

## *Halorubrum luteum* sp. nov., isolated from Lake Chagannor, Inner Mongolia, China

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A novel halophilic archaeon, strain CGSA15<sup>T</sup>, was isolated from water of Lake Chagannor in China. The strain grew optimally at 33–37 °C, pH 9.5–10.0 and 4.0–4.3 M NaCl. The major polar lipids were phosphatidylglycerol and phosphatidylglycerol phosphate methyl ester. The genomic DNA G + C content of strain CGSA15<sup>T</sup> was 60.2 mol%. Phylogenetic analysis based on 16S rRNA gene sequences revealed that strain CGSA15<sup>T</sup> was a member of the genus *Halorubrum* and was related most closely to *Halorubrum alkaliphilum* AS 1.3528<sup>T</sup> (96.1% similarity) and *Halorubrum tibetense* AS 1.3239<sup>T</sup> (96.9%). Levels of DNA–DNA relatedness between strain CGSA15<sup>T</sup> and *Hrr. alkaliphilum* AS 1.3528<sup>T</sup> and *Hrr. tibetense* AS 1.3239<sup>T</sup> were 36.7 and 28.9%, respectively. According to the phenotypic and genotypic data presented, strain CGSA15<sup>T</sup> is considered to represent a novel species of the genus *Halorubrum*, for which the name *Halorubrum luteum* sp. nov. is proposed. The type strain is CGSA15<sup>T</sup> (=CGMCC 1.6783<sup>T</sup> =CECT 7303<sup>T</sup>).

The genus *Halorubrum* was first proposed by McGenity & Grant (1995) to accommodate several species previously included in the genus *Halobacterium*: *Halorubrum saccharovororum* (Tomlinson & Hochstein, 1976), *Halorubrum sodomense* (Oren, 1983), *Halorubrum trapanicum* (Petter, 1931) and *Halorubrum lacusprofundi* (Franzmann *et al.*, 1988). At the time of writing, *Halorubrum* is the largest genus within the family *Halobacteriaceae*, with 19 recognized species, three of which are alkaliphilic and 16 neutrophilic (Cui *et al.*, 2007; Xu *et al.*, 2007; Castillo *et al.*, 2007). Of the three alkaliphilic species, *Halorubrum vacuolatum* was isolated from Lake Magadi, Kenya (Mwatha & Grant, 1993), *Halorubrum tibetense* from Lake Zabuye, Tibet, China (Fan *et al.*, 2004), and

*Halorubrum alkaliphilum* from a soda lake of Xinjiang, China (Feng *et al.*, 2005). Here, we report on the taxonomic characterization of a novel alkaliphilic species of the genus *Halorubrum*, which was isolated from a soda lake of Inner Mongolia, China.

Strain CGSA15<sup>T</sup> was isolated from a water sample of Lake Chagannor (43° 16' 13" N 112° 55' 63" E) located in Inner Mongolia Autonomous Region, China. At the time of sampling (September 2003), the water of the lake had a temperature of 17 °C, a pH of 10.5 and a salinity of 18%. The strain was isolated by a direct dilution plate method and was purified by repeated streaking. The medium used for isolation and cultivation contained (per litre distilled water): 7.5 g Casamino acids (Difco), 10.0 g yeast extract (Difco), 3.0 g trisodium citrate, 2.0 g MgSO<sub>4</sub>·7H<sub>2</sub>O, 10.0 g KCl, trace Fe<sup>2+</sup> and Mn<sup>2+</sup>, 200 g NaCl and 10.0 g Na<sub>2</sub>CO<sub>3</sub>. When grown on agar medium with 4.0 M NaCl at 37 °C for 7 days, strain CGSA15<sup>T</sup> formed orange, convex, entire and circular colonies. Cells of strain CGSA15<sup>T</sup> were motile and pleomorphic in liquid cultures,

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain CGSA15<sup>T</sup> is DQ987877.

Micrographs of cells of strain CGSA15<sup>T</sup> and figure showing the polar lipid pattern of strain CGSA15<sup>T</sup> are available as supplementary material with the online version of this paper.

as determined by phase-contrast microscopy without fixation and by Gram staining with acetic acid fixation (Dussault, 1955). Peritrichous tufts of flagella were observed by negative staining (Kodaka *et al.*, 1982) and transmission electron microscopy of exponentially growing liquid cultures. Micrographs of cells of strain CGSA15<sup>T</sup> are available as Supplementary Fig. S1 in IJSEM Online.

Phenotypic tests were carried out in accordance with the proposed minimal standards for the description of new taxa in the order *Halobacteriales* (Oren *et al.*, 1997) and included anaerobic growth in the presence of nitrate or arginine, catalase and oxidase activities, hydrolysis of starch, Tween 80, gelatin and casein, nitrate and nitrite reduction, H<sub>2</sub>S and indole formation and the utilization of sugars, alcohols, amino acids and organic acids. Antibiotic sensitivity tests were performed by spreading bacterial suspensions on culture plates and applying discs impregnated with the antibiotics to be tested. The type strains of *Hrr. tibetense*, *Hrr. alkaliphilum* and *Hrr. vacuolatum* were used as reference strains. Detailed results of the physiological and biochemical tests as well as the antibiotic susceptibility tests are given in the species description below. Differential characteristics between strain CGSA15<sup>T</sup> and other alkaliphilic members of the genus *Halorubrum* are shown in Table 1.

