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ORIGINAL PAPER

Description of *Erythrobacter mangrovi* sp. nov., an aerobic bacterium from rhizosphere soil of mangrove plant (*Kandelia candel*)

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Abstract A novel Gram-stain negative, aerobic, non-motile, rod-shaped bacterium, designated as strain EB310^T, was isolated from rhizosphere soil of mangrove plant *Kandelia candel* in Fugong village, Zhangzhou, China. Phylogenetic analysis based on 16S rRNA gene sequences revealed that strain EB310^T belonged to the genus *Erythrobacter*, clustering with *Erythrobacter pelagi* JCM 17468^T, *Erythrobacter lutimaris* KCTC 42109^T and *Erythrobacter marisflavi* KCTC 62896^T, and showed the highest 16S rRNA gene sequence similarity of 97.5% to *Erythrobacter pelagi* JCM 17468^T. The genomic average nucleotide identity and in silico DNA–DNA hybridization values between strain EB310^T and the reference strains were 71.0–75.5% and 19.8–20.0%, respectively. Growth ranges of the isolate occurred at 10–45 °C (optimum 28–30 °C), pH 5.5–9.5 (optimum pH 7.5) and 0–9.0% NaCl concentrations (optimum 2.0%, w/v). The strain did not produce bacteriochlorophyll *a* and flexirubin, but produced carotenoids. The strain contained Q-10 as the predominant ubiquinone and summed feature 3 ($C_{16:1}$ $\omega7c$/$C_{16:1}$ $\omega6c$) and summed feature 8 ($C_{18:1}$ $\omega6c$/$C_{18:1}$ $\omega7c$) as the major fatty acids. The major polar lipids were sphingoglycolipid, phosphatidylglycerol, phosphatidylethanolamine, diphosphatidylglycerol and phosphatidylcholine. Differential phenotypic characteristics, together with chemotaxonomic, phylogenetic and genomic distinctiveness, indicated that strain EB310^T is distinguishable from other members of the genus *Erythrobacter*. On the basis of the data exhibited, strain EB310^T is considered to represent a novel species of the genus *Erythrobacter*, for which the name *Erythrobacter mangrovi* sp. nov., is

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain EB310^T are MT522623. Whole Genome Shotgun project of strain EB310^T has been deposited at DDBJ/ENA/GenBank under the accession CP053921.

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proposed. The type strain is EB310^T (= KCTC 72109^T = MCCC 1K03690^T). The genomic DNA G + C content is 62.9 mol%.

Keywords *Erythrobacter mangrovi* · Mangrove plant · Polyphasic taxonomy · Environmental adaptation

Abbreviations

ANI	Average nucleotide identity
*is*DDH	In silico DNA–DNA hybridization
BGCs	Biosynthetic gene clusters
MA	Marine agar 2216
MB	Marine broth 2216

Introduction

The genus *Erythrobacter* was first proposed by Shiba and Simidu with the description of a single species of *Erythrobacter longus* (1982), and belonged to the family *Erythrobacteraceae* of class *Alphaproteobacteria*. At the time of writing, 26 species of the genus *Erythrobacter* with validly published names have been described according to List of Prokaryotic names with Standing in Nomenclature (https://lpsn.dsmz.de/genus/erythrobacter). Among these, *Erythrobacter luteolus* has been reclassified as *Altererythrobacter luteolus* by Kwon et al. (2007). Members within this genus are Gram-stain-negative, aerobic chemoorganotrophs and distributed in the multifarious marine environments including seawater (Yoon 2017; Lee et al. 2010; Jung et al. 2012; Wu et al. 2012; Li et al. 2017), ocean sediment (Zhuang et al. 2015; Tang et al. 2019; Xu et al. 2010; Fang et al. 2019), tidal flat (Jung et al. 2014; Yoon et al. 2005; Park et al. 2020), mangrove (Lei et al. 2015), halobios (Ivanova et al. 2005; Zhuang et al. 2019) etc., which exhibiting the excellent adaptation of the genus *Erythrobacter* strains to marine environment. In order to investigate the taxonomic position of strain EB310^T, which was isolated from a rhizosphere soil sample of mangrove plant, the polyphasic taxonomy approach, including phylogenetic, gemomic, phenotypic and chemotaxonomic characterization, was performed in this study. In addition, according to the genomic data mining of strain EB310^T, numerous genes related to xenobiotics degradation, signal transduction and stress reaction of extreme environment were discovered, which provided new insight of the strain into the adaptation to the complex mangrove habitats and the potential of biological applications at the genetic level.

