Erythrobacter pelagi sp. nov., a member of the family *Erythrobacteraceae* isolated from the Red Sea

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A novel Gram-negative, aerobic, catalase- and oxidase-positive, non-sporulating, non-motile, rod-shaped bacterium, designated strain UST081027-248^T, was isolated from seawater of the Red Sea. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain UST081027-248^T fell within the genus *Erythrobacter*. Levels of 16S rRNA gene sequence similarity between the novel strain and the type strains of *Erythrobacter* species ranged from 95.3% (with *Erythrobacter gangjinensis*) to 98.2% (with *Erythrobacter citreus*). However, levels of DNA–DNA relatedness between strain UST081027-248^T and the type strains of closely related species were below 70%. Optimal growth of the isolate occurred in the presence of 2.0% NaCl, at pH 8.0–9.0 and at 28–36 °C. The isolate did not produce bacteriochlorophyll *a*. The predominant cellular fatty acids were C_{17:1ω6c}, summed feature 8 (C_{18:1ω6c} and/or C_{18:1ω7c}) and C_{15:0} 2-OH. The genomic DNA G+C content of strain UST081027-248^T was 60.4 mol%. Phenotypic properties and phylogenetic distinctiveness clearly indicated that strain UST081027-248^T represents a novel species of the genus *Erythrobacter*, for which the name *Erythrobacter pelagi* sp. nov. is proposed. The type strain is UST081027-248^T (=JCM 17468^T=NRRL 59511^T).

The genus *Erythrobacter*, proposed by Shiba & Simidu (1982), belongs to the family *Erythrobacteraceae* (Lee *et al.*, 2005), which contains three other genera, *Altererythrobacter* (Kwon *et al.*, 2007), *Porphyrobacter* (Fuerst *et al.*, 1993) and *Erythromicrobium* (Lee *et al.*, 2005). Most members of the family have been isolated from aquatic environments such as freshwater, seawater, marine mats or marine sediment; members of the genus *Erythrobacter* have been solely isolated from marine habitats. All members of the genus *Erythrobacter* produce pigments and some produce bacteriochlorophyll (BChl) *a*. At the time of writing, the genus comprised ten recognized species: *Erythrobacter longus* (Shiba & Simidu, 1982), *E. litoralis* (Yurkov *et al.*, 1994), *E. citreus* (Denner *et al.*, 2002), *E. flavus* (Yoon *et al.*, 2003), *E. aquimaris* (Yoon *et al.*, 2004), *E. seohaensis* (Yoon *et al.*, 2005), *E. gaetbuli* (Yoon *et al.*, 2005), *E. vulgaris* (Ivanova *et al.*, 2005), *E.*

nanhaisediminis (Xu *et al.*, 2010) and *E. gangjinensis* (Lee *et al.*, 2010). In the present study, a novel, slightly halophilic, orange-pigmented strain, designated UST081027-248^T, isolated from the Red Sea is described. On the basis of polyphasic analysis of phenotypic and phylogenetic data, we propose that strain UST081027-248^T represents a novel species of the genus *Erythrobacter*.

During a study of the bacterial diversity and resources of brine pools in the Red Sea and the seawater lying above them, strain UST081027-248^T was isolated from a seawater sample. The sample was collected in October 2008 with a Niskin bottle at 20 m water depth above the Discovery Deep brine pool (21° 16.976' N 38° 02.985' E). Strain UST081027-248^T was isolated by the standard dilution-plating technique on marine agar 2216 (MA; Difco) and incubated at room temperature (25 °C). The strain was purified by repeated streaking and observed under a light microscope (Leica MZ6). The purified strain was stored at –80 °C in marine broth 2216 (MB; Difco) with 30% (v/v) glycerol. Unless otherwise specified, all characteristics described hereafter were based on cultures incubated on MA or in MB for 3 days at room temperature. *E. aquimaris* JCM 12189^T and *E. nanhaisediminis* JCM 16125^T, obtained

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Abbreviation: BChl *a*, bacteriochlorophyll *a*.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain UST081027-248^T is HQ203045.

Two supplementary figures are available with the online version of this paper.

from the Japan Collection of Microorganisms (JCM), *E. longus* DSM 6997^T, *E. citreus* DSM 14432^T, *E. flavus* DSM 16421^T, *E. seohaensis* DSM 16221^T and *E. vulgaris* DSM 17792^T, obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ), were cultured under the same conditions for comparison.

