Altererythrobacter marinus sp. nov., isolated from deep seawater

Qiliang Lai, Jun Yuan and Zongze Shao

Correspondence
Zongze Shao
shaozz@163.com

Key Laboratory of Marine Biogenetic Resources, Third Institute of Oceanography, State Oceanic Administration, PR China

A taxonomic study was carried out on strain H32^T, which was isolated from a crude-oil-degrading consortium enriched from deep seawater of the Indian Ocean. The 16S rRNA gene sequence of strain H32^T showed highest similarity to that of *Altererythrobacter luteolus* SW-109^T (96.7%); lower similarities were observed with other members of the genus *Altererythrobacter* (94.7–95.7%) and with members of the genera *Erythrobacter* (94.5–96.3%), *Erythromicrobium* (94.9%) and *Porphyrobacter* (94.3–95.3%). Phylogenetic analyses of all members of the family *Erythrobacteraceae* and several members of the family *Sphingomonadaceae* revealed that strain H32^T formed a clade within the genus *Altererythrobacter*. The dominant fatty acids were C_{16:0} (6.7%), C_{18:1ω7c} (44.3%) and 11-methyl C_{18:1ω7c} (25.4%). The major respiratory quinone was ubiquinone 10. The DNA G+C content was 66.5 mol%. Strain H32^T did not contain bacteriochlorophyll *a*. These characteristics are in good agreement with those of members of the genus *Altererythrobacter*. Therefore, according to its morphology, physiology, fatty acid composition and 16S rRNA gene sequence data, strain H32^T belongs to the genus *Altererythrobacter*, but can be readily distinguished from known *Altererythrobacter* species. It is therefore proposed that strain H32^T represents a novel species, *Altererythrobacter marinus* sp. nov., with H32^T (=CCTCC AB 208229^T=LMG 24629^T=MCCC 1A01070^T) as the type strain.

In an attempt to investigate crude-oil-degrading bacteria in deep seawater of the Indian Ocean, many bacterial strains have been isolated and characterized taxonomically (Lai *et al.*, 2009). This study focuses on one of these isolates, designated strain H32^T. Comparative 16S rRNA gene sequence analysis indicated that strain H32^T formed a clade within the genus *Altererythrobacter*. The genus *Altererythrobacter* was first proposed by Kwon *et al.* (2007) and comprises three species with validly published names: *Altererythrobacter epoxidivorans* (Kwon *et al.*, 2007), *A. luteolus* (Yoon *et al.*, 2005b; Kwon *et al.*, 2007) and *A. indicus* (Kumar *et al.*, 2008). Members of the genus *Altererythrobacter* are non-motile and do not contain bacteriochlorophyll *a* (Bchl *a*) as a photosynthetic pigment. The dominant fatty acid and respiratory quinone are C_{18:1ω7c} and ubiquinone-10, respectively. Accordingly, the aim of the present work was to determine the exact taxonomic position of strain H32^T by using a polyphasic approach including determination of phenotypic prop-

erties and detailed phylogenetic analysis based on 16S rRNA gene sequences.

Seawater was sampled from a water depth of 1468 m (1020 m above the sea floor) at the site of IR-CTD9 (25.3217° S 70.0405° E) on the south-west Indian Ridge in December 2005 during cruise DY-105A of R/V *Da-Yang Yi-Hao*. The sample was enriched with crude oil and strains were isolated on 216L marine agar medium as described by Lai *et al.* (2009). Strain H32^T was also cultivated on 216L medium for morphological and biochemical characterization.

The Gram reaction, catalase, oxidase and lipase (Tween 80) activities, H₂S production, hydrolysis of aesculin and starch, optimal growth temperature and pH, tolerance of NaCl, antibiotic susceptibility, general cell morphology and electron microscopy (Supplementary Fig. S1 available in IJSEM Online) were investigated as described by Lai *et al.* (2009). Bchl *a* was examined by registration of the *in vitro* absorption spectrum of a methanol extract of cells at 768–769 nm as described previously by Yoon *et al.* (2003). Other biochemical tests were carried out using API 20NE and API ZYM strips (bioMérieux) and Biolog GN2 MicroPlate panels according to the manufacturers' instructions, with the adjustment of NaCl concentration to 3.0% in all tests. *A. indicus* LMG 23789^T, *A. epoxidivorans* JCM 13815^T and *A. luteolus* JCM 12599^T were also tested using

Abbreviation: Bchl *a*, bacteriochlorophyll *a*.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain H32^T is EU726272.

