Altererythrobacter dongtanensis sp. nov., isolated from a tidal flat

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A Gram-negative, rod-shaped and non-spore-forming bacterial strain, JM27^T, was isolated from a tidal flat of Dongtan Wetland, Chongming Island, China. The strain formed smooth yellow colonies on R2A plates. Growth occurred at 10–37 °C (optimum, 30–37 °C), at pH 6.0–10.0 (optimum, pH 7.0–9.0) and in the presence of 0–1 % NaCl (optimum, 0 %). Catalase test was positive and oxidase test was negative. Ubiquinone 10 (Q10) was the major respiratory quinone. C_{18:1}ω7c and C_{17:1}ω6c were the most abundant fatty acids. Diphosphatidylglycerol, phosphatidylethanolamine and phosphatidylglycerol were the major polar lipids. The DNA G+C content of strain JM27^T was 66.4 mol%. The 16S rRNA gene sequence of the isolate showed highest similarity to that of *Altererythrobacter marinus* H32^T (96.4 %). Phylogenetic analysis based on 16S rRNA gene sequences indicated that the strain belonged to the genus *Altererythrobacter* of the family *Erythrobacteraceae* of the class *Alphaproteobacteria*. On the basis of phylogenetic analysis, whole-cell fatty acids, polar lipid compositions, and biochemical and physiological characteristics, strain JM27^T is proposed to represent a novel species of the genus *Altererythrobacter* for which the name *Altererythrobacter dongtanensis* sp. nov. is proposed. The type strain is JM27^T (=KCTC 22672^T =CCTCC AB 209199^T).

The genus *Altererythrobacter* belongs to the family *Erythrobacteraceae* in the phylum *Proteobacteria*. It was first proposed by Kwon *et al.* (2007) and at the time of writing the genus includes seven species: *Altererythrobacter epoxidivorans* (Kwon *et al.*, 2007), *A. luteolus* (Kwon *et al.*, 2007), *A. indicus* (Kumar *et al.*, 2008), *A. marinus* (Lai *et al.*, 2009), *A. marensis* (Seo & Lee, 2010), *A. namhicola* and *A. aestuarii* (Park *et al.*, 2011). Members of the genus *Altererythrobacter* have been isolated from seawater (Seo & Lee, 2010; Park *et al.*, 2011), marine sediment (Kwon *et al.*, 2007), the rhizosphere of wild rice associated with mangrove (Kumar *et al.*, 2008) and a tidal flat of the Yellow Sea (Yoon *et al.*, 2005).

Strain JM27^T was isolated from a tidal flat of Dongtan wetland, Chongming Island, China, by the dilution-plating technique on R2A (Difco) plates supplemented with 1 % NaCl and solidified with 1.5 % gellan. Gram-staining was performed as described by Gerhardt *et al.* (1994). Cell morphology was examined by light microscopy (YS-2; Nikon) and transmission electron microscopy (H600;

Hitachi) after negative staining with 1 % (w/v) phosphotungstic acid, using cells grown on R2A agar after 2 days of incubation at 30 °C. Motility was determined by phase-contrast microscopy (Eclipse TS 100; Nikon). Growth at different salt concentrations and pH was monitored in R2A broth incubated for up to 7 days in the presence of 0, 1 and 2 % (w/v) NaCl and at pH 5.0–11.0 (intervals of 1.0 pH unit). pH was adjusted by addition of 1M HCl or 1M NaOH. Growth at different temperatures was monitored on R2A agar incubated for 7 days at 4, 10, 20, 30, 37 and 42 °C. The production of flexirubin-type pigments on R2A agar was determined by using the bathochromic shift test with 20 % (w/v) KOH (Fautz & Reichenbach, 1980). Catalase activity was determined by bubble production in 3 % (v/v) hydrogen peroxide solution. Oxidase activity was determined by oxidation of 1 % (w/v) tetramethyl *p*-phenylenediamine (Merck). Hydrolysis of starch and Tween 80, utilization of glucose, fructose, acetate, pyruvate, glutamate, succinate, malate and formate as sole carbon sources, and endospore formation were determined as described by Cowan & Steel (1993). H₂S production was determined on Kligler iron agar (MacFaddin, 1985). Other physiological, biochemical and enzyme activity tests were

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain JM27^T is GU166344.

conducted using API 20NE and API ZYM strips (bioMérieux) according to the manufacturer's instructions.

