinjiang, China, and 16S rRNA gene sequence analysis indicated that this isolate formed a clade within the genus *Altererythrobacter*. In this report, isolation and classification of strain S3-63T using a polyphasic approach are described.

A novel member of the genus *Altererythrobacter*, strain S3-63T, was isolated from the desert of Xinjiang, China (41° N 88° E). The sand sample was diluted with sterile water and dilutions were plated onto tenfold-diluted tryptic soy broth (TSB/10; Difco) agar. The strain was isolated after incubation at 30 °C for 5 days. It showed poor growth on 0.1 % TSB agar and could only form single colonies. Tests using various standard bacteriological media showed that the isolate could grow well on 0.3 % marine agar 2216 (MA; Difco) plates. The type strains of four *Altererythrobacter* species were used as reference strains; *A. marensis* KCTC 22370T and *A. luteolus* KCTC 12311T were obtained from the Korean Collection for Type Cultures (KCTC), *A. epoxidivorans* KACC 14100T was obtained from the Korean Agricultural Culture Collection (KACC) and *A. marinus* H32T was obtained from China Center for Type Culture Collection (CCTCC).

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain S3-63T is HM028673.

Four supplementary figures and one supplementary table are available with the online version of this paper.
Cell morphology was examined by phase-contrast microscopy (Olympus BX51) and transmission electron microscopy (Hitachi-8100). Cells were prepared for transmission electron microscopy as described by Golyshina et al. (2000). Gram staining was carried out according to the classical Gram-staining procedure described by Doetsch (1981). Motility was examined according to the hanging drop technique, as recommended by Bernardet et al. (2002). Growth at various temperatures (4, 10, 15, 20, 28, 30, 37 and 42 °C) and pH 5.0–10.0 (in increments of 0.5 pH units) was assessed on 0.3× MA for 5 days. Oxidase activity was evaluated by oxidation of 1% p-aminodi-methylaniline oxalate and catalase activity was determined by measurements of bubble production after the application of 3% (v/v) hydrogen peroxide solution. Tolerance to NaCl [0–5% (w/v) in increments of 1%] was determined on R2A (Difco) for 5 days. Bacteriochlorophyll a content was determined as described by Biebl et al. (2005). Cell pigments were extracted with methanol from a culture grown on 0.3× MA and spectra were measured with a spectrophotometer (UV-2100Pro; Amersham). Further physiological and biochemical characteristics were determined using the API 20NE and API ZYM systems (bioMérieux) and Biolog GN2 MicroPlate panels, according to the instructions of the manufacturer.

Cells of strain S3-63T were Gram-stain-negative, rod-shaped (1.1–1.2×1.3–1.6 μm; see Supplementary Fig. S1, available in IJSEM Online) and non-motile. The isolate grew aerobically at 20–37 °C, at pH 7.0–9.0 and in NaCl concentrations of 0–3% (w/v), with optimum growth at 30 °C, at pH 8.0 and in 1% NaCl. Bacterial colonies were yellow, smooth, circular and about 1 mm in diameter after growth at 30 °C for 4 days. The isolate tested positive for catalase and negative for oxidase. It was able to hydrolyse casein and cellulose, but not starch. Detailed differential phenotypic properties of strain S3-63T and related strains are listed in Table 1 and other characters are given in the species description.

### Table 1. Differential phenotypic characteristics of strain S3-63T and type strains of phylogenetically related species