Cell morphology was determined by using light microscopy and transmission electron microscopy (Tecnai 12). Gram staining was performed according to Collins *et al.* (1989). Gliding and swimming motility were observed under a phase-contrast light microscope (Olympus BX51; 100× magnification) after growing the isolate on MB solidified with 0.4 % agar (Bowman, 2000). The presence of BChl *a* was determined based on the absorption spectrum of the methanol extract of cells according to Yoon *et al.* (2003).

Genomic DNA was extracted from the cells by using the MiniBEST Bacterial Genomic DNA Extraction kit (TaKaRa). The 16S rRNA gene of UST081027-248^T was amplified by using primer pair 8F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1525R (5'-AAGGAGTGTCCARCC-3') (Shrout *et al.*, 2005) with Vent DNA polymerase (NEB) and was sequenced by using an Applied Biosystems 3100 automated DNA sequencer as previously described (Lau *et al.*, 2005). The resulting 16S rRNA gene sequence was compared with sequences obtained from GenBank by using BLAST searches (Altschul *et al.*, 1997) to determine its approximate phylogenetic position. Sequences of the novel isolate and those of related species were aligned with CLUSTAL X (Thompson *et al.*, 1997), and then manually edited with the BioEdit sequence alignment editor. Phylogenetic analysis was performed by using the software package MEGA version 5.0 (Tamura *et al.*, 2011). Evolutionary distances were computed according to Kimura's two-parameter model (Kimura, 1980) and phylogenetic trees were generated by using the neighbour-joining (Saitou & Nei, 1987), maximum-likelihood (Felsenstein, 1981) and minimum-evolution (Felsenstein, 1997) methods. Bootstrap analysis was used to evaluate the tree topology of the neighbour-joining data by means of 1000 resamplings (Felsenstein, 1985). DNA–DNA hybridization was performed in triplicate by using the membrane filter method as described by Denhardt (1966) and De Ley & Tijtgaat (1970) with the modifications suggested by Cano *et al.* (1992). The DNA G+C content was determined by using the HPLC method performed in triplicate (Mesbah *et al.*, 1989).

For determination of the cellular fatty acid composition, cells were grown in MB in flasks on a rotary shaker (220 r.p.m.) at 30 °C for 3 days and the fatty acids were then extracted and methylated as described by Sasser (1990). The profile of methylated fatty acids was determined by using the MIDI Sherlock Microbial Identification System (Microbial ID, Sherlock version 6.1, TSBA6 method). Polar lipids were extracted and analysed on silica gel plates (Kieselgel 60 F; Merck) by two-dimensional TLC (Kates, 1986). Polar lipids from *E. citreus* DSM 14432^T, *E. seohaensis* DSM 16221^T and *E. nanhaisediminis* JCM 16125^T were used as reference strains. Respiratory lipoquinones were extracted and

analysed by HPLC (Collins, 1985). Ubiquinone-10 (Q-10) from Sigma was used as a reference.

The phenotypic characteristics of strain UST081027-248^T, including growth conditions, hydrolysis of various substrates, susceptibility to antibiotics, reduction of nitrate, production of H₂S, enzymes, indole and acetoin, and utilization of and acid production from carbon sources, were tested. The requirement of oxygen for growth was examined by using the Oxoid anaerobic system on YP-SW agar (Lau *et al.*, 2005). Growth at 4–52 °C and pH 5.0–10.0 (pH adjusted with 1 M HCl or NaOH) was observed after up to 8 days of incubation. The requirement for and tolerance to various salt concentrations were tested on 1.2 % agar medium containing (per litre) 5.9 g MgCl₂, 3.24 g Na₂SO₄, 1.8 g CaCl₂, 0.55 g KCl, 5.0 g peptone, 0.1 g ferric citrate and 1.0 g yeast extract (pH 7.6) in the presence of 0–18 % (w/v) NaCl (intervals of 1 %) (ZoBell, 1941). Sensitivity to kanamycin (30 µg), tetracycline (30 µg), ampicillin (30 µg), chloramphenicol (30 µg), streptomycin (30 µg) and penicillin (20 IU) were tested by using the method described by Acar (1980). Catalase and oxidase activities were determined as described by Chen *et al.* (2007). Hydrolysis of Tween 80, starch and gelatin were determined according to Smibert & Krieg (1994). Other enzyme activities, substrate utilization patterns, nitrate reduction and production of H₂S, indole and acetoin were tested by using the API 20E, API 20NE, API 50CH and API ZYM commercial systems (bioMérieux) according to the manufacturer's instructions, with 2.2 % NaCl salt in the suspension (MacDonell *et al.*, 1982). Growth on formate, glycerol, dextrin, succinate, lactate, L-alanine, maltose, sucrose, D-sorbitol, D-galactose, starch, pyruvate, D-glutamate and D-glucose as sole carbon sources was also tested by using medium containing 0.2 g NaNO₃, 0.2 g NH₄Cl, 0.05 g yeast extract and 0.4 % (w/v) carbon source per litre of artificial seawater at 3.5 % salinity (Nedashkovskaya *et al.*, 2003).