Materials and methods

Isolation and cultivation

During our work focused on microbial diversity of mangrove ecosystem, strain EB310^T was isolated from a rhizosphere soil sample of the mangrove plant named *Kandelia candel* in Fugong village, Zhangzhou (117° 57′ N 24° 24′ E), in Fujian, China. Rhizosphere soil were collected and placed into clean plastic buckets in 2017 and stored at 4 °C until use. Serially diluted (tenfold dilutions each) samples were made and spread on three kinds of selective isolation media by the traditional dilution-plating method: HV (humic acid-vitamin agar medium), M7 (glycerine-peptone agar medium) and MA (marine agar 2216 medium). All of the media were supplemented with 25 mg/L of nalidixic acid and 50 mg/L of nystatin. After 7 days of incubation at 30 °C, an orange-pigmented colony was picked from MA plates and named as strain EB310^T. After repeated plate streaking on the same medium, pure strain was obtained from individual colony and preserved as suspension with 25% (v/v) glycerol at -80 °C for further use. The reference strains selected in this study including *E. pelagi* JCM 17468^T, *E. longus* JCM 6170^T and *E. lutimaris* KCTC 42109^T, *E. marisflavi* KCTC 62896^T, were purchased from the Japan Collection of Microorganisms (JCM) and the Korean Collection for Type Cultures (KCTC), respectively.

16S rRNA gene sequencing and phylogenetic analysis

Strain EB310^T was incubated in marine broth (MB) medium at 28 °C for 3 days for the phylogenetic analysis. The amplification of 16S rRNA gene of the isolate was performed by using universal primers 27F [5′-AGAGTTTGATCCTGGCTCAG-3′] and 1492R [5′-ACGGCTACCTTGTTACGACTT-3′] (Anzai et al. 2000). Amplification reactions were prepared in a 25 μL final reaction volume comprising 0.5 μL of each primer, 0.5 μL of extracted DNA template, 11 μL

of distilled water and 12.5 μL of PCR SuperMix. PCR procedure was proceeded under the following conditions: 30 cycles of 94 °C/5 min, 94 °C/30 s, 55 °C/30 s, 72 °C/75 s and a final extension of 72 °C/10 min. PCR products were ligated to vector pMD 19-T (TaKaRa) and cloned into *E. coli* DH5*a* for sequencing, then the almost-complete 16S rRNA gene sequence was obtained. The sequence was assembled with DNASTAR SeqMan (LaserGene, Madison, WI), and analyzed with other 16S rDNA sequences by submitting to the NCBI (http://www.blast.ncbi.nlm.nih.gov/Blast.cgi) (Altschul et al. 1997) and the EzTaxon-e server (http://eztaxon-e.ezbiocloud.net) (Yoon et al. 2017). The multiple sequences of the isolate and other closely related strains were aligned with Clustal W (Thompson et al. 1994). Furthermore, phylogenetic trees were constructed with the MEGA 5.0 software package (Tamura et al. 2011) by using neighbour-joining (Saitou and Nei 1987), minimum-evolution (Rzhetsky and Nei 1992) and maximum-likelihood (Felsenstein 1981) methods. Bootstrap analysis (1000 replicates) was used to evaluate the tree's topology. Kimura two-parameter model was used for phylogeny construction and evolutionary distances analysis (Kimura 1980).

Genome sequencing and data mining analysis

For DNA isolation, strain EB310^T was inoculated into MB medium and grown at 28 °C with shaking (180 r.p.m) for 5 days. High-quality genomic DNA was extracted using the Bacteria Genomic DNA Extraction Kit (DongSheng Biotech) according to the manufacturer's instructions. The draft genome of strain EB310^T was sequenced by the Illumina HiSeq 2000 platform (Beijing Genomics Institute) with Solexa PE150 sequencing technology to generate sub-reads set (approximate 878-fold genome coverage). To improve the accuracy of the genome sequence, the GATK (https://www.broadinstitute.org/gatk/) and SOAP (SOAP2, SOAPsnp, SOAPindel) tool packages were used to make single-base corrections. The *de-novo* assembly of the reads was performed by using ABySS 1.5.2(Simpson et al. 2009). The assembly k-value was tested from 32 to 64 to find the optimal k-value using abyss-perl script. The sub-reads with length shorter than 2000 bp were removed. The quality of the genome was assessed by using Check M (Parks et al. 2015). The gene prediction was performed by the Glimmer 3 (http://www.cbcb.umd.edu/software/glimmer/) with Hidden Markov models. The open reading frames (ORFs) were annotated by the Rapid Annotation using Subsystem Technology (RAST) server online (Overbeek et al. 2014). The Kyoto Encyclopedia of Genes and Genomes (KEGG) (Kanehisa et al. 2016) and the Clusters of Orthologous Groups (COG) (Tatusov et al. 2001) databases were used for general function annotation. For the identification of secondary metabolism gene clusters, the antiSMASH 5.0 program was used (Blin et al. 2019). The genome sequences of the reference strains in this study were obtained from the NCBI database (http://www.ncbi.nlm.nih.gov) under the GenBank accession numbers: *E. pelagi* JCM 17468^T (WTYD00000000), *E. longus* JCM 6170^T (JMIW00000000), *E. lutimaris* KCTC 42109^T (QRBB00000000) and *E. marisflavi* KCTC 62896^T (VCAO00000000) to investigate the genomic average nucleotide identity (ANI) and in silico DNA–DNA hybridization (*is*DDH) values with strain EB310^T. The genomic ANI values were calculated using the OrthoANIu algorithm of the Chun lab's online Average Nucleotide Identity calculator in the EZBioCloud (Lee et al. 2016). In silico DNA–DNA hybridization (*is*DDH) values were calculated by genome-to-genome distance calculator (GGDC) according to Meier-Kolthoff et al. (2013).

