**Erythrobacter atlanticus** sp. nov., a bacterium from ocean sediment able to degrade polycyclic aromatic hydrocarbons

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A Gram-stain-negative, motile, rod-shaped, orange-pigmented bacterium able to degrade polycyclic aromatic hydrocarbons was isolated from deep-sea sediment of the Atlantic Ocean and subjected to a polyphasic taxonomic study. The strain, designated s21-N3T, could grow at 4–37 °C (optimum 28 °C), at pH 5–10 (optimum pH 7–8) and with 1–7 % (w/v) NaCl (optimum 2–3 %). Strain s21-N3T was positive for nitrate reduction, denitrification, aesculin hydrolysis, oxidase and catalase, but negative for indole production and urease. Phylogenetic analyses based on 16S rRNA gene sequences showed that strain s21-N3T formed a distinct branch within the genus *Erythrobacter*, sharing high similarities with three closely related strains, *Erythrobacter marinus* HWDM-33T (98.67 %), *Erythrobacter luteus* KA37 (97.80 %) and *Erythrobacter gangjinensis* K7-2T (97.59 %). The similarities between strain s21-N3T and other type strains of recognized species within the genus *Erythrobacter* ranged from 95.00 to 96.47 %. The digital DNA–DNA hybridization values and average nucleotide identity (ANI) values between strain s21-N3T and the three closely related strains *Erythrobacter marinus* HWDM-33T, *Erythrobacter luteus* KA37 and *Erythrobacter gangjinensis* K7-2T were 18.60, 18.00 and 18.50 % and 74.24, 72.49 and 72.54 %, respectively. The principal fatty acids were summed feature 8 (C18:1ω7c/ω6c) and summed feature 3 (C16:1ω7c/ω6c). The respiratory lipoquinone was identified as Q-10. The major polar lipids comprised sphingoglycolipid, phosphadityldecanolamine, phosphaditylglycerol, phosphaditylcholine and diphosphatidylglycerol. The G+C content of the chromosomal DNA was determined to be 58.18 mol%. The combined genotypic and phenotypic distinctiveness demonstrated that strain s21-N3T represents a novel species of the genus *Erythrobacter*, for which the name *Erythrobacter atlanticus* sp. nov. is proposed, with the type strain s21-N3T (=MCCC 1A00519T=KCTC 42897T).

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*Erythrobacter*, the type genus of the family *Erythrobacteraceae*, was originally proposed by Shiba & Simidu (1982).

†These authors contributed equally to the paper.

**Abbreviations:** ANI, average nucleotide identity; dDDH, digital DNA–DNA hybridization; PAH, polycyclic aromatic hydrocarbon.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene and complete genome sequences of *Erythrobacter atlanticus* s21-N3T are KP094305 and CP011310, respectively. Accession numbers for the draft genome sequences of *E. marinus* HWDM-33T, *E. luteus* KA37 and *E. gangjinensis* K7-2T are LBHU00000000, LBHB00000000 and LBHC00000000, respectively.

Two supplementary figures are available with the online Supplementary Material.

Members of this genus are Gram-stain-negative, orange- or pink-pigmented bacteria. At the time of writing, the genus *Erythrobacter* comprises 17 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. gaebuli* (Yoon et al., 2005), *E. seohaensis* (Yoon et al., 2005), *E. vulgaris* (Ivanova et al., 2005), *E. gangjinensis* (Lee et al., 2010), *E. nanhaiasediminis* (Xu et al., 2010), *E. marinus* (Jung et al., 2012), *E. pelagi* (Wu et al., 2012), *E. westpacificensis* (Wei et al., 2013; name not validly published), *E. jejuensis* (Yoon et al., 2013), *E. odishensis* (Subhash et al., 2013), *E. lutimaris* (Jung et al., 2014) and the recently described ‘E. luteus’ (Lei et al., 2015). In the present study, the aim...
was to determine the exact taxonomic position of strain s21-N3\textsuperscript{T} using a polyphasic approach.