The cultural, physiological and biochemical characteristics of strain JM27^T are given in the species description and in Table 1. Strain JM27^T was positive for catalase activity, but negative for hydrolysis of gelatin, H₂S production and nitrate reduction; these are common characteristics of the genus *Altererythrobacter*. The novel strain could be readily differentiated from related species in the genus *Altererythrobacter* on the basis of several phenotypic properties: it is negative for hydrolysis of Tween 80, and positive for utilization of fructose and succinate as sole carbon sources.

The 16S rRNA gene of strain JM27^T was amplified by PCR using the universal primer pair 27F and 1512R as described previously (Quan *et al.*, 2005). The typical 16S rRNA gene sequences of related taxa were obtained from EzTaxon (Chun *et al.*, 2007) and multiple alignments were performed using the CLUSTAL X program (Thompson *et al.*, 1997). Phylogenetic trees were reconstructed based on the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Swofford, 1993) algorithms using the software package MEGA4 (Tamura *et al.*, 2007) with bootstrap values

based on 1000 replications (Felsenstein, 1985). Distance matrices were calculated using the method of Jukes & Cantor (1969).

The partial 16S rRNA gene sequence of strain JM27^T (1436 bp) was obtained. Strain JM27^T shared highest 16S rRNA gene sequence similarities with *A. marinus* H32^T (96.4%) and *Novosphingobium tardaugens* ARI-1^T (95.9%). In the phylogenetic tree (Fig. 1), the isolate was clearly grouped in the genus *Altererythrobacter*, suggesting that it represents a novel species of the genus *Altererythrobacter*. Since the highest similarity was lower than 97%, there was no need to perform DNA–DNA hybridization (Stackebrandt & Goebel, 1994).

Isoprenoid quinones were extracted and analysed as described by Komagata & Suzuki (1987). For quantitative analysis of the cellular fatty acid composition, 40 mg wet cell material was harvested from Marine 2216 agar (MA; Difco) plates after 3 days of incubation at 30 °C and the cellular fatty acids were extracted, saponified and methylated according to the protocol of the Sherlock Microbial Identification System (MIDI). The fatty acids were analysed by a gas chromatograph (Hewlett Packard 6890) and identified

Table 1. Phenotypic characteristics of strain JM27^T and the type strains of other species in the genera *Altererythrobacter* and *Croceicoccus*

Strains: 1, JM27^T (data from this study); 2, *A. namhicola* KYW48^T (data from Park *et al.*, 2011); 3, *A. aestuarii* KYW147^T (Park *et al.*, 2011); 4, *A. epoxidivorans* JCS350^T (Kwon *et al.*, 2007); 5, *A. indicus* MSSRF26^T (Kumar *et al.*, 2008); 6, *A. marensis* MSW-14^T (Seo & Lee, 2010); 7, *A. marinus* H32^T (Lai *et al.*, 2009); 8, *A. luteolus* SW-109^T (Yoon *et al.*, 2005); 9, *Croceicoccus marinus* E4A9^T (Xu *et al.*, 2009). All strains were positive for catalase activity and negative for H₂S production, nitrate reduction, urease activity and utilization of citrate as a sole carbon source. +, Positive; –, negative; w, weakly positive; ND, not determined/no data available.

Characteristic	1	2	3	4	5	6	7	8	9
Cell shape	Rod	Rod	Rod	Ovoid–rod	Rod	Rod	Rod	Rod	Coccoid
Oxidase	–	+	+	+	–	+	–	+	–
Motility	–	–	–	–	–	+	–	–	+
pH range (optimum)	6.0–10.0 (7.0–9.0)	5.0–11.0 (6.0–7.0)	5.0–11.0 (6.0–8.0)	6–8.5 (6.5)	(7.0)	6.1–11.1 (7.1)	ND	5.5–8.0 (7.0–8.0)	6.0–9.0 (7.0)
Temperature range (optimum) (°C)	10–37 (30)	15–37 (30)	10–40 (30)	20–40 (35)	4–42 (25–30)	4–42 (30)	10–42 (25–37)	4–36 (30)	4–42 (25)
Hydrolysis of:									
Aesculin	–	+	+	–	+	ND	+	+	+
Gelatin	–	–	–	–	ND	–	–	–	+
Starch	–	+	–	ND	–	–	–	w	+
Tween 80	–	+	+	+	ND	+	+	+	+
Utilization of:									
Glucose	+	–	–	–	+	–	–	+	+
Fructose	+	–	–	–	–	ND	–	–	–
Acetate	+	–	–	–	w	ND	+	–	+
Formate	–	ND	ND	–	ND	ND	ND	–	–
Pyruvate	+	ND	ND	–	–	ND	+	+	+
Glutamate	+	ND	ND	–	–	ND	+	–	+
Succinate	+	ND	ND	–	–	ND	–	–	+
Malate	+	+	–	–	+	+	–	–	+
DNA G + C content (mol%)	66.4	63.8	67.2	54.5	66.8	63.1	66.5	60.3	71.5