Morphological, physiological and biochemical analysis

Cell morphology and ultra-structure were detected by using optical microscopy (BX40, Olympus) and transmission electron microscopy (JEM1230, JEOL) as described by Ye et al. (2019). Gram stain reaction was conducted using a Gram stain kit (Hangzhou Tianhe Micro-organism Reagent) according to the manufacturer's instructions. Anaerobic growth was tested on MA medium in an anaerobic chamber (N_2: CO_2: H_2 = 86: 7: 7; 1029, Forma) at 28 °C for 10–15 days. Motility of the isolate was tested on semi-solid MA medium (according to MA formula, but agar concentration was decreased to 0.5%, w/v) using hanging drop method (Gerhardt et al. 1994). The temperature range for growth was investigated at 4, 10, 15, 20, 25, 28, 30, 32, 35, 37, 40, 45 and 50 °C. The pH range for growth (pH 5.0–10.0, with intervals of 0.5 pH units) was detected by using the appropriate biological buffers: citrate/phosphate (pH 5.0 and 5.5),

MOPS (Sigma) (pH 6.0–8.0), boric acid/borax (pH 8.5 and 9.0), borax/NaOH (pH 9.5 and 10.0), and each at a final concentration of 40 mM. Growth at various NaCl concentrations (0–12.0%, w/v, at intervals of 0.5%) was observed in NaCl-free MB medium (according to MB formula, but without NaCl). The pigment was extracted from freeze-dried cells with acetone and methanol (1: 1, v/v) and the pigment absorption spectrum was analyzed by using a Varian CARY 300 BIO spectrophotometer (Varian, Cary, NC, USA) according to Zhuang et al. (2019). Oxidase and catalase activities were examined by the addition of *p*-aminodimethylaniline oxalate (1%, w/v) and H_2O_2 solution (3%, w/v), respectively (Cappuccino and Sherman 2002). H_2S production, nitrate reduction and hydrolysis of starch, casein, tyrosine, CM-cellulose, xanthine, hypoxanthine and Tweens (20, 40, 60 and 80) (1.0%, w/v) were tested based on the methods given in Dong and Cai (2001). Antibiotics sensitivity was investigated by spreading bacterial suspension on MA medium and applying filter papers impregnated with the following antibiotics: chloramphenicol (100 μg), novobiocin (5 μg), lincomycin (15 μg), polymyxin B (100 IU), streptomycin (50 μg), ampicillin (10 μg), tetracycline (30 μg), cefalotin (30 μg), kanamycin (30 μg), carbenicillin (100 μg), neomycin (30 μg), oleandomycin (15 μg), penicillin G (20 IU) and gentamicin (30 μg) (Jung et al. 2014). Utilization of carbon source was detected by using various filter-sterilized nutrients as sole carbon and energy source in modified MB medium (according to MB formula, but without peptone, and yeast extract concentration was decreased to 0.01%, w/v) (Farmer et al. 2005). Other physiological and biochemical characteristics tests were performed in API 20NE, API ZYM and API 50CH systems (bioMérieux) according to the manufacturers' instructions. Unless otherwise specified, strain EB310^T and all of the reference strains were cultured for 3 days in MB medium at 28 °C for the phenotypic properties analysis.

Chemotaxonomic characteristics

Fatty acid methyl esters (FAME) were analyzed according to the standard protocol of the Microbial Identification System (MIDI) with the MIS Library Generation software version 4.5 (Microbial ID) using a gas chromatograph (6850, Agilent) (Paisley 1996; Miller 1982). Strain EB310^T and four reference strains were cultivated on MA medium at 28 °C for 3 days to harvest and freeze-dry the cells at the late exponential stage for the FAME analysis. Then cellular fatty acids were saponified, methylated and extracted based on the reported methods (Sasser 1990; Anwar et al. 2016) and identified with the database TSBA6 (Sherlock version 6.0). For the analysis of respiratory quinones and polar lipids of strain EB310^T, biomass was obtained when cells were grown in MB medium for 72 h at 28 °C. Respiratory quinones of the isolate were extracted according to the procedure described by Minnikin et al. (1984), and analyzed by using HPLC–MS (Agilent 1200 and Thermo Finnigan LCQ DECA XP MAX mass spectrometer). Polar lipids were extracted from 2.4 g freeze-dried cells, then separated by two-dimensional TLC on silica gel 60F_{254} plates (Merck) and identified according to the published methods (Minnikin et al. 1984; Su et al. 2016).