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxidase activity</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Hydrolysis of:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Casein</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Starch</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>API ZYM:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Valine arylamidase</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>α-Chymotrypsin</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Acid phosphatase</td>
<td>+</td>
<td>w</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>β-Galactosidase</td>
<td>–</td>
<td>–</td>
<td>w</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>β-Galactosidase</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>w</td>
<td>–</td>
</tr>
<tr>
<td>β-Glucosidase</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>API 20NE:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gelatin</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>d-Glucose</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>L-Arabinose</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>d-Mannitol</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>N-Acetylglucosamine</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Maltose</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Potassium gluconate</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Capric acid</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Adipic acid</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Malic acid</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Trisodium citrate</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>
Genomic DNA was extracted with a Bacteria Genomic DNA isolation kit (Shanghai Chaoshi Bio Technologies). The 16S rRNA gene was amplified by PCR with bacterial universal primers 27F (5'-GAGTTTGATCCTGGCTCAG-3') and 1527R (5'-AGAAAGGAGGTGATCCAGCC-3') (Rainey et al., 1996). The PCR product was linked to the pGEM-T vector and sequenced by Invitrogen Biotechnology. The 16S rRNA gene sequence of strain S3-63T was a continuous stretch of 1444 bp. Sequence similarity was calculated by pairwise alignment obtained from the EzTaxon server (http://www.eztaxon.org/; Chun et al., 2007). Strain S3-63T showed highest similarity to type strains of the genus *Altererythrobacter*: *A. marinus* H32T (97.2% similarity), *A. marensis* MSW-14T (95.9%), *A. aestuarii* KCTC 22735T (95.5%), *A. epoxidivorans* JCS350T (95.1%), *A. namhidica* KCTC 22736T (95.1%), *A. luteolis* SW-109T (95.0%) and *A. indicus* LMG 23789T (93.5%). Multiple alignments with sequences of the most closely related *Altererythrobacter* and calculations of sequence similarities were carried out using CLUSTAL_X (Thompson et al., 1997). Phylogenetic trees were constructed by the neighbour-joining (Saitou & Nei, 1987) (Fig. 1), maximum-parsimony (Fitch, 1971) (Supplementary Fig. S2, available in IJSEM Online) and maximum-likelihood (Felsenstein, 1981) (Supplementary Fig. S3, available in IJSEM Online) tree-making algorithm by using the software packages MEGA version 4.0 (Tamura et al., 2007) and PHYLIP version 3.6. A maximum-likelihood tree was also constructed online (http://www.atgc-montpellier.fr/phylm/).

**Fig. 1.** Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the positions of strain S3-63T and related strains (all *Altererythrobacter* species and members of closely related genera). Bootstrap values (%) based on 1000 replications are given at branch points; only values ≥50% are shown. Bar, 0.01 substitutions per nucleotide position.
Guindon & Gascuel, 2003; Supplementary Fig. S4, available in IJSEM Online).

Evolutionary distances for the neighbour-joining algorithm were calculated with the Kimura two-parameter model and close-neighbour-interchange (search level=2, random additions=100) was applied in the maximum-parsimony analysis. In each case, bootstrap values were calculated based on 1000 replications (Felsenstein, 1985). Phylogenetic analyses with all members of the family Erythrobacteraceae and several members of the family Sphingomonadaceae revealed that strain S3-63T formed a distinct branch within the genus Altererythrobacter.

Levels of DNA–DNA relatedness were determined between strain S3-63T and the type strain of the phylogenetically most closely related species (A. marinus H32T) using the microplate method, as described previously by Ezaki et al. (1989) and Willems et al. (2001). The mean DNA–DNA relatedness value between the novel isolate and A. marinus H32T was 54.5 ± 2 %, a value well below the 70 % cut-off point recommended by Tindall et al. (2010) for the delineation of genomic species.

To measure the G+C content of the chromosomal DNA, genomic DNA from the isolate was extracted and purified as described by Moore & Dowhan (1995), then degraded enzymically into nucleosides. The G+C content was determined as described by Mesbah et al. (1989) using reversed-phase HPLC (UltiMate 3000; Dionex). Respiratory quinones were isolated from lyophilized cells as described by Collins et al. (1977) and analysed by HPLC as described by Xie & Yokota (2003). For cellular fatty acid analysis, strain S3-63T and other related Altererythrobacter species were cultivated on 0.3 × MA plates at 30 °C for 4 days before harvesting cell mass. Fatty acid methyl esters were tested according to the protocol of the Sherlock Microbial Identification System (MIDI system) and analysed by GC (Agilent 6890N) with the MIDI Sherlock TSBA6 version of the database (Sasser, 1990).

The DNA G+C content of strain S3-63T was 64.6 mol%, which is similar to those of members of the genus Altererythrobacter. The major isoprenoid quinone of strain S3-63T was ubiquinone-10 (Q-10), which corresponds to the characteristic quinone found in members of the genus Altererythrobacter. The fatty acid pattern of strain S3-63T is shown in Supplementary Table S1 (available in IJSEM Online). Predominant fatty acids were C18:1ω7c (50.8 %), summed feature 3 (C16:1ω7c and/or C16:1ω6c; 12.6 %), C16:0 (12.3 %), C14:0 2- OH (7.3 %) and C17:0ω6c (4.5 %). Although the major fatty acids were in agreement with those found in members of the genus Altererythrobacter, the profile of the isolate differed in terms of content and composition; compared with related type strains, strain S3-63T lacked 11-methyl-C18:1ω7c and C18:1ω5c, and had higher proportions of C16:0 (12.3 %).

