

## *Altererythrobacter buctense* sp. nov., isolated from mudstone core

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**Abstract** A yellow-pigmented, Gram-negative, non-flagellated, rod-shaped bacterial strain, designated M0322<sup>T</sup>, was isolated from a mudstone core sample of the Mohe Basin, China. Growth of strain M0322<sup>T</sup> was observed at 15–40 °C (optimum, 30 °C), at pH 5.0–10.0, (optimum, pH 6.0–7.0) and in the presence 0–4 % NaCl (optimum, 0 %). Phylogenetic analyses of 16S rRNA gene sequences revealed that strain M0322<sup>T</sup> formed a distinct phyletic lineage with the members of the genus *Altererythrobacter* and is closely related to *Altererythrobacter aestuarii* JCM 16339<sup>T</sup> (96.1 %) and *Altererythrobacter namhicola* JCM 16345<sup>T</sup> (95.7 %). The only isoprenoid quinone was identified as ubiquinone 10 (Q-10), major polar lipids were determined to be phosphatidylethanolamine, phosphatidylglycerol, phosphatidylcholine, one unidentified glycolipid and three unidentified phospholipids, while major cellular fatty acids were summed feature 8 (C<sub>18:1</sub>ω6c and/or C<sub>18:1</sub>ω7c), summed feature 3 (C<sub>16:1</sub>ω6c and/or

C<sub>16:1</sub>ω7c) and 11-Methyl C<sub>18:1</sub>ω7c. The DNA G+C content of strain M0322<sup>T</sup> was determined to be 64.6 mol%. Based on the results of the polyphasic taxonomic study, strain M0322<sup>T</sup> is considered to represent a novel species of the genus *Altererythrobacter*, for which the name *Altererythrobacter buctense* sp. nov. is proposed. The type strain is M0322<sup>T</sup> (=CGMCC 1.12871<sup>T</sup> = JCM 30112<sup>T</sup>).

**Keywords** Polyphasic approach ·  
*Altererythrobacter* · Novel species · Mohe basin

### Introduction

The genus *Altererythrobacter*, a member of the family *Erythrobacteraceae* (Lee et al. 2005), was first proposed by Kwon et al. (2007). Currently, the genus includes 16 species (<http://www.bacterio.net/altererythrobacter.html>). The members of the genus *Altererythrobacter* are characterised as yellow-pigmented, Gram-negative and rod-shaped bacteria, which are lacking of BChl *a* as a photosynthetic pigment; C<sub>18:1</sub>ω7c is the major fatty acid and Q-10 is the main respiratory quinone (Kwon et al. 2007). In this work, a yellow-pigmented strain M0322<sup>T</sup>, which was isolated from a subterranean sediment sample of Mohe basin permafrost in northeast of China, was subjected to a polyphasic taxonomic investigation in order to establish its taxonomic status,

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## Materials and methods

### Isolation of the bacterial strain

In 2011, 123 drilling core samples were collected from the Mohe basin scientific well MK-2 (53°28′37.80″N, 122°19′23.40″E). Well MK-2 is located in Mohe basin permafrost in northeast of China. With exogenous microbial contamination eliminated by fluorescent microspheres (Colwell et al. 1992), a mudstone core sample (depth, 60.0 m) was separated and ground to powder under liquid nitrogen (SPEX Sample Prep 6870, USA) and sterile conditions (Huang et al. 2014). A portion of the core sample powder (1 g) was serially diluted in a saline solution (0.85 %, w/v), spread on R2A agar (Difco, US) and incubated for one week at 25 °C aerobically. A yellow-pigmented strain M0322<sup>T</sup> was isolated from the mudstone core sample. The isolate was routinely grown under the same conditions and preserved in R2A broth (without agar) containing glycerol (15 %, v/v) at –80 °C.