Results and discussion

16S rRNA gene sequencing and phylogenetic analysis

The 16S rRNA gene sequence of strain EB310^T comprised 1446 bp (NCBI accession number: MT522623). In the phylogenetic trees reconstructed using the neighbour-joining (Fig. 1), maximum-parsimony (Fig. S1) and maximum-likelihood (Fig. S2) algorithms, strain EB310^T fell within the clade of the genus *Erythrobacter*, clustering with *E. pelagi* JCM 17468^T by a moderate bootstrap resampling value of 49%. Strain EB310^T exhibited the 16S rRNA gene sequence similarity values of 97.5% to *E. pelagi* JCM 17468^T, of 97.0% to *E. marisflavi* KCTC 62896^T, of 96.1% to *E. lutimaris* KCTC 42109^T, of 94.9% to *E. longus* JCM 6170^T, respectively, and of 93.5–97.3% to the other type strain of the genus *Erythrobacter*.

Genome sequencing and data mining analysis

The genome completeness of strain EB310^T was 99.48% with 0.73% contamination, which considered as a good quality of genome for deeper genome analysis ($\geq$ 95% completeness, $\leq$ 5% contamination). Whole genome sequence comprised 3,058,693 bp. The contig and N_{50} value of the genome were 2 and 2,281,280 bp, respectively. A total of 3034