Transmission electron micrographs of cells of strain H32^T and cellular fatty acid contents and API 20NE/API ZYM data and antibiotic susceptibility characteristics of strain H32^T and related strains are available as supplementary material with the online version of this paper.

API 20NE and API ZYM strips and for antibiotic susceptibility at the same time as strain H32^T. These results are given in the species description, Table 1 and Supplementary Table S2.

Genomic DNA preparation, 16S rRNA gene amplification and phylogenetic analysis using MEGA version 4 (Tamura *et al.*, 2007) and DNAMAN (version 5.1; Lynnon Biosoft) were carried out as described by Lai *et al.* (2009). Evolutionary distances, calculated using the algorithm of Kimura's two-parameter model (Kimura, 1980), and clustering with the neighbour-joining method of Saitou & Nei (1987) and the minimum-evolution method of Rzhetsky & Nei (1992, 1993) (data not shown, as results were similar to those observed with the neighbour-joining method) were determined by using bootstrap values based on 1000 replications.

A nearly full-length 16S rRNA gene sequence (1451 nt) of strain H32^T was determined and about 1325 nt were used

for sequence alignment and phylogenetic analysis. The 16S rRNA gene sequence of strain H32^T showed the highest similarity to *A. luteolus* SW-109^T (96.7%). 16S rRNA gene sequence similarities were lower to other members of the genus *Altererythrobacter* (94.7–95.7%) and members of the genera *Erythrobacter* (94.5–96.3%), *Erythromicrobium* (94.9%) and *Porphyrobacter* (94.3–95.3%). A tree constructed using the neighbour-joining method is shown in Fig. 1. Phylogenetic analyses with all members of the family *Erythrobacteraceae* and several members of the family *Sphingomonadaceae* revealed that strain H32^T formed a distinct clade within the genus *Altererythrobacter*. 16S rRNA gene sequence divergences between strain H32^T and the recognized species were all >3.3%; thus, the data support the view that strain H32^T represents a novel species (Stackebrandt & Goebel, 1994).

The major respiratory quinone of strain H32^T was ubiquinone 10, as determined by HPLC analysis according to Collins (1985). This trait is in accordance with the

Table 1. Characteristics that differentiate strain H32^T from type strains of *Altererythrobacter* species and from related genera of the family *Erythrobacteraceae*

Taxa: 1, H32^T (data from this study); 2, *A. indicus* LMG 23789^T (unless indicated, data from Kumar *et al.*, 2008); 3, *A. epoxidivorans* JCM 13815^T (Kwon *et al.*, 2007; Kumar *et al.*, 2008); 4, *A. luteolus* JCM 12599^T (Yoon *et al.*, 2005b; Kwon *et al.*, 2007); 5, *Porphyrobacter* (Hiraishi & Imhoff, 2005; Yoon *et al.*, 2006); 6, *Erythromicrobium ramosum* DSM 8510^T (Yurkov, 2005); 7, *Erythrobacter* (Yoon *et al.*, 2004, 2005a; Shiba & Imhoff, 2005). +, Positive; –, negative; w, weakly positive; v, variable reaction; v (+/–), variable, with the response of the majority of strains in parentheses; ND, no data available; ND (–), no data available for the majority of strains, but negative for those for which data are available. All taxa are catalase-positive.

Characteristic	1	2	3	4	5	6	7
Cell shape	Rod	Rod	Ovoid-rod	Rod	Pleomorphic	Rod, branched	Rod
Oxidase	–	–	+	+	v (+)	+	+
Motility	–	–	–	–	v (+)	+	v (–)
Presence of BChl <i>a</i>	–	–	–	–	+	+	v (–)
Hydrolysis of:							
Aesculin	+	+	–*	+	v (+)	ND	+
Gelatin	–	–†	–†	–†	v (–)	–	v (–)
Starch	–	–	–*	w	v (+)	–	v (–)
Tween 80	+	–†	–	+	v (+)	–	+
H ₂ S production	–	–†	–	–	ND (–)	ND	ND (–)
Utilization of:							
Glucose	–	+	–‡	+	+	+	v (+)
Fructose	–	–	–*	–	v (–)	+	v (–)
Acetate	+	w	–‡	–	v (–)	+	+
Citrate	–	–	–‡	–	–	+	v (–)
Pyruvate	+	–	–*	+	v (+)	+	v (+)
Glutamate	+	–	–	+	v	+	v (–)
Succinate	–	–	–	–	v	+	v (+)
Malate	–	+	–*	–	v (–)	+	v (–)
DNA G + C content (mol%)	66.5	66.8	54.5	60.3	63.8–66.8	64.2	60–67