### Morphological and physiological characteristics

Cell morphology and presence of flagella were observed by the phase-contrast microscopy (Leica DMLS, Germany), transmission (JEM-2100; JEOL) and scanning (SU1510; HITACHI) electron microscopy with cells grown on R2A agar for 3 days at 30 °C. Gliding motility was determined as described by Bernardet et al. (2002). The Gram reaction was performed with 2 % (w/v) crystal violet (Gerhardt 1994). The growth condition was examined on MR agar (per liter distilled water: peptone, 2.75 g; yeast extract, 0.75 g; ferric citrate, 0.05 g; NaCl, 9.72 g; MgCl<sub>2</sub>, 2.95 g; MgSO<sub>4</sub>, 1.65 g; CaCl<sub>2</sub>, 0.90 g; KCl, 0.28 g; casamino acids 0.25 g; glucose, 0.25 g; soluble starch, 0.25 g; Na-pyruvate, 0.15 g; K<sub>2</sub>HPO<sub>4</sub>, 0.15 g; NaHCO<sub>3</sub>, 0.08 g; KBr, 0.04 g; SrCl<sub>2</sub>, 17.0 mg; boric acid, 11.0 mg; Na<sub>2</sub>SiO<sub>4</sub>, 2 mg; NaF, 1.2 mg; NH<sub>4</sub>NO<sub>3</sub>, 0.8 mg; Na<sub>2</sub>HPO<sub>4</sub>, 4 mg; agar, 15 g; pH 7.2 ± 0.2 at 25 °C), R2A agar, marine broth agar (MA, Difco, US), trypticase soy broth agar (TSBA, Difco, US), nutrient agar (NA, Difco, US) and Luria–Bertani agar (LB, Difco, US) for 5 days at 30 °C. The optimum temperature for growth was determined in R2A broth in triplicate at 4, 10, 15, 20, 25, 30, 35, 37, 40 and 42 °C for 3 days on a rotary incubator. The pH range for growth was also determined in R2A broth in

triplicate at 30 °C for 3 days on a rotary incubator model, conditions. The optimal pH for growth was tested in R2A broth at pH 4.5–10.0 (at intervals of pH 0.5) by using the following buffer system: pH 4.0–5.0, 0.1 M citric acid/0.1 M sodium citrate; pH 6.0–8.0, 0.1 M KH<sub>2</sub>PO<sub>4</sub>/0.1 M NaOH; pH 9.0–10.0, 0.1 M NaHCO<sub>3</sub>/0.1 M Na<sub>2</sub>CO<sub>3</sub>; pH 11.0–12.0, 0.05 M Na<sub>2</sub>HPO<sub>4</sub>/0.1 M NaOH. Salt tolerance was examined in R2A broth supplemented with 0–7 % (w/v, at intervals of 1 %) at 30 °C for 3 days. The growth turbidity was recorded using a spectrophotometer (O.D.<sub>600</sub> nm; UV-2450, Shimadzu). Anaerobic growth was assessed on R2A agar after incubation for up to 5 day in an anaerobic chamber (CO<sub>2</sub>/H<sub>2</sub>/N<sub>2</sub>, 5:5:90; YQX-II CIMO). Bacteriochlorophyll (BChl) *a* was detected by using Cary 100 Bio UV–visible Spectrophotometer (Varian, USA), according to the method described by Gich and Overmann (2006). The oxidase and catalase activities were tested using 1 % tetramethyl-*p*-phenylenediamine (oxidase test strips; bioMérieux) and 3 % (v/v) H<sub>2</sub>O<sub>2</sub>, respectively. H<sub>2</sub>S production was assessed using the methods of Barrow and Feltham (1993). Tests for hydrolysis of starch, casein, gelatin, urea, chitin, Tween 20, and Tween 80 were assessed as described elsewhere (Yuan et al. 2014). Additional physiological and biochemical analyses, including the enzyme activities and carbon source assimilation, were performed in parallel with *A. aestuarii* JCM 16339<sup>T</sup> and *A. namhicola* JCM 16345<sup>T</sup> by using the API 20 NE, API 20E, API ZYM and API 50CH kits (bioMérieux) according to the instructions of manufacturer, except that the all strips were incubated at 30 °C.