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the phylogenetic position of strain JM27^T within the family Erythrobacteraceae. Bootstrap values (expressed as percentages of 1000 replications) greater than 50 are shown at branching points. Filled circles indicate branches that were also recovered by using the maximum-parsimony algorithm. Bar, 1 substitution per 100 nt positions.

by the Microbial Identification software package (Sasser, 1990). Polar lipids were isolated by using standard procedures (Komagata & Suzuki, 1987) and separated by TLC according to the method of Minnikin *et al.* (1984). The G + C content of the DNA was determined by the fluorescence monitoring method (Xu *et al.*, 2000; Gonzalez & Saiz-Jimenez, 2002) with a real-time thermal cycler (Mx3000p; Stratagene). Genomic DNA of *Escherichia coli* B (Sigma-Aldrich) and *Pseudomonas aeruginosa* KCTC 1750^T was used as calibration references.

The major respiratory quinone was ubiquinone 10 (Q10), as in all members of the genus *Altererythrobacter*. The fatty acid profile of strain JM27^T and other members of the genus *Altererythrobacter* are shown in Table 2. The major fatty acids were C_{18:1}ω7c (34.0 %), C_{17:1}ω6c (19.8 %) and C_{16:0} 2-OH (12.5 %) with smaller amounts of C_{15:0} 2-OH (7.4 %), C_{16:0} (7.1 %), summed feature 3 (6.1 %, comprising C_{16:1}ω7c and/or iso-C_{15:0} 2-OH), C_{14:0} 2-OH (5.6 %), C_{16:1}ω5c (2.8 %), 11-methyl C_{18:1}ω7c (2.2 %), C_{18:1} 2-OH (1.6 %) and iso-C_{16:0} 3-OH (0.9 %). C_{18:1}ω7c is the most abundant fatty acid among members of the genus *Altererythrobacter*. Diphosphatidylglycerol, phosphatidylethanolamine and phosphatidylglycerol were the major polar lipids. These

three polar lipids were also the major polar lipids in other species of the genus *Altererythrobacter* such as *A. aestuarii* (Park *et al.*, 2011) and *A. indicus* (Kumar *et al.*, 2008). The DNA G + C content of strain JM27^T was 66.4 mol%, which is similar to those of *A. indicus* MSSRF26^T (66.8 mol%, Kumar *et al.*, 2008), *A. marinus* H32^T (66.5 mol%, Lai *et al.*, 2009) and *A. aestuarii* KYW147^T (67.2 mol%, Park *et al.*, 2011).

On the basis of the phylogenetic tree, and biochemical and physiological characteristics, it is proposed that strain JM27^T belongs to the genus *Altererythrobacter*. Due to the large phylogenetic distance and different phenotypic characteristics of the new strain compared with those of the type strains of members of the genus *Altererythrobacter*, strain JM27^T should be classified as a novel species of the genus *Altererythrobacter*, for which the name *Altererythrobacter dongtanensis* sp. nov. is proposed.

Description of *Altererythrobacter dongtanensis* sp. nov.

Altererythrobacter dongtanensis (dong.ta.nen'sis. N.L. masc. adj. *dongtanensis* pertaining to Dongtan, a wetland region in Chongming Island, Shanghai, China).

Table 2. Fatty acid patterns (%) of strain JM27^T and the type strains of other species of the genus *Altererythrobacter*

Strains: 1, JM27^T (data from this study); 2, *A. namhicola* KYW48^T; 3, *A. aestuarii* KYW147^T; 4, *A. epoxidivorans* JCS350^T; 5, *A. indicus* MSSRF26^T; 6, *A. marensis* MSW-14^T; 7, *A. marinus* H32^T; 8, *A. luteolus* SW-109^T. Data for reference strains were taken from Park *et al.* (2011). All cells were grown on MA plates for 3 days at 30 °C. —, Not detected.