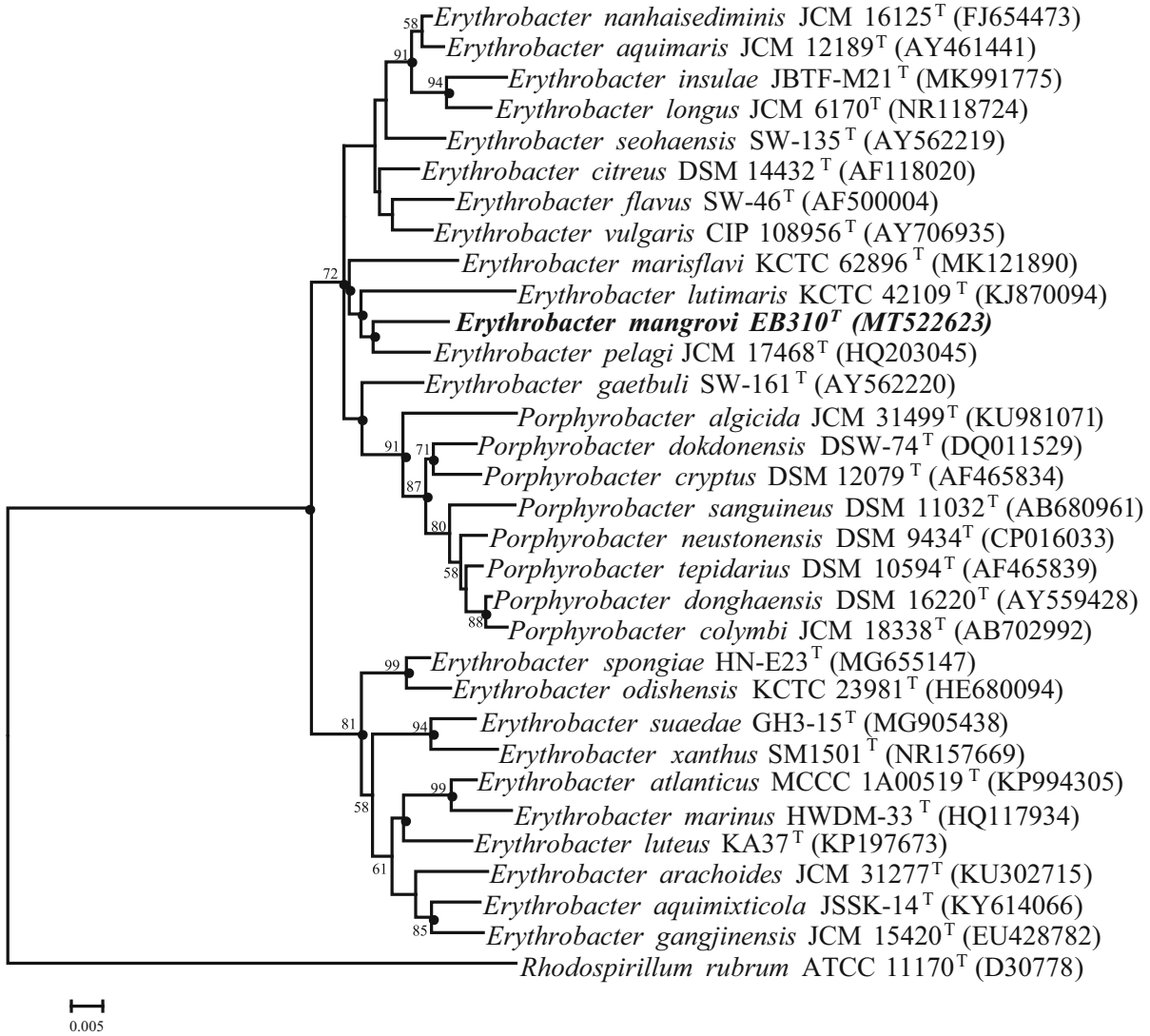

Fig. 1 Neighbour-joining tree based on 16S rRNA gene sequences, showing the phylogenetic relationship between strain EB310^T and other related species of the genus *Erythrobacter*. Bootstrap values were expressed as a percentage of 1000 replicates and only those higher than 50% were shown. Filled circles indicate that the corresponding nodes were also recovered in the maximum-likelihood tree. *Rhodospirillum rubrum* ATCC 11170^T were used as outgroup. Bar, 0.005 substitutions per nucleotide position

protein-coding genes were predicted. Of these, 1644 (54.2%) and 2516 (82.9%) genes were annotated by querying the KEGG and COG databases, respectively. The major categories by the COG analysis (Table S1) were amino acid transport and metabolism (5.2%), energy production and conversion (5.0%), translation, ribosomal structure and biogenesis (4.7%), replication, recombination and repair (4.6%), transcription (4.5%) and carbohydrate transport and metabolism (4.4%). As detected by the antiSMASH 5.0 software (Blin et al. 2019), the genome of strain EB310^T involved two putative secondary metabolites biosynthetic gene clusters (BGCs), including one terpene type and one bacteriocin type. The BGCs analysis of the isolate indicated that the terpene gene cluster was probably involved in the production of a compound structurally similar to a carotenoid based on its high similarity of 95% to the carotenoid biosynthetic gene cluster (NCBI accession number: D90087) from *Erwinia uredovora* ATCC 19321^T (Misawa et al. 1990),

which is in accordance with the result of pigment absorption spectrum analysis. The genomic average nucleotide identity (ANI) values between strain EB310^T and *E. pelagi* JCM 17468^T, *E. lutimaris* KCTC 42109^T, *E. marisflavi* KCTC 62896^T and *E. longus* JCM 6170^T were 75.5%, 74.8%, 73.4% and 71.0%, respectively. In silico DNA–DNA hybridization (*is*DDH) values between strain EB310^T and *E. pelagi* JCM 17468^T, *E. lutimaris* KCTC 42109^T, *E. marisflavi* KCTC 62896^T and *E. longus* JCM 6170^T were 19.9%, 19.8%, 20.0% and 20.0%, respectively. Both the ANI and *is*DDH values were supported the proposed threshold values of less than 95–96% to ANI and of less than 70% to *is*DDH for the delineation on species level (Chun et al. 2018). The genomic DNA G + C content of strain EB310^T was 62.9 mol%, which was within the range (57.0–67.2 mol%) of previously reported for recognized species in the genus *Erythrobacter*.

Mangrove covers about 60–75% of the world's tropical and subtropical coastlines, in saline sediment habitats at transition zones with ocean, freshwater and land (Holguin et al. 2001). The tidal action in these areas cause large changes in temperature, oxygen and salinity level during the day, which lead to the formation of complex mangrove soil environment (Kathiresan and Bingham 2001). On basis of the genome annotation and the KEGG analysis, a considerable number of genes of strain EB310^T associated with environmental adaptation were found. 54 genes responsible for xenobiotics degradation were annotated in the genome, including 8 genes for benzoate degradation, 5 genes for chloroalkane and chloroalkene degradation, 5 genes for styrene degradation, 13 genes for drug metabolism etc. The existence of these genes revealed the excellent potential of strain EB310^T on bioremediation, particularly in benzoate degradation and drug metabolism (Su et al. 2019). Additionally, the strain contained 18 genes encoding for histidine kinase and 20 genes encoding for GGDEF domain-containing proteins to promote the ability to respond effectively to environmental signals, such as, heavy metal resistance, EPS production, phage resistance and biofilm formation (Jeon et al. 2019). 27 genes related to the glutathione metabolism were discovered, together with 10 other functional genes to deal with oxidative stress (Mishra and Imlay 2012). Four cold shock proteins and three heat shock proteins genes were also discovered in the genome, which could protect the cell from the extreme temperatures. All together, the genomic data mining could help to improve our understanding of the adaptation of strain EB310^T to the complex mangrove habitats and encourage further exploration and experimental identification of the strain for biological applications.