### Phylogenetic analysis by the 16S rRNA sequencing and DNA base content

To determine 16S rRNA gene sequences, bacterial DNA was extracted and purified using a bacterial DNA kit (OMEGA, USA). The 16S rRNA gene was amplified by PCR using primers 27F (5′-AGAGTTT-GATCCTGGCTCAG-3′) and 1492R (5′-AAG-GAGGTGATCCAGCC-3′). The PCR product was purified using a PCR purification kit (OMEGA) and then ligated into the pMD18-T vector (TaKaRa). The ligation product was transformed into *Escherichia coli* strain DH5 $\alpha$ . The cloned 16S rRNA gene sequence was then sequenced on an ABI 3730XL 96-capillary DNA analyser (Applied Biosystems). The nearly complete 16S rRNA gene sequence (1449 bases) of

strain M0322<sup>T</sup> was determined. The search for phylogenetic neighbours based on 16S rRNA gene sequence similarity was performed using the EzTaxon server (<http://www.eztaxon.org/>; Kim et al. 2012). Alignment was performed using ARB release 6.0.2 (Ludwig et al. 2004) based on the complete SSU rRNA ‘All-Species Living Tree’ Project (LTP) database (Yarza et al. 2008) release 121 (July 2015). Sequences not included in the LTP database were imported into the LTP database, automatically aligned with the Fast Aligner tool. The alignment of sequences was controlled manually based on secondary structure prediction information. Evolutionary distances were calculated using the algorithm of Kimura’s two-parameter model (Kimura 1980). Distances (distance options according to Kimura’s two-parameter model) and clustering with the maximum-likelihood (Kishino and Hasegawa 1989), neighbour-joining (Saitou and Nei 1987) and maximum-parsimony (Fitch 1971) methods in MEGA release 6.06 (Tamura et al. 2011) were determined by using bootstrap values based on 1000 replications (Felsenstein 1985). The DNA G+C content of strain M0322<sup>T</sup> was determined as described previously (Zhang et al. 2014).

#### Analyses of biochemical and chemotaxonomic characteristics

For analyses of biochemical and chemotaxonomic characteristics, *A. aestuarii* JCM 16339<sup>T</sup> and *A. namhicola* JCM 16345<sup>T</sup> were selected as reference strains. Strain M0322<sup>T</sup>, *A. aestuarii* JCM 16339<sup>T</sup> and *A. namhicola* JCM 16345<sup>T</sup> did not grow well on R2A agar and MA medium (Table 1), so a modified MA medium (MR medium) was used as basal medium to grow strain M0322<sup>T</sup>. Fatty acid analysis was performed as described by Zhang et al. (2014). Bacterial strains were grown on MR at 30 °C at late-exponential phase. Fatty acids were saponified, methylated and extracted from bacterial cells using the standard protocol (MIDI, Sherlock Microbial Identification System, version 6.0). The fatty acids were analyzed by using GC-spell out (6890N; Hewlett Packard) and identified using the TSBA6 database of the Microbial Identification System (Sasser 1990). Polar lipids and isoprenoid quinones were integrally extracted from 100 mg of freeze-dried cell according to the methods described by Minnikin et al. (1984), except that cells were grown on MR agar at 30 °C for 3 days. The polar

lipids and isoprenoid quinone were determined as described elsewhere (Yuan et al. 2014).