Fatty acid	1	2	3	4	5	6	7	8
Straight-chain								
C _{15:0}	—	3.2	—	—	—	0.8	—	—
C _{16:0}	7.1	5.8	14.6	4.9	7.0	9.0	5.3	4.7
C _{18:0}	—	—	—	—	0.5	—	—	—
Unsaturated								
C _{16:1ω5c}	2.8	—	—	3.2	—	1.9	1.1	3.1
C _{17:1ω6c}	19.8	19.9	2.9	2.1	5.4	3.8	5.0	1.8
C _{17:1ω8c}	—	2.1	—	—	1.2	—	0.7	—
C _{18:1ω5c}	—	—	—	1.2	1.0	—	1.9	1.1
C _{18:1ω7c}	34.0	35.2	36.1	42.6	63.6	40.4	48.8	41.7
C _{18:1ω9c}	—	—	7.9	—	—	—	—	—
11-Methyl C _{18:1ω7c}	2.2	—	11.0	6.0	—	18.1	23.7	5.0
Cyclic								
C _{19:0ω8c} cyclo	—	—	—	—	—	—	0.7	—
Hydroxy								
C _{14:0} 2-OH	5.6	7.5	—	3.7	13.8	5.7	3.2	3.9
C _{15:0} 2-OH	7.4	3.5	—	—	1.2	—	—	—
C _{16:0} 2-OH	12.5	—	9.0	2.1	0.8	3.0	1.8	2.1
C _{17:0} 3-OH	—	—	—	3.2	4.5	5.4	2.7	5.3
iso-C _{16:0} 3-OH	0.9	—	—	—	—	—	—	—
C _{16:1} 2-OH	—	—	—	1.2	—	—	—	1.2
C _{18:1} 2-OH	1.6	—	—	—	—	—	—	—
Summed feature 3*	6.1	22.7	18.4	29.8	1.1	12.7	4.6	29.1

*Summed feature 3 comprised C_{16:1ω7c} and/or iso-C_{15:0} 2-OH.

Cells are Gram-negative, aerobic, non-spore-forming rods approximately 0.4–0.5 × 0.5–1.1 µm in size; no flagellum is detected. Visible yellow colonies are formed after 2 days of incubation at 30 °C on R2A agar. Colonies are smooth, circular and wet. Cells do not produce flexirubin-type pigment on R2A agar. Growth occurs between 10 and 37 °C (optimum 30–37 °C), at pH 6.0–10.0 (optimum pH 7.0–9.0) and in the presence of 0–1 % NaCl (optimum 0 %). Positive for catalase and negative for oxidase. Positive for esterase (C4), esterase lipase (C8), naphthol-AS-BI-phosphohydrolase and β-galactosidase, but negative for urease, leucine arylamidase, α-glucosidase, β-glucosidase, lipase (C14), arginine dihydrolase, α-chymotrypsin, α-galactosidase, alkaline phosphatase, acid phosphatase, valine arylamidase, cystine arylamidase, β-glucuronidase, α-fucosidase, α-mannosidase and N-acetyl-β-glucosaminidase. Negative for acidification of D-glucose, production of indole and H₂S, trypsin activity and hydrolysis of starch, aesculin, gelatin and Tween 80. Nitrate and nitrite are not reduced under anaerobic

conditions. The following substrates are not utilized as sole carbon source: caprate, adipate, arabinose, citrate, gluconate, maltose, mannitol, mannose, N-acetylglucosamine or phenylacetate. The following substrates are utilized as sole carbon source: D-glucose, fructose, acetate, pyruvate, glutamate, succinate and malate. Ubiquinone 10 (Q10) is the major respiratory quinone. The major fatty acids are C_{18:1ω7c}, C_{17:1ω6c} and C_{16:0} 2-OH. Diphosphatidylglycerol, phosphatidylethanolamine and phosphatidylglycerol are the major polar lipids. The DNA G+C content of the type strain is 66.4 mol%.

The type strain, JM27^T (=KCTC 22672^T =CCTCC AB 209199^T), was isolated from a tidal flat (Dongtan Wetland, Chongming Island, Shanghai, China).