*Data from this study; also reported as negative by Kumar *et al.* (2008).

†Data from this study.

‡Data from this study; reported as positive by Kumar *et al.* (2008).

Fig. 1. Neighbour-joining tree showing the phylogenetic positions of strain H32^T and representatives of some other related taxa based on 16S rRNA gene sequences. Bootstrap values (expressed as percentages of 1000 replications) are shown at branch points. Bar, 0.005 nucleotide substitution rate (K_{nuc}).

properties of its closest relatives (Denner *et al.*, 2002; Yoon *et al.*, 2003, 2004, 2005a, b; Kwon *et al.*, 2007; Kumar *et al.*, 2008). Fatty acids in whole cells grown on 216L plate medium at 28 °C for 48 h were extracted, saponified and esterified; this was followed by GC analysis of the fatty acid methyl esters according to the instructions of the MIDI system (Sasser, 1990). As showed in Supplementary Table S1, the major fatty acids of strain H32^T were C_{18:1}ω7c (44.3 %), 11-methyl C_{18:1}ω7c (25.4 %) and C_{16:0} (6.7 %). All the *Altererythrobacter* type strains studied and strain H32^T contained C_{14:0} 2-OH as the major hydroxylated fatty acid (2.8–7.9 %). The fatty acids C_{16:0} 2-OH and 11-methyl C_{18:1}ω7c were detected in strain H32^T, *A. luteolus* JCM 12599^T and *A. epoxidivorans* JCM 13815^T, but not in *A. indicus* LMG 23789^T. However, strain H32^T had a greater proportion of 11-methyl C_{18:1}ω7c (25.4 %) than the other two type strains (<8 %).

The G+C content of the chromosomal DNA was determined according to Mesbah & Whitman (1989) using reversed-phase HPLC. The DNA G+C content of strain H32^T was 66.5 mol%, which is close to that of *A. indicus* LMG 23789^T (66.8 mol%).

Phylogenetic analysis based on 16S rRNA gene sequences confirmed that strain H32^T belonged to the genus *Altererythrobacter*. The clade of the genus *Altererythrobacter* was separated from other genera of the family *Erythrobacteraceae* with a bootstrap value of 46 %. This was supported by morphological, physiological and chemotaxonomic characteristics. Strain H32^T can be differen-

tiated from members of the two closely related genera *Porphyrobacter* and *Erythromicrobium* by its cell shape, motility and the absence of BChl *a*. Table 1 shows the detailed characteristics used to distinguish strain H32^T from related species. As shown in Supplementary Table S2, strain H32^T can be further differentiated from the three species of the genus *Altererythrobacter* by API 20NE and API ZYM data and by antibiotic susceptibility results. On the basis of its unique phenotypic traits, strain H32^T represents a novel species within the genus *Altererythrobacter*, for which the name *Altererythrobacter marinus* sp. nov. is proposed.

Description of *Altererythrobacter marinus* sp. nov.

Altererythrobacter marinus (ma.ri' nus. L. masc. adj. *marinus* of the sea, marine).