## Results and discussion

### Morphological and physiological characteristics

Strain M0322<sup>T</sup> was observed to be Gram-stain negative, facultative anaerobic, non-motile, non-flagellated short rods. The scanning electron micrograph of strain M0322<sup>T</sup> is included in Supplementary Fig. S1. It was found that strain M0322<sup>T</sup> does not contain BChl *a* as a photosynthetic pigment, is positive for  $\beta$ -glucosidase, aesculin hydrolysis and  $\beta$ -galactosidase, but negative for reduction of nitrate to nitrite, indole production, D-glucose fermentation, arginine dihydrolase, urease activity, gelatin hydrolysis and utilization of D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetylglucosamine, maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. According to API 20E, strain M0322<sup>T</sup> was found to be positive for  $\beta$ -galactosidase and acetoin production, negative for lysine decarboxylase, ornithine decarboxylase, citrate utilization, H<sub>2</sub>S production, urease activity, tryptophan deaminase activity, indole production, gelatinase and acid production from glucose, mannitol, inositol, sorbitol, rhamnose, sucrose melibiose, amygdalin and L-arabinose. According to API 50CH, strain M0322<sup>T</sup> was found to be positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, cystine arylamidase, trypsin,  $\alpha$ -chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase,  $\beta$ -galactosidase,  $\beta$ -glucuronidase,  $\alpha$ -glucosidase and  $\beta$ -glucosidase; weakly positive for valine arylamidase; negative for lipase (C14),  $\alpha$ -galactosidase, *N*-acetyl- $\beta$ -glucosaminidase,  $\alpha$ -mannosidase and  $\beta$ -fucosidase (API ZYM). Acid is only produced from aesculin ferric citrate (API 50CH). Other morphological, physiological and biochemical characteristics of strain M0322<sup>T</sup> are given in the species description. The differential morphological, physiological, and biochemical characteristics between strain M0322<sup>T</sup> and reference strains are shown in Table 1.

### Chemotaxonomic characteristics

The whole-cell fatty acid compositions of strain M0322<sup>T</sup> and the reference strains are shown in

**Table 1** Differential characteristics of strain M0322<sup>T</sup> and phylogenetically close species of the genus *Altererythrobaacter*

Characteristic	1	2	3
Cell shape	Rod	Rod*	Ovoid rod*
Colony colour	Yellow	Yellow	Orange
Source of isolation	Mudstone core	Seawater*	Seawater*
Growth on			
R2A agar	+	+	–
Marine broth agar	w	+	+
Enzyme activity (API ZYM)			
Valine arylamidase	w	–	+
$\beta$ -Galactosidase	+	–	–
$\beta$ -Glucuronidase	+	–	–
$\beta$ -Glucosidase	+	+	–
$\beta$ -Fucosidase	–	+	–
API 20NE results			
$\beta$ -Galactosidase	+	–	–
D-mannitol	–	+	–
API 20E results			
Acetoin production	+	–	–
Acid production from (API 50CH)			
Aesculin ferric citrate	+	–	–
D-raffinose	–	–	+
DNA G+C content (mol%)	64.6	67.2*	63.8*

Strains 1 M0322<sup>T</sup>; 2 *A. aestuarii* JCM 16339<sup>T</sup> 3 *A. namhicola* JCM 16345<sup>T</sup>

All strains are positive for the following characteristics: alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, cystine arylamidase, trypsin,  $\alpha$ -chymotrypsin, Acid phosphatase, naphthol-AS-BI-phosphohydrolase,  $\alpha$ -glucosidase and  $\beta$ -glucosidase (aesculin hydrolysis) activities. All strains are negative for the following characteristics: lipase (C14),  $\alpha$ -galactosidase, *N*-acetyl- $\beta$ -glucosaminidase,  $\alpha$ -mannosidase; reduction of nitrate to nitrite, indole production, D-glucose fermentation, arginine dihydrolase, urease activity, gelatin hydrolysis, lysine decarboxylase, ornithine decarboxylase, H<sub>2</sub>S production, tryptophan deaminase activity, gelatinase; assimilation of D-glucose, L-arabinose, D-mannose, *N*-acetylglucosamine, maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid; acid production from glycerol, erythritol, D-arabinose, L-arabinose, D-ribose, D-xylose, L-xylose, D-adonitol, methyl- $\beta$ -D-xylopyranoside, D-galactose, D-glucose, D-fructose, D-mannose, L-sorbose, L-rhamnose, dulcitol, inositol, D-Mannitol, D-sorbitol, methyl- $\alpha$ -D-mannopyranoside, methyl- $\alpha$ -D-glucopyranoside, *N*-acetylglucosamine, amygdalin, arbutin, salicin, D-Cellobiose, D-Maltose, D-lactose (bovine origin), D-melibiose, sucrose, D-trehalose, inulin, D-melezitose, starch, glycogen, xylitol, gentiobiose, D-turanose, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, potassium gluconate, potassium 2-ketogluconate and potassium 5-ketogluconate. Data are taken from this study unless otherwise indicated