Acknowledgements

This work was supported by National Key Technology R&D Program of China (no. 2006BAJ05A11).

References

- Chun, J., Lee, J.-H., Jung, Y., Kim, M., Kim, S., Kim, B. K. & Lim, Y.-W. (2007). EzTaxon: a web-based tool for the identification of prokaryotes based on 16S ribosomal RNA gene sequences. *Int J Syst Evol Microbiol* 57, 2259–2261.
- Cowan, S. T. & Steel, K. J. (1993). *Manual for the Identification of Medical Bacteria*, 3rd edn. Edited by G. I. Barrow & R. K. A. Feltham. Cambridge: Cambridge University Press.
- Fautz, E. & Reichenbach, H. (1980). A simple test for flexirubin-type pigments. *FEMS Microbiol Lett* 8, 87–91.
- Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39, 783–791.
- Gerhardt, P., Murray, R. G. E., Wood, W. A. & Krieg, N. R. (editors) (1994). *Methods for General and Molecular Bacteriology*. Washington, DC: American Society for Microbiology.
- Gonzalez, J. M. & Saiz-Jimenez, C. (2002). A fluorimetric method for the estimation of G+C mol% content in microorganisms by thermal denaturation temperature. *Environ Microbiol* 4, 770–773.
- Jukes, T. H. & Cantor, C. R. (1969). Evolution of protein molecules. In *Mammalian Protein Metabolism*, pp. 21–132. Edited by H. N. Munro. New York: Academic Press.
- Komagata, K. & Suzuki, K. (1987). Lipid and cell-wall analysis in bacterial systematics. *Methods Microbiol* 19, 161–207.
- 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. L., Yuan, J. & Shao, Z. Z. (2009). *Altererythrobacter marinus* sp. nov., isolated from deep seawater. *Int J Syst Evol Microbiol* 59, 2973–2976.
- MacFaddin, J. F. (1985). *Media for Isolation–Cultivation–Identification–Maintenance of Medical Bacteria*, vol. 1. Baltimore: Williams & Wilkins.

- Minnikin, D. E., O'Donnell, A. G., Goodfellow, M., Alderson, G., Athalye, M., Schaal, A. & Parlett, J. H. (1984). An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. *J Microbiol Methods* **2**, 233–241.
- Park, S. C., Baik, K. S., Choe, H. N., Lim, C. H., Kim, H. J., Ka, J. O. & Seong, C. N. (2011). *Altererythrobacter namhicola* sp. nov. and *Altererythrobacter aestuarii* sp. nov., isolated from seawater. *Int J Syst Evol Microbiol* **61**, 709–715.
- Quan, Z. X., Bae, H. S., Baek, J. H., Chen, W. F., Im, W. T. & Lee, S. T. (2005). *Rhizobium daejeonense* sp. nov. isolated from a cyanide treatment bioreactor. *Int J Syst Evol Microbiol* **55**, 2543–2549.
- 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.
- Seo, S. H. & Lee, S. D. (2010). *Altererythrobacter marensis* sp. nov., isolated from seawater. *Int J Syst Evol Microbiol* **60**, 307–311.
- 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.
- Swofford, D. L. (1993). PAUP. Phylogenetic analysis using parsimony, version 3.1.1. Champaign, IL: Illinois Natural History Survey.
- 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.
- Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997). The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* **25**, 4876–4882.
- Xu, H.-X., Kawamura, Y., Li, N., Zhao, L., Li, T.-M., Li, Z.-Y., Shu, S. & Ezaki, T. (2000). A rapid method for determining the G + C content of bacterial chromosomes by monitoring fluorescence intensity during DNA denaturation in a capillary tube. *Int J Syst Evol Microbiol* **50**, 1463–1469.
- Xu, X.-W., Wu, Y.-H., Wang, C.-S., Wang, X.-G., Oren, A. & Wu, M. (2009). *Croceicoccus marinus* gen. nov., sp. nov., a yellow-pigmented bacterium from deep-sea sediment, and emended description of the family Erythrobacteraceae. *Int J Syst Evol Microbiol* **59**, 2247–2253.
- Yoon, J.-H., Kang, K. H., Yeo, S.-H. & Oh, T.-K. (2005). *Erythrobacter luteolus* sp. nov., isolated from a tidal flat of the Yellow Sea in Korea. *Int J Syst Evol Microbiol* **55**, 1167–1170.