Strain S3-63T was isolated from the desert of Xinjiang, China. Other members of the genus Altererythrobacter have been isolated from seawater environments. Unlike related type strains of the genus Altererythrobacter, strain S3-63T cannot tolerate high NaCl concentrations, is weakly positive for β-glucosidase, can hydrolyse casein, and can assimilate D-glucose, L-arabinose, D-mannitol, capric acid and trisodium citrate.

Thus, based on phylogenetic analysis and the physiological tests, strain S3-63T is a phylogenetically and physiologically distinct strain that represents a novel species in the genus Altererythrobacter, for which the name Altererythrobacter xinjiangensis sp. nov. is proposed.

**Emended description of the genus Altererythrobacter**

Main characteristics are as given in the genus description of Kwon et al. (2007), with the following amendments. Cells are motile or non-motile. Positive or negative for oxidase. Methanol-soluble pigments are species-dependent. Some species require NaCl for growth, whereas other species do not require NaCl. The major polar lipids are phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol and sphingoglycolipid. The DNA G+C content is 54.3–67.2 mol%.

**Description of Altererythrobacter xinjiangensis sp. nov.**

_Altererythrobacter xinjiangensis_ (xin.ji.ang.en’sis. N.L. masc. adj. _xinjiangensis_ of or pertaining to Xinjiang, an autonomous region in north-west China).

Cells are Gram-negative, aerobic, non-motile and rod-shaped (1.1–1.2 × 1.3–1.6 μm). Colonies on 0.3 × MA plates are yellow, smooth, circular and about 1 mm in diameter after growth at 30 °C for 4 days. Bacterial chlorophyll a is not detected. Methanol-soluble pigment is characterized by absorption maxima at 447 and 473 nm. Growth occurs on 0.3 × MA at 20–37 °C, at pH 7–9 and in 0–3 % (v/v) NaCl. Optimum growth occurs at 30 °C, at pH 8.0 and in 1 % (v/v) NaCl. Oxidase-negative and catalase-positive. Hydrolyses casein and cellulose, but not starch. In API 20NE and API ZYM test strips: positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, z-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphoamidase and β-galactosidase; weakly positive for cystine arylamidase, trypsin, z-glucosidase and β-glucosidase; and negative for lipase (C14), β-galactosidase, β-glucuronidase, N-acetyl-β-glucosaminidase, z-mannosidase, z-fucosidase, nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease and gelatin hydrolysis. In API 20NE test strips, D-glucose, L-arabinose, D-mannitol, maltose, potassium gluconate, capric acid, adipic acid, malic acid and trisodium citrate are assimilated. Does not assimilate D-mannose, N-acetylglucosamine or phenylacetic acid. Of the 95 carbon substrates in the Biolog system (Biolog GN2), positive for z-cyclodextrin, dextrin, glycogen, Tween 40, D-fructose, D-psicose, raffinose, pyruvic acid
methyl ester, succinic acid monomethyl ester, acetic acid, α-ketoglutaric acid, succinic acid, L-asparagine, L-ornithine, DL-α-glycerol phosphate, α-D-glucose 1-phosphate and D-glucose 6-phosphate. The major respiratory quinone is ubiquinone-10 and the predominant fatty acids are \( C_{16:0} \; \text{i107C} \), summed feature 3 (\( C_{16:1} \; \text{i106C} \) and/or \( C_{16:0} \; \text{i106C} \), \( C_{14:0} \; 2\text{-OH} \) and \( C_{17:0} \; \text{i106C} \) (Supplementary Table S1, available in IJSEM Online).

The type strain is S3-63\(^T\) (=CCTCC AB 207166\(^T\)=CIP 110123\(^T\)), isolated from sand from the desert of Xinjiang, China. The DNA G+C content of the type strain is 64.6 mol%.

## Acknowledgements

This work was supported by the R & D Infrastructure and Facility Development Program from the Ministry of Science and Technology of the People’s Republic of China (grant no. 2005DKA21208) and KIZJ Foundation of Xinjiang Uyghur Autonomous Region (Grant No. 201091236). We thank Dr Jung-Soon Lee from the KCTC for offering us Altererythrobacter marenis KCTC 22370\(^\text{T} \) and Altererythrobacter luteolus KCTC 12311\(^T\) for taxonomy study. We also thank Dr Soon-Wo Kwon from the KACC for offering us Altererythrobacter epoxidivorans KACC 14100\(^T\) for taxonomy study.

## References