\* Date from Park et al. (2011)

Symbol: + positive; – negative; w weakly positive

Table 2. The major fatty acids (>10 % of the total fatty acids) of strain M0322<sup>T</sup> were determined to be summed feature 3 (29.5 %, C<sub>16:1</sub>  $\omega$ 6c and/or C<sub>16:1</sub>  $\omega$ 7c), summed feature 8 (27.8 %, C<sub>18:1</sub>  $\omega$ 6c and/or C<sub>18:1</sub>  $\omega$ 7c) and 11-Methyl C<sub>18:1</sub>  $\omega$ 7c (22.0 %). Strain M0322<sup>T</sup> was found to possess summed feature 3 and summed feature 8 as the major fatty acids, which are similar to those detected in *A. aestuarii* JCM 16339<sup>T</sup> and *A. namhicola* JCM 16345<sup>T</sup>. However, 11-Methyl

C<sub>18:1</sub>  $\omega$ 7c was found to be abundant in strain M0322<sup>T</sup>, while the proportion of C<sub>17:1</sub>  $\omega$ 6c is higher in *A. namhicola* JCM 16345<sup>T</sup>. The only isoprenoid quinone of strain M0322<sup>T</sup> was determined to be ubiquinone 10 (Q-10), which is the characteristic ubiquinone for the genus *Altererythrobaacter*. The polar lipid profile of M0322<sup>T</sup> was found to consist of phosphatidylethanolamine, phosphatidylglycerol, phosphatidylcholine, one unidentified glycolipid and

**Table 2** Cellular fatty acid contents of strains M0322<sup>T</sup> and phylogenetically close species of the genus *Altererythrobacter*

Fatty acid	1	2	3
Saturated fatty acids			
C <sub>14:0</sub>	0.4	0.9	0.5
C <sub>16:0</sub>	2.7	7.5	5.6
C <sub>17:0</sub>	–	–	0.9
C <sub>18:0</sub>	1.6	0.2	–
Unsaturated fatty acids			
C <sub>15:1</sub> ω6c	0.5	1.4	–
C <sub>16:1</sub> ω5c	0.7	–	1.5
C <sub>17:1</sub> ω6c	6.6	3.3	16.0
C <sub>17:1</sub> ω7c	–	2.1	–
C <sub>17:1</sub> ω8c	2.6	–	2.5
C <sub>18:1</sub> ω5c	0.8	1.1	0.4
11-Methyl C <sub>18:1</sub> ω7c	22.0	4.2	2.3
Hydroxy fatty acids			
C <sub>13:0</sub> 2-OH	–	–	0.4
C <sub>14:0</sub> 2-OH	1.2	1.4	7.0
C <sub>15:0</sub> 2-OH	1.0	0.4	2.3
C <sub>16:0</sub> 2-OH	2.8	2.9	–
C <sub>16:1</sub> 2-OH	–	–	0.6
Summed feature 3*	29.4	28.1	29.0
Summed feature 8*	27.7	46.5	31.0

Strains 1 M0322<sup>T</sup>; 2 *A.aestuarii* JCM 16339<sup>T</sup>; 3 *A.namhicola* JCM 16345<sup>T</sup>