Cells of strain UST081027-248^T were Gram-negative, long rods (2.5–3.0 µm long and 0.5–0.6 µm wide) (see Fig. S1 in IJSEM Online) that grew only under aerobic conditions and showed no gliding or flagellar motility. Pure colonies were orange, raised and circular (1.5–2.5 mm in diameter) with an entire edge and smooth surface. BChl *a* was absent. Detailed physiological and biochemical characteristics of strain UST081027-248^T are listed in Table 1 and in the species description below.

Phylogenetic analysis of the nearly complete 16S rRNA gene sequence (1453 bp) showed that strain UST081027-248^T exhibited closest affiliation with species of the genus *Erythrobacter*, family *Erythrobacteraceae*. Levels of 16S rRNA gene sequence similarity between strain UST081027-248^T and the type strains of recognized *Erythrobacter* species ranged from 95.3 % (with *E. gangjinensis*) to 98.2 % (with *E. citreus*). In the neighbour-joining phylogenetic tree based on 16S rRNA gene sequences, strain UST081027-248^T was closely related to members of the genus *Erythrobacter* (Fig. 1). Similar tree topologies were also found in the

Table 1. Differential phenotypic characteristics between strain UST081027-248^T and the type strains of phylogenetically related *Erythrobacter* species.

Strains: 1, UST081027-248^T; 2, *E. citreus* RE35F/1^T (data from Denner *et al.*, 2002, except where indicated); 3, *E. aquimaris* SW-110^T (Yoon *et al.*, 2004); 4, *E. flavus* SW-46^T (Yoon *et al.*, 2003); 5, *E. seohaensis* SW-135^T (Yoon *et al.*, 2005); 6, *E. vulgaris* 022-2-10^T (Ivanova *et al.*, 2005); 7, *E. longus* OCh101^T (Yoon *et al.*, 2003); 8, *E. nanhaisediminis* T30^T (Xu *et al.*, 2010). All strains are susceptible to chloramphenicol but resistant to streptomycin, and catalase- and oxidase-positive.

Characteristic	1*	2	3	4	5	6	7	8
Colony colour	Orange	Yellow	Orange	Yellow	Orange-yellow	Yellow	Orange	Orange
Motility	—	—	—	+	—	—	+	+
Presence of BChl <i>a</i>	—	—	—	—	—	—	+	—
Nitrate reduction*	+	+	—	—	—	—	+	—
Tolerance to NaCl (%)	8	10	10	14	9	8	8	10
Hydrolysis of:*								
Gelatin	—	—†	—	—	—	—	+	—
Starch	—	—†	+	+	—	—	—	—
Tween 80	—	—†	+†	+†	+†	+	+	+
Utilization of:*								
D-Glucose	+	+	+	—	+	—	+	+
Fructose	+	—	—	—	—	—	—†	—
Acetate	+	+	+	+	+	—†	+	+
Citrate	—	+	—	—	—	—	+	—
Pyruvate	+	—	+	+	+	—	+	+†
Glutamate	+	+	—	—	—	—	+	+†
Succinate	+	+†	+	—	+	+	—	+
Malate	+	—	+	—	—	—	—	+
Lactate	—	—	—	+	—	—	—	—
Maltose	+	—	—	+	—	—	+	+
Sucrose	+	—	—	—	+	—	+	—
Susceptibility to:*								
Ampicillin	+	—	—†	—†	—†	—†	+†	—†
Tetracycline	+	—	—†	—†	—†	—	—	—†
Kanamycin	+	—	—†	+†	—†	+†	—†	—
Growth temperature range (°C)*	12–40	4–37	10–41	10–42	10–40	10–40	10–35†	4–45
DNA G + C content (mol%)	60.4	62.0	62.2	64.0	62.2	61–62	60.7	59.5

*Data are from this study.