Morphological, physiologial and biochemical analysis

Strain EB310^T formed circular, convex, smooth, glistening, orange in colour colonies with a diameter of 0.5–1.5 mm after 72 h of incubation on MA medium at 28 °C. Cells of strain EB310^T showed rod-shaped without flagellum (Fig. S3). The isolate were Gram-stain negative, aerobic growth, and positive for catalase and oxidase activities, which were in accordance with the properties of the *Erythrobacter* strains. Acetone and methanol (1: 1, v/v) extracts of strain EB310^T showed the presence of carotenoid-type pigments with maximum absorption peak at 465 nm, but absence of bacteriochlorophyll α- and flexirubin-type pigments. The detailed phenotypic characteristics of strain EB310^T are summarized in the species description. As shown in Table 1, several physiological and biochemical features could be used to distinguish strain EB310^T from other closely related type strains.

Chemotaxonomic characteristics

The major fatty acids (> 10.0% of the total fatty acids) found in strain EB310^T were summed feature 3 ($C_{16:1}$ ω7c/$C_{16:1}$ ω6c, 31.9%) and summed feature 8 ($C_{18:1}$ ω6c/$C_{18:1}$ ω7c, 33.9%). As shown in Table 2, the fatty acid profiles of strain EB310^T were similar to the four phylogenetically closely related *Erythrobacter* species, although there were differences in the proportions of major fatty acids, such as higher amounts of iso-$C_{17:0}$, summed feature 1 (iso-$C_{15:1}$ H/$C_{13:0}$ 3-OH) and summed feature 3 ($C_{16:1}$ ω7c/$C_{16:1}$ ω6c) and lower amounts of $C_{17:1}$ ω6c and $C_{14:0}$ 2-OH in strain EB310^T. The predominant respiratory quinone detected in strain EB310^T was ubiquinone-10 (Q-10), which is consistent with that shown in the *Erythrobacter* strains (Park et al. 2019). Strain EB310^T exhibited major polar lipids profile including sphingoglycolipid (SGL), phosphatidylglycerol (PG), phosphatidylethanolamine (PE), diphosphatidylglycerol (DPG) and phosphatidylcholine (PC), and five

Table 1 Differential phenotypic characteristics of strain EB310^T and its closely related species

Characteristic	1	2	3	4	5
Cell shape	Rods	Rods	Cocci, ovals	Short-rods	Rods
Colony colour (on MA medium)	Orange	Orange	Yellow	Yellow	Orange
Motility	−	−[a]	−[b]	−[c]	+[d]
Presence of bacteriochlorophyll *a*	–	−[a]	−[b]	−[c]	+[d]
Presence of carotenoid	+	−[a]	+[b]	−[c]	+[d]
Temperature range for growth (optimum) (°C)	10–45 (28–30)	10–42 (20–36)[a]	10–40 (30)[b]	4–35 (25)[c]	NA (30)[d]
pH range for growth (optimum)	5.5–9.5 (7.5)	5.0–10.0 (8.0–9.0)[a]	5.0–NA (7.0–8.0)[b]	5.5–NA (7.0–8.0)[c]	NA (7.0)[d]
NaCl range for growth (optimum) (%, w/v)	0–9.0 (2.0)	0–10.0 (2.0–3.0)[a]	0–10.0 (2.0–3.0)[b]	0.5–5.0 (1.0–2.0)[c]	NA (1.7-2.6)[d]
DNA G + C content (mol %)	62.9	60.4[a]	66.0[b]	61.7[c]	60.7[d]
(mol %)					
Nitrate reduction	+	+	−	−	+
Indole production	w	+	−	+	−
Hydrolysis of					
Casein	+	−	−	−	−
Tween 60	+	−	+	−	+
Tween 80	−	−	+	+	+
Gelatin	−	−	−	+	−
Utilization of					
D-sorbitol	+	+	−	w	+
D-ribose	−	+	−	+	+
Sodium acetate	+	+	−	−	+
L-arabinose	−	+	+	−	+
D-mannose	+	−	−	−	−
N-acetyl-glucosamine	+	+	−	w	+
D-maltose	−	+	−	−	+
Potassium gluconate	+	−	−	−	−
Adipic acid	w	−	−	+	−
Enzyme activity (API ZYM)					
Esterase (C4)	+	w	−	+	+
Trypsin	−	+	−	+	+
α-chymotrypsin	−	+	+	−	+
Naphthol-AS-BI-phosphatase	−	+	+	+	+
β-galactosidase	−	−	−	+	+
α-glucosidase	+	+	−	−	−
N-acetyl-β-glucosaminidase	−	−	+	−	+
Acid production (API 50CH)					
D-galactose	+	−	−	−	−
D-mannitol	−	+	−	−	+
D-melibiose	+	+	−	−	+
Glycogen	+	−	−	−	−
L-rhamnose	−	+	−	w	+
Myo-inositol	+	+	−	−	−