All data are obtained in this study. \* Summed features are groups of two or three fatty acids that cannot be separated by GLC with the MIDI system. Summed feature 3 consists of C<sub>16:1</sub> ω6c and/or C<sub>16:1</sub> ω7c, Summed feature 8 consists of C<sub>18:1</sub> ω6c and/or C<sub>18:1</sub> ω7c. Values are percentages of total fatty acids; – not present

three unidentified phospholipids (Fig. S3). The polar lipid profile of strain M0322<sup>T</sup> was found to be similar to that of *A.aestuarii* JCM 16339<sup>T</sup>, but different to that of *A.namhicola* JCM 16345<sup>T</sup> (Fig. S3).

#### Phylogenetic analysis of 16S rRNA sequences

The 16S rRNA gene sequence analysis of the almost complete 16S rRNA gene sequence (1449 bp) of strain M0322<sup>T</sup> indicated that strain M0322<sup>T</sup> formed a weak cluster (below 50 % of bootstrap support) with two species of the genus *Altererythrobacter*, *A.aestuarii* JCM 16339<sup>T</sup> (96.1 %) and *A.namhicola* JCM 16345<sup>T</sup> (95.7 %). Analysis of the maximum-likelihood tree (Fig. 1) and neighbor-joining tree (Fig. S2) showed

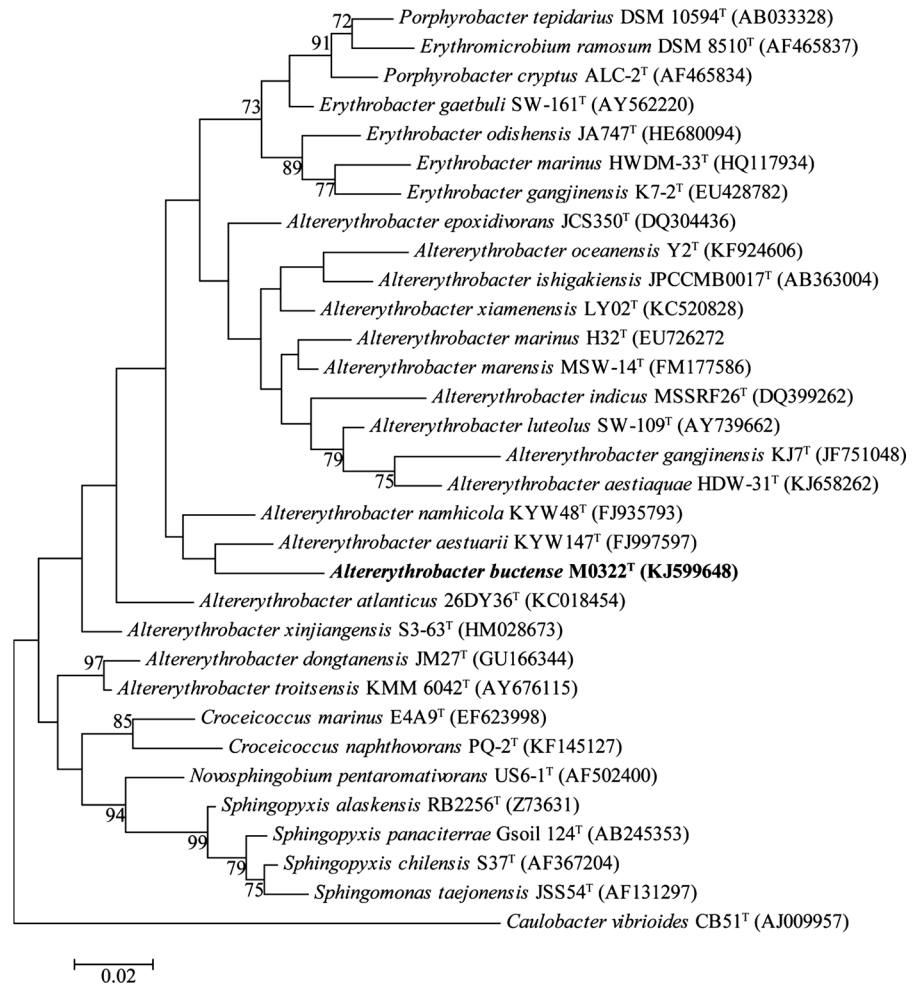
that the genus *Altererythrobacter* is not monophyletic. The species of the genus *Altererythrobacter* are grouped with bacteria of the genera *Croceicoccus*, *Sphingopyxis* and *Novosphingobium* and with bacteria belonging to the family *Sphingomonadaceae* (the genera *Novosphingobium* and *Sphingopyxis*). However, the characteristics of the novel isolate are consistent with the description of the genus *Altererythrobacter* with regard to morphological, biochemical and chemotaxonomic properties supporting the affiliation of strain M0322<sup>T</sup> with the genus *Altererythrobacter*. At the same time on the basis of phylogenetic distances below 97 % between strain M0322<sup>T</sup> and two phylogenetically closest species, *A.aestuarii* JCM 16339<sup>T</sup> and *A.namhicola* JCM 16345<sup>T</sup>, it can be concluded that strain M0322<sup>T</sup> represents a distinct species of the genus *Altererythrobacter*. Furthermore, strain M0322<sup>T</sup> can be readily distinguished from other *Altererythrobacter* species by the combination of many of the physiological and biochemical features (Table 1). Therefore, strain M0322<sup>T</sup> is considered to represent a novel species within the genus *Altererythrobacter*, for which the name *Altererythrobacter buctense* sp. nov. is proposed.