Cells are rod-shaped (1.0–1.2 µm long and 0.4 µm wide) and non-motile. On 216L agar, forms smooth yellow colonies with regular edges that are 1–2 mm in diameter after 72 h incubation at 28 °C and slightly raised in the centre. Positive for catalase, lipase (Tween 80) and hydrolysis of aesculin, but negative for Gram reaction, oxidase, amylase, urease, gelatinase, arginine dihydrolase, indole production, H₂S production, glucose fermentation and reduction of nitrate to nitrite. Growth, assessed over 7 days, is observed in 0.5–5.0 % NaCl (optimum 3 %) and at 10–42 °C (optimum 25–37 °C), but not at 4 or 45 °C. The major respiratory quinone is ubiquinone 10. BChl *a* is not detected. Principal fatty acids are C_{18:1}ω7c, 11-methyl C_{18:1}ω7c and C_{16:0}. Sensitive to ampicillin (10 µg per disc), carbenicillin (100 µg), cefalexin (30 µg), cefazolin (30 µg), cefobid (30 µg), cephradine (30 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), co-trimoxazole (25 µg), erythromycin (15 µg), kanamycin (30 µg), penicillin G (10 µg), piperacillin (100 µg), rifampicin (5 µg), rocephin (30 µg), tetracycline (30 µg), vancomycin (30 µg) and vibramycin (30 µg). Resistant to clindamycin (2 µg), furazolidone (15 µg), gentamicin (10 µg), lincomycin (2 µg), metronidazole (5 µg), minomycin (30 µg), neomycin (10 µg), norfloxacin (10 µg), ofloxacin (5 µg), oxacillin (1 µg), polymyxin B (30 U) or streptomycin (10 µg). In API ZYM tests, positive for alkaline phosphatase, esterase lipase (C8), leucine aminopeptidase, trypsin, valine aminopeptidase and α-chymotrypsin; weakly positive for acid phosphatase, cystine aminopeptidase, esterase (C4), lipase (C14), naphthol-AS-BI-phosphoamidase, α-glucosidase and β-glucosidase; and negative for N-acetyl-β-glucosaminidase, α-fucosidase, α-galactosidase, α-mannosidase, β-galactosidase and β-glucuronidase. Of the 12 carbon substrates in the API 20NE strip, can only utilize maltose. Of the 95 carbon substrates in the Biolog system, positive for acetic acid, L-asparagine, L-aspartic acid, L-ornithine, L-phenylalanine, L-threonine, α-cyclodextrin, Tweens 40 and 80, maltose, methylpyruvate, monomethyl succinate, β-hydroxybutyric acid, α-ketovaleric acid,

L-glutamic acid, glycyl L-glutamic acid, L-leucine, L-proline and L-serine.

The type strain is H32^T (=CCTCC AB 208229^T =LMG 24629^T =MCCC 1A01070^T), isolated from deep seawater of the Indian Ocean. The DNA G + C content of the type strain is 66.5 mol%.

Acknowledgements

This work was financially supported by the Scientific Research Foundation of the Third Institute of Oceanography, SOA (no. 2009065), the COMRA program (no. DYXM115-02-2-05), the National Infrastructure of Natural Resources for Science and Technology Program of China (no. 2005DKA21209), the National Basic Research Program of China (no. 2004CB719601) and the National Natural Science Foundation of China (no. 30670051).