†Data absent or different from previous studies.

trees constructed with the minimum-evolution and maximum-likelihood algorithms. The closest relatives of strain UST081027-248^T were the type strains of *E. citreus* (98.2% 16S rRNA gene sequence similarity), *E. vulgaris* (97.7%), *E. flavus* (97.5%), *E. nanhaisediminis* (97.3%) and *E. seohaensis* (97.2%). However, the results of DNA–DNA hybridization experiments demonstrated that strain UST081027-248^T represented a novel species in the genus, with levels of relatedness of the novel strain to the type strains of the above species being below 70%. Mean levels of DNA–DNA relatedness between strain UST081027-248^T and *E. citreus* DSM 14432^T, *E. vulgaris* DSM 17792^T, *E. flavus* DSM 16421^T, *E. nanhaisediminis* DSM 16125^T and *E. seohaensis* DSM 16221^T were 62, 14, 24, 14 and 48%, respectively, with a standard deviation of 2.5%. The DNA G + C content of strain UST081027-248^T was 60.4 mol% (Table 1), which was within

the range previously found for recognized species in the genus *Erythrobacter* (59.5–67.0 mol%).

The cellular fatty acid composition of UST081027-248^T was similar to that of the reference strains in the genus, with a very high proportion of unsaturated fatty acids, C_{17:1}ω6c, summed feature 8 (C_{18:1}ω6c and/or C_{18:1}ω7c), and the hydroxyl fatty acid C_{15:0} 2-OH. However, the content of C_{17:1}ω6c in strain UST081027-248^T was four-fold higher than in the reference strains, whereas the content of summed feature 8 was only half that of the reference strains. Therefore, strain UST081027-248^T shared common major cellular fatty acids with members of the genus *Erythrobacter* but was subtly different in certain components. The detailed cellular fatty acid composition of strain UST081027-248^T is shown in Table 2. The major polar lipids were similar to those of the reference strains

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the position of strain UST081027-248^T among related taxa. Numbers at nodes are bootstrap values (percentages of 1000 resamplings); only values >50% are shown. Asterisks indicate nodes that are also found with both the maximum-likelihood and the minimum-evolution methods; hashes indicate nodes that are also found with the minimum-evolution method. *Rhodospirillum rubrum* ATCC 11170^T was used as an outgroup. Bar, 0.02 substitutions per site.

and were identified as phosphatidylcholine, phosphatidylethanolamine, diphosphatidylglycerol and phosphatidylglycerol (Fig. S2). The predominant lipoquinone was ubiquinone Q-10.

On the basis of the data presented, strain UST081027-248^T is clearly a member of the genus *Erythrobacter* but is distinguishable from all described species in this genus. Therefore, strain UST081027-248^T is considered to represent a novel species of the genus *Erythrobacter*, for which the name *Erythrobacter pelagi* sp. nov. is proposed.

Description of *Erythrobacter pelagi* sp. nov.

Erythrobacter pelagi (pe'la.gi. L. gen. n. *pelagi* of/from the sea, reflecting isolation of the type strain from seawater of the Red Sea).

Cells are Gram-negative, long rods (2.5–3.0 µm long and 0.5–0.6 µm wide) (Fig. S1) that grow only under aerobic conditions. No gliding or flagellar motility. Colonies grown on MA at 28 °C for 5 days are orange, 1.5–2.5 mm in diameter, circular and convex with a smooth surface and an entire edge. Flexirubin-type or diffusible pigments are not produced. Optimal pH for growth is 8.0–9.0; no growth is observed below pH 5.0 or above pH 10.0. Growth occurs at 10–42 °C (optimal, 20–36 °C). Optimal growth occurs in the presence of 2–3 % (w/v) NaCl; growth does not occur in the absence of NaCl or above 10 % (w/v) NaCl. Cells do not produce BChl *a*. Does not hydrolyse DNA, gelatin, aesculin, starch, casein, cellulose or Tween