Polar lipids were extracted and analysed by the methods of Kamekura & Kates (1988) by using two-dimensional TLC (Merck DC silica gel 60 F<sub>254</sub> plates, layer thickness 0.2 mm; art. 5554). The first direction was developed in chloroform/methanol/water (65:25:4, by volume) and the second in chloroform/methanol/acetic acid/water (80:12:15:4, by volume). Phospholipids were detected as blue spots by spraying with Zinzadze reagent. All other lipids were visualized by spraying with sulfuric acid/ethanol (1:1, v/v), followed by heating at 150 °C. The major polar lipids of strain CGSA15<sup>T</sup> were phosphatidylglycerol and methylated phosphatidylglycerol phosphate (Supplementary Fig. S2). Glycolipid and phosphatidylglycerol sulfate were absent. This characteristically simple polar-lipid pattern is shared with other alkaliphilic members of the genus *Halorubrum* (Mwatha & Grant, 1993; Fan *et al.*, 2004; Feng *et al.*, 2005).

The 16S rRNA gene of strain CGSA15<sup>T</sup> was amplified by PCR by using the modified primers described by Yang *et al.* (2007) and was directly sequenced on an ABI 373A DNA sequencer. Phylogenetic trees based on 16S rRNA gene sequences were constructed by using the neighbour-joining, minimum-evolution and maximum-parsimony methods in the MEGA3 program package (Kumar *et al.*, 2004). The stability of relationships was assessed by bootstrap analysis (1000 replications). The tree based on the neighbour-joining

**Table 1.** Differential characteristics between strain CGSA15<sup>T</sup> and other alkaliphilic *Halorubrum* species

Data are from Mwatha & Grant (1993), Fan *et al.* (2004), Feng *et al.* (2005) and the present study. +, Positive; -, negative; w+, weakly positive; ND, no data available.

Characteristic	Strain CGSA15 <sup>T</sup>	<i>Hrr. alkaliphilum</i> AS 1.3528 <sup>T</sup>	<i>Hrr. tibetense</i> AS 1.3239 <sup>T</sup>	<i>Hrr. vacuolatum</i> JCM 9060 <sup>T</sup>
Cell shape	Pleomorphic rods	Short rods	Irregular rods	Pleomorphic short rods
Motility	+	+	-	-
Colony colour	Orange	Red	Red	Bright pink
NaCl concentration for growth (M)				
Range	2.5–5.2	1.8–5.2	1.7–5.2	2.5–5.2
Optimum	4.0–4.3	3.9–4.3	3.0–3.4	3.5
Growth temperature (°C)				
Optimum	33–37	38	37–40	35–40
Range	17–41	20–44	22–45	20–50
pH range for growth	7.5–10.5	8.0–10.5	8.0–10.5	8.5–10.5
H <sub>2</sub> S formation	+	+	-	ND
Indole formation	+	+	-	ND
Tween 80 hydrolysis	-	-	+	ND
Utilization of:				
Fructose	-	+	ND	-
Galactose	-	-	-	+
Sucrose	-	-	+	+
Maltose	+	+	+	-
Lactose	w+	-	+	ND
Mannitol	w+	-	+	-
Succinate	+	-	+	+
Acetate	-	-	+	+
DNA G+C content (mol%)	60.2	62.1	63.3	62.7

method (Fig. 1) indicated that strain CGSA15<sup>T</sup> was phylogenetically related to the genus *Halorubrum*, and was related most closely to *Hrr. alkaliphilum* AS 1.3528<sup>T</sup> (96.1% 16S rRNA gene sequence similarity) and *Hrr. tibetense* AS 1.3239<sup>T</sup> (96.9%). Similar tree topologies were obtained with the minimum-evolution and maximum-parsimony algorithms (data not shown).

Genomic DNA was extracted by using the method of Ng *et al.* (1995). The DNA G+C content of strain CGSA15<sup>T</sup> was 60.2 mol%, as determined by thermal denaturation (Marmur & Doty, 1962). DNA–DNA hybridizations were performed with the thermal denaturation and renaturation method (De Ley *et al.*, 1970; Huß *et al.*, 1983) by using a Beckman DU 800 spectrophotometer. Levels of DNA–DNA relatedness between strain CGSA15<sup>T</sup> and *Hrr. alkaliphilum* AS 1.3528<sup>T</sup> and *Hrr. tibetense* AS 1.3239<sup>T</sup> (its closest relatives as judged from 16S rRNA gene sequence analyses) were 36.7 and 28.9%, respectively. Taking these results in combination with the levels of 16S rRNA gene sequence similarity, strain CGSA15<sup>T</sup> is thus not genotypically related to any recognized species of the genus *Halorubrum* (Goebel & Stackebrandt, 1994; Oren *et al.*, 1997).