References

- Collins, M. D. (1985).** Isoprenoid quinone analysis in bacterial classification and identification. In *Chemical Methods in Bacterial Systematics*, pp. 267–287. Edited by M. Goodfellow & D. E. Minnikin. London: Academic Press.
- Denner, E. B. M., Vybiral, D., Koblížek, M., Kämpfer, P., Busse, H.-J. & Velimirov, B. (2002).** *Erythrobacter citreus* sp. nov., a yellow-pigmented bacterium that lacks bacteriochlorophyll *a*, isolated from the western Mediterranean Sea. *Int J Syst Evol Microbiol* **52**, 1655–1661.
- Hiraishi, A. & Imhoff, J. F. (2005).** Genus VII. *Porphyrobacter* Fuerst, Hawkins, Holmes, Sly, Moore and Stackebrandt 1993, 132^{VP}. In *Bergey's Manual of Systematic Bacteriology*, 2nd edn, vol. 2, part C, pp. 275–279. Edited by D. J. Brenner, N. R. Krieg, J. T. Staley & G. M. Garrity. New York: Springer.
- Kimura, M. (1980).** A simple model for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* **16**, 111–120.
- Kumar, N. R., Nair, S., Langer, S., Busse, H.-J. & Kämpfer, P. (2008).** *Altererythrobacter indicus* sp. nov., isolated from wild rice (*Porteresia coarctata* Tateoka). *Int J Syst Evol Microbiol* **58**, 839–844.
- Kwon, K. K., Woo, J.-H., Yang, S.-H., Kang, J.-H., Kang, S. G., Kim, S.-J., Sato, T. & Kato, C. (2007).** *Altererythrobacter epoxidivorans* gen. nov., sp. nov., an epoxide hydrolase-active, mesophilic marine bacterium isolated from cold-seep sediment, and reclassification of *Erythrobacter luteolus* Yoon *et al.* 2005 as *Altererythrobacter luteolus* comb. nov. *Int J Syst Evol Microbiol* **57**, 2207–2211.
- Lai, Q., Yuan, J., Gu, L. & Shao, Z. (2009).** *Marispirillum indicum* gen. nov., sp. nov., isolated from a deep-sea environment. *Int J Syst Evol Microbiol* **59**, 1278–1281.
- Mesbah, M. & Whitman, W. B. (1989).** Measurement of deoxyguanosine/thymidine ratios in complex mixtures by high-performance liquid chromatography for determination of the mole percentage guanine + cytosine of DNA. *J Chromatogr* **479**, 297–306.
- Rzhetsky, A. & Nei, M. (1992).** A simple method for estimating and testing minimum-evolution trees. *Mol Biol Evol* **9**, 945–967.
- Rzhetsky, A. & Nei, M. (1993).** Theoretical foundation of the minimum-evolution method of phylogenetic inference. *Mol Biol Evol* **10**, 1073–1095.
- Saitou, N. & Nei, M. (1987).** The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Sasser, M. (1990).** *Identification of bacteria by gas chromatography of cellular fatty acids*. MIDI Technical Note 101. Newark, DE: MIDI Inc.
- Shiba, T. & Imhoff, J. F. (2005).** Genus IV. *Erythrobacter* Shiba and Simidu 1982, 215^{VP}. In *Bergey's Manual of Systematic Bacteriology*, 2nd edn, vol. 2, part C, pp. 267–268. Edited by D. J. Brenner, N. R. Krieg, J. T. Staley & G. M. Garrity. New York: Springer.
- Stackebrandt, E. & Goebel, B. M. (1994).** Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int J Syst Bacteriol* **44**, 846–849.
- Tamura, K., Dudley, J., Nei, M. & Kumar, S. (2007).** MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. *Mol Biol Evol* **24**, 1596–1599.
- Yoon, J.-H., Kim, H., Kim, I.-G., Kang, K. H. & Park, Y.-H. (2003).** *Erythrobacter flavus* sp. nov., a slight halophile from the East Sea in Korea. *Int J Syst Evol Microbiol* **53**, 1169–1174.
- Yoon, J.-H., Kang, K. H., Oh, T.-K. & Park, Y.-H. (2004).** *Erythrobacter aquimaris* sp. nov., isolated from sea water of a tidal flat of the Yellow Sea in Korea. *Int J Syst Evol Microbiol* **54**, 1981–1985.
- Yoon, J.-H., Oh, T.-K. & Park, Y.-H. (2005a).** *Erythrobacter seohaensis* sp. nov. and *Erythrobacter gaetbuli* sp. nov., isolated from a tidal flat of the Yellow Sea in Korea. *Int J Syst Evol Microbiol* **55**, 71–75.
- Yoon, J.-H., Kang, K. H., Yeo, S.-H. & Oh, T.-K. (2005b).** *Erythrobacter luteolus* sp. nov., isolated from a tidal flat of the Yellow Sea in Korea. *Int J Syst Evol Microbiol* **55**, 1167–1170.
- Yoon, J.-H., Kang, S.-J., Lee, M.-H., Oh, H. W. & Oh, T.-K. (2006).** *Porphyrobacter dokdonensis* sp. nov., isolated from sea water. *Int J Syst Evol Microbiol* **56**, 1079–1083.
- Yurkov, V. V. (2005).** Genus V. *Erythromicrobium* Yurkov, Stackebrandt, Holmes, Fuerst, Hugenholtz, Golecki, Gad'on, Gorlenko, Kompantseva and Drews 1994c, 432^{VP}. In *Bergey's Manual of Systematic Bacteriology*, 2nd edn, vol. 2, part C, pp. 268–273. Edited by D. J. Brenner, N. R. Krieg, J. T. Staley & G. M. Garrity. New York: Springer.