80. Positive for indole production and nitrate reduction, but negative for H₂S production. Positive for catalase, oxidase, alkaline phosphatase, esterase lipase (C8), trypsin, valine arylamidase, α-chymotrypsin, naphthol-AS-BI-phosphohydrolase, α-glucosidase, leucine arylamidase and acid phosphatase, and weakly positive for esterase (C4) and cystine arylamidase. Negative for lipase (C14), urease, *N*-acetyl-β-glucosaminidase, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, α-galactosidase, β-galactosidase, β-glucuronidase, β-glucosidase, α-mannosidase, α-fucosidase, gelatinase and tryptophan deaminase activities. Acid is produced from L-sorbose, L-arabinose, inositol, D-rhamnose, D-sorbitol, dulcitol, D-fructose, D-galactose, D-glucose, D-ribose, D-xylose and D-mannitol, but not from fucose, erythritol, amygdalin, methyl β-D-xylopyranoside, melezitose, starch, arbutin, salicin, cellobiose, inulin, xylitol, gentiobiose, glycogen, turanose, citrate, D-lyxose, D-tagatose or arabinol. Utilization of glycerol, pyruvate, glutamate, succinate, maltose, sucrose, dextrin and L-alanine as sole carbon sources is observed on agar medium supplemented with 0.4 % (w/v) carbon source while utilization of D-glucose, adonitol, *N*-acetylglucosamine, aesculin, lactose, maltose, melibiose, sucrose, trehalose and raffinose is observed in the API 20E and 50CH systems. No utilization of formate, starch or lactate is observed. Susceptible to chloramphenicol, kanamycin, tetracycline, ampicillin and penicillin, but resistant to streptomycin. The predominant fatty acids are C_{17:1ω6c}, summed feature 8 (C_{18:1ω6c} and/or C_{18:1ω7c})

Table 2. Cellular fatty acid compositions (%) of strain UST081027-248^T and reference type strains of *Erythrobacter* species

Strains: 1, UST081027-248^T; 2, *E. citreus* DSM 14432^T; 3, *E. seohaensis* DSM 16221^T; 4, *E. vulgaris* DSM 17792^T; 5, *E. longus* DSM 6997^T; 6, *E. nanhaediminis* DSM 16125^T. Only fatty acids with a relative abundance of more than 0.5% in any of the strains are shown. —, Not detected. All data are from this study.

Fatty acid	1	2	3	4	5	6
Straight-chain						
C _{14:0}	—	—	0.4	0.4	—	0.6
C _{16:0}	4.1	5.7	5.3	3.7	4.7	8.3
C _{17:0}	1.5	—	—	1.4	1.6	0.7
C _{18:0}	1.3	0.9	0.3	0.2	0.6	0.6
Branched						
iso-C _{17:0}	—	1.2	—	—	1.8	—
Unsaturated						
C _{16:1} ω5c	—	1.7	1.2	—	—	0.7
C _{17:1} ω6c	39.2	9.0	9.6	3.9	3.2	9.7
C _{17:1} ω8c	4.1	0.8	1.2	2.2	4.8	1.0
C _{18:1} ω5c	—	1.8	0.5	—	—	1.0
C _{18:1} ω9c	1.6	—	—	—	—	1.0
11-Methyl C _{18:1} ω7c	—	3.2	2.1	2.4	—	6.1
Hydroxy						
C _{13:0} 2-OH	2.2	—	—	—	1.4	—
C _{14:0} 2-OH	5.1	2.9	7.1	5.2	9.0	8.2
C _{15:0} 2-OH	10.6	1.8	1.6	6.6	8.3	5.2
C _{16:0} 2-OH	—	3.5	0.9	0.9	0.6	2.2
C _{16:1} 2-OH	—	—	0.4	1.3	—	—
C _{18:1} 2-OH	—	0.9	0.7	1.0	—	0.8
iso-C _{16:0} 3-OH	—	1.5	0.5	—	—	0.8
Summed features*						
3	2.4	8.8	16.5	12.7	—	5.8
8	28.1	56.5	51.7	58.2	64.1	46.5

*Summed feature 3 comprises C_{16:1}ω6c and/or C_{16:1}ω7c; summed feature 8 comprises C_{18:1}ω6c and/or C_{18:1}ω7c.

and C_{15:0} 2-OH. Major polar lipids are phosphatidylcholine, phosphatidylethanolamine, diphosphatidylglycerol and phosphatidylglycerol while the predominant respiratory lipoquinone is ubiquinone Q-10.

The type strain, UST081027-248^T (=JCM 17468^T=NRRL 59511^T), was isolated from shallow seawater collected from the middle of the Red Sea. The DNA G+C content of the type strain is 60.4 mol%.

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