On the basis of the data described above, strain CGSA15<sup>T</sup> should be placed in the genus *Halorubrum* as representing a novel species. Strain CGSA15<sup>T</sup> can be distinguished easily from other alkaliphilic members of the genus *Halorubrum* by its production of orange pigments. The name proposed for this novel organism is *Halorubrum luteum* sp. nov.

### Description of *Halorubrum luteum* sp. nov.

*Halorubrum luteum* (lu.te'um. L. neut. adj. *luteum* orange coloured).

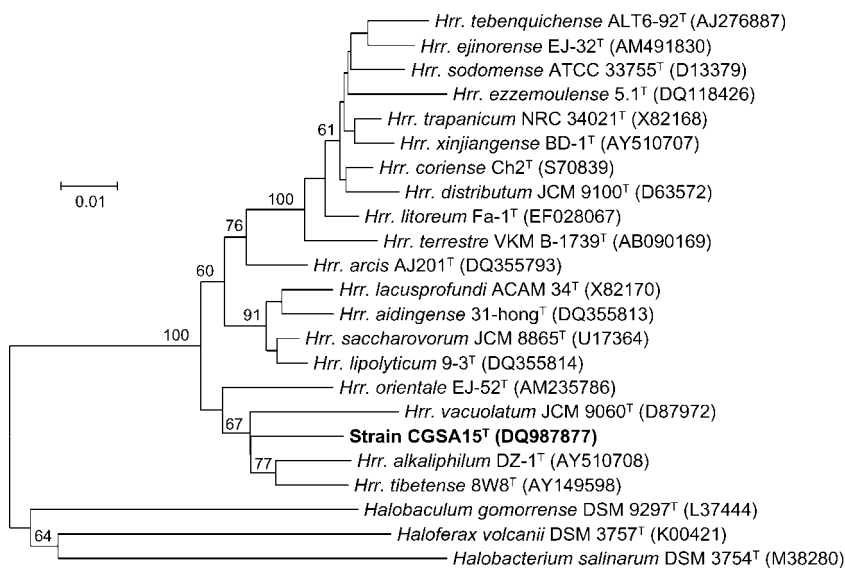
Cells are Gram-negative, pleomorphic (0.5–0.9 × 0.6–1.5 µm) and motile. Colonies growing on agar medium containing 4.0 M NaCl are small (1–2 mm in diameter),

entire, smooth, round and orange. Chemo-organotrophic and aerobic. Growth occurs at NaCl concentrations of 2.5–5.2 M, at pH 7.5–10.5 and at 17–41 °C. Optimal NaCl concentration, pH and temperature for growth are 4.0–4.3 M, pH 9.5–10.0 and 33–37 °C. Magnesium is not required for growth. Cells lyse in distilled water. Catalase- and oxidase-positive. Anaerobic growth with nitrate, arginine or DMSO does not occur. Nitrate reduction to nitrite is observed. H<sub>2</sub>S is produced from Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>. Indole formation is positive. Tween 80, casein and starch are not hydrolysed. Gelatin is not liquefied. Glucose, mannose, maltose, lactose, mannitol and D-sorbitol are utilized but acid production is not clearly observed. Growth occurs on succinate, L-aspartic acid, pyruvate, glycerol, DL-lactate, L-malate, fumarate, citrate, glycine, L-alanine, L-glutamate and L-ornithine. The following compounds are not used as sole carbon and energy sources: fructose, xylose, sorbose, galactose, D-ribose, sucrose, L-lysine, acetate, starch and L-arginine. Sensitive to erythromycin (15 µg) and novobiocin (30 µg). Resistant to neomycin (30 µg), rifampicin (5 µg), chloramphenicol (30 µg), bacitracin (0.04 IU), ampicillin (10 µg), penicillin G (10 IU), norfloxacin (10 µg), ciprofloxacin (5 µg), streptomycin (10 µg), kanamycin (30 µg), tetracycline (30 µg) and vancomycin (30 µg). Cells contain phosphatidylglycerol and phosphatidylglycerol phosphate methyl ester, but lack phosphatidylglycerol sulfate and glycolipids. The G+C content of the DNA of the type strain is 60.2 mol% (*T<sub>m</sub>*).

The type strain, CGSA15<sup>T</sup> (=CGMCC 1.6783<sup>T</sup> =CECT 7303<sup>T</sup>), was isolated from Lake Chagannor in Inner Mongolia, China.

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**Fig. 1.** Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the relationship of strain CGSA15<sup>T</sup> amongst recognized members of the genus *Halorubrum*. Bootstrap values (%) are based on 1000 replicates and are shown for branches with ≥60% bootstrap support. Bar, 0.01 expected changes per site.

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