Table 1 continued

Characteristic	1	2	3	4	5
D-lactose	−	+	−	−	+
D-melezitose	−	−	−	+	+
D-raffinose	+	+	−	−	+
D-turanose	+	−	−	−	−
Susceptibility to					
Ampicillin	−	+	−	−	−
Tetracycline	+	+	−	−	+
Cefalotin	−	−	+	−	+
Kanamycin	−	−	+	+	−
Carbenicillin	−	+	−	+	+
Neomycin	+	−	+	w	−
Oleandomycin	−	w	+	−	+
Penicillin G	−	−	−	−	+
Gentamicin	+	−	−	+	−

Strains: 1, *Erythrobacter mangrovi* EB310^T; 2, *Erythrobacter pelagi* JCM 17468^T; 3, *Erythrobacter lutimaris* KCTC 42109^T; 4, *Erythrobacter marisflavi* KCTC 62896^T; 5, *Erythrobacter longus* JCM 6170^T

All strains were positive for the following: catalase and oxidase, hydrolysis of tyrosine, Tween 20 and Tween 40, utilization of D-glucose, dextrin and malic acid, acid production from D-xylose and D-glucose, susceptibility to chloramphenicol and novobiocin, and activity of alkaline phosphatase, esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase and acid phosphatase. All strains are negative for the following: anaerobic growth, Gram staining, motility, hydrolysis of CM-cellulose, urea, hypoxanthine, starch and xanthine, utilization of D-fructose, sucrose, trehalose, benzoate, trisodium citrate, formate, L-glutamate, salicin, capric acid and phenylacetic acid, acid production from gentiobiose, L-fucose and arabitol, susceptibility to lincomycin, polymyxin B and streptomycin, and activity of arginine dihydrolase, lipase (C14), α-galactosidase, β-glucuronidase, β-glucosidase, α-mannosidase and α-fucosidase. +, positive; -, negative; W, weakly positive; NA, no data available. Unless otherwise stated, all data were obtained from this study

[abcd]Data were taken from Wu et al. 2012; Jung et al. 2014; Park et al. 2019; Shiba and Simidu 1982, respectively

unidentified lipids (L1–L5) were also detected as minor ones (Fig. S4). The polar lipids profile was similar to those of *E. pelagi* JCM 17468^T, *E. lutimaris* KCTC 42109^T, *E. marisflavi* KCTC 62896^T and *E. longus* JCM 6170^T in which PC, PG and SGL were major polar lipids, but distinguishable from *E. lutimaris* KCTC 42109^T by the presence of PE as a major component, distinguishable from *E. pelagi* JCM 17468^T and *E. marisflavi* KCTC 62896^T by the absence of one unidentified glycolipid as a major component and distinguishable from *E. longus* JCM 6170^T by the absence of phosphatidyl-monomethylethanolamine (PME) as major component (Jung et al. 2014; Park et al. 2019).

Taxonomic conclusion

On basis of the combined results obtained from phylogenetic, genomic, phenotypic and chemotaxonomic analysis, it is reasonable to classify strain EB310^T as a member of the genus *Erythrobacter* (Figs. 1 and S1–S4; Tables 1, 2). Strain EB310^T was distinguished from *E. pelagi* JCM 17468^T, *E. lutimaris* KCTC 42109^T, *E. marisflavi* KCTC 62896^T and *E. longus* JCM 6170^T by differences in several phenotypic features. The novel isolate could hydrolyze casein, utilize D-mannose and potassium gluconate as carbon and energy sources and produce acid from D-galactose, glycogen and D-turanose, but *E. pelagi* JCM 17468^T, *E. lutimaris* KCTC 42109^T, *E. marisflavi* KCTC 62896^T and *E. longus* JCM 6170^T could not (Table 1). Strain EB310^T was inactive for naphthol-AS-BI-phosphatase, whereas the three reference strains were active for naphthol-AS-BI-phosphatase (Table 1). The distinguished phenotypic and chemotaxonomic characteristics, 16S rRNA gene sequence similarities, the phylogenetic trees, the DNA G + C content and genomic distinctiveness by *is*DDH and

Table 2 Fatty acid profiles of strain EB310^T and type strains of phylogenetically related species

Fatty acids	Percentages of total fatty acids				
	1	2	3	4	5
$C_{14:0}$	1.3	–	2.7	–	–
$C_{16:0}$	7.5	6.1	5.0	13.4	8.4
$C_{17:0}$	7.7	tr	3.2	tr	7.5
$C_{18:0}$	tr	1.3	–	–	1.1
$C_{16:1}$ $\omega5c$	2.5	3.1	–	tr	–
$C_{17:1}$ $\omega6c$	**tr**	**30.2**	**27.4**	**13.0**	**2.7**
$C_{17:1}$ $\omega8c$	1.9	4.5	3.5	1.6	5.6
$C_{13:0}$ 2-OH	tr	2.6	1.8	–	tr
$C_{14:0}$ 2-OH	**–**	**3.6**	**4.5**	**5.9**	**4.7**
$C_{15:0}$ 2-OH	2.3	7.9	8.2	3.1	9.0
iso-$C_{17:0}$	**2.8**	**tr**	**–**	**–**	**1.3**
11-Methyl $C_{18:1}$ $\omega7c$	–	1.4	1.1	6.7	–
Summed feature 1[a]	**1.3**	**tr**	**tr**	**–**	**–**
Summed feature 3[a]	**31.9**	**11.5**	**2.3**	**24.8**	**tr**
Summed feature 8[a]	**33.9**	**24.6**	**36.5**	**26.3**	**56.6**