#### Description of *Altererythrobacter buctense* sp. nov

*Altererythrobacter buctense* (buc.ten'se. N.L. masc. adj. Referring to the acronym BUCT, Beijing University of Chemical Technology, where the strain was identified).

Cells are Gram-negative, facultative anaerobic, non-motile, non-flagellated, short rods (approximately 0.2–0.4 × 0.5–0.9 μm). Colonies on R2A are yellow-pigmented, smooth, circular and convex. Grows at 15–40 °C (optimum, 30 °C), pH 5.0–10.0 (optimum, pH 6.0–7.0) and in the presence of 0–4.0 % NaCl (optimum, 0 %). Does not contain BChl *a* as a photosynthetic pigment. Methanol-soluble pigment is characterised by absorption maxima at 449 and 478 nm. Grows well on MR agar, R2A agar, LB and TSBA, but poorly on MA and does not grow on NA. Positive for hydrolysis of Tween 20 and urea, oxidase and catalase activities. Negative for hydrolysis of starch, tyrosine, gelatin, cellulose, chitin and Tween 80. H<sub>2</sub>S is not produced. The only isoprenoid quinone is identified to be Q-10. The major polar lipids are phosphatidylethanolamine, phosphatidylglycerol, phosphatidylcholine, one unidentified glycolipid and three

**Fig. 1** A maximum-likelihood (ML) phylogenetic tree based on the 16S rRNA gene sequences of strain M0322<sup>T</sup> and representatives of related taxa. Numbers at the nodes indicate the bootstrap values (greater than 50 %) expressed as a percentage of 1000 replicates. Bar represents 0.02 substitutions per nucleotide position



unidentified phospholipids. The major fatty acids (>10 %) are Summed Feature 3 (*C*<sub>16:1</sub> ω6*c* and/or *C*<sub>16:1</sub> ω7*c*), Summed Feature 8 (*C*<sub>18:1</sub> ω6*c* and/or *C*<sub>18:1</sub> ω7*c*) and 11-Methyl *C*<sub>18:1</sub> ω7*c*. The DNA G+C content of the type strain is 64.6 mol%.

The type strain, M0322<sup>T</sup> (=CGMCC 1.12871<sup>T</sup> = JCM 30112<sup>T</sup>), was isolated from the Mohe Basin, China. The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain M0322<sup>T</sup> is KJ599648.

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