Strains: 1, *Erythrobacter mangrovi* EB310^T; 2, *Erythrobacter pelagi* JCM 17468^T; 3, *Erythrobacter lutimaris* KCTC 42109^T; 4, *Erythrobacter marisflavi* KCTC 62896^T; 5, *Erythrobacter longus* JCM 6170^T

[a]Summed features represent groups of two or three fatty acids that cannot be separated by GLC with the MIDI system. Summed feature 1 consists of iso-$C_{15:1}$ H/$C_{13:0}$ 3-OH; Summed feature 3 consists of $C_{16:1}$ $\omega7c$/$C_{16:1}$ $\omega6c$ and summed feature 8 consists of $C_{18:1}$ $\omega6c$/$C_{18:1}$ $\omega7c$. tr, Trace (less than 1.0%); – no detected. All data were obtained from this study

Bold indicate the major differences between strain EB310^T and type strains of phylogenetically related species in fatty acids

ANI data suggest that strain EB310^T is separated from other members of the genus *Erythrobacter*. Therefore, according to the polyphasic taxonomic data presented here, strain EB310^T is considered to represent a novel species of the genus *Erythrobacter*, for which the name *Erythrobacter mangrovi* sp. nov. is proposed.

Description of *Erythrobacter mangrovi* sp. nov

Erythrobacter mangrovi (man.gro'vi. N.L. gen. n. *mangrovi* of a mangrove, referring to the isolation of the type strain from mangrove soil).

Cells are Gram stain negative, aerobic growth, non-motile, non-spore-forming and rod-shaped in size of 0.2–0.7 × 0.8–1.6 (μm). After incubation on MA medium at 28 °C for 3 days, the colonies are circular, convex, smooth, glistening, orange in colour and 0.5–1.5 mm in diameter. The temperature, NaCl and pH ranges for growth were 10–45 °C (optimum, 28–30 °C), 0–9.0% NaCl concentrations (optimum, 2.0%, w/v) and pH 5.5–9.5 (optimum, pH 7.5), respectively. Positive for catalase, oxidase, nitrate reduction, indole, carotenoid-type pigments production and hydrolysis of tyrosine, casein and Tweens 20, 40 and 60. Negative for H_2S, bacteriochlorophyll α- and flexirubin-type pigments production and hydrolysis of CM-cellulose, xanthine, hypoxanthine, Tween 80, starch, urea, aesculin and gelatin. D-glucose, dextrin, D-sorbitol, sodium acetate, D-lactose, D-mannose, D-mannitol, N-acetyl-glucosamine, potassium gluconate, malic acid and adipic acid are utilized as carbon and energy sources, but D-fructose, sucrose, trehalose, benzoate, trisodium citrate, formate, L-glutamate, salicin, capric acid and phenylacetic acid, D-ribose, L-arabinose and D-maltose are not. In assays with the API ZYM system, activity of alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, acid phosphatase and α-glucosidase is present, but activity of other enzymes is absent. Acid is produced oxidatively from D-xylose, D-glucose, D-galactose, D-melibiose, glycogen, *myo*-inositol, D-sorbitol, D-raffinose and D-turanose, but not from the other substrates of the API 50CH system. Susceptible to chloramphenicol, novobiocin, tetracycline, neomycin and gentamicin, but insusceptible to lincomycin, polymyxin B, streptomycin, ampicillin, cefalotin, kanamycin, carbenicillin, oleandomycin and penicillin G. The predominant ubiquinone is Q-10. The major fatty acids (> 10.0% of the total fatty acids) are summed feature 3 ($C_{16:1}$ $\omega7c$/$C_{16:1}$ $\omega6c$) and summed feature 8 ($C_{18:1}$ $\omega6c$/$C_{18:1}$ $\omega7c$). The major polar lipids are sphingoglycolipid, phosphatidylglycerol, phosphatidylethanolamine, diphosphatidylglycerol, phosphatidylcholine, and five unidentified lipids were also detected as minor ones.

The type strain EB310^T (= KCTC 72109^T = MCCC 1K03690^T), was isolated from rhizosphere soil of mangrove plant (*Kandelia candel*) in FuGong village, Zhangzhou, Fujian, China. The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain EB310^T is MT522623. Whole Genome Shotgun project of strain EB310^T has been deposited at DDBJ/ENA/GenBank under the

accession CP053921. The genomic DNA G + C content is 62.9 mol %.

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Compliance with ethical standards

Conflict of interest The authors declare that there are no conflicts of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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