During an investigation of the polycyclic-aromatic-hydrocarbon-degrading bacterial diversity, strain s21-N3\textsuperscript{T} was isolated from a sediment sample. The sample was collected in May 2011 at 2076 m water depth of the Atlantic Ocean (20.89° S 11.42° W). The sediment was enriched by mixed polycyclic aromatic hydrocarbons (PAHs). Strain s21-N3\textsuperscript{T} was isolated subsequently by the standard dilution plating technique on marine agar 2216 (MA; BD) and incubated at 28 °C for 1 week. The purified strain was stored at −80 °C in marine broth 2216 (MB; BD) with 30% (v/v) glycerol. Multiple growth experiments indicated that strain s21-N3\textsuperscript{T} was able to utilize a variety of PAHs as sole carbon sources, such as naphthalene, phenanthrene, pyrene, etc.; in contrast, the three closely related strains described below were unable to utilize them. Therefore, strain s21-N3\textsuperscript{T} was an outstanding PAH-degrading marine bacterium. Unless otherwise noted, all characteristics described hereafter were determined following incubation on MA or in MB at 28 °C for 72 h. Three closely related strains, E. marinus HWDM-33\textsuperscript{T}, ‘E. luteus’ KA37 and E. gangjinensis K7-2\textsuperscript{T}, were obtained from the Marine Culture Collection of China (MCCC) and were used as reference strains for fatty acid analysis and other tests.

Cell morphology, size and flagellation pattern were observed by transmission electron microscopy (JEM-1230, JEOL) using cells grown on MA at 28 °C for 72 h and negatively stained with phosphotungstic acid. The Gram reaction was determined using a Gram stain kit (Hangzhou Tianhe Micro-organism Reagent) according to the manufacturer’s instructions. Anaerobic growth was tested on MA in an anaerobic chamber (1029, Forma; N\textsubscript{2}/CO\textsubscript{2}/H\textsubscript{2}, 86:7:7 %) at 28 °C for 2 weeks. Growth at 4, 15, 22, 28, 37 and 40 °C was measured on MA. The range of and optimum NaCl concentration for growth were determined using various concentrations of NaCl (0–8 %, w/v) according to a previously described method (Yoon \textit{et al.}, 2013). The pH range for growth was tested using MB that had been adjusted to various pH values (pH 3–11, at intervals of 1 pH unit) with the appropriate biological buffers, citrate/phosphate (pH 3.0–7.0), Tris/HCl (pH 8.0–9.0) or sodium carbonate/NaOH (pH 10.0–11.0). Activities of catalase and oxidase, hydrolysis of starch, casein, and Tween 20 and 40 were performed as described by Hansen & Sørheim (1991). Other physiologic properties were confirmed using API ZYM, 20NE and 20E strips (bioMérieux) according to the manufacturer’s instructions. For spectral analysis of \textit{in vivo} pigment absorption, strain s21-N3\textsuperscript{T} was cultivated aerobically in the dark at 28 °C in MB. The culture was washed twice by using centrifugation with a MOPS buffer (0.01 mol MOPS/NaOH 1 \textsuperscript{−1}; 0.1 mol KCl 1 \textsuperscript{−1}; 0.001 mol MgCl\textsubscript{2} 1 \textsuperscript{−1}; pH 7.5) and disrupted by means of sonication (Vibra Cell-Sonics). After removal of cell debris by centrifugation, the absorption spectrum of the supernatant was examined on a Bio-Rad Smartspe plus spectrophotometer (Yoon \textit{et al.}, 2005).

Strain s21-N3\textsuperscript{T}, was cultivated at 28 °C for 48 h in MB to obtain the cell biomass required for DNA extraction and for analyses of isoprenoid quinones and polar lipids. Genomic DNA was extracted according to using the AxyPrep Bacterial Genomic DNA Miniprep kit (Axygen Biosciences) according to the manufacturer’s instructions. The 16S rRNA gene was amplified using bacteria-specific universal primers 27F and 1492R (Lane, 1991) and then sequenced by using the ABI3730xl platform (Shanghai Majorbio Bio-pharm Technology, China). The 16S rRNA gene sequence similarity was determined using the EzTaxon-e server (Kim \textit{et al.}, 2012). Sequences of related taxa were obtained from the GenBank database. Phylogenetic analysis was performed using MEGA version 5.0 (Tamura \textit{et al.}, 2011) with distance options according to the Kimura two-parameter model and clustering with the neighbour-joining (Saitou & Nei, 1987), maximum-likelihood (Felsenstein, 1981) and minimum evolution (Rzhetsky & Nei, 1992) methods, and supported with bootstrap values based on 1000 replications.

For fatty acid analysis, cells were harvested from the third quadrants on MA medium, after cultivation for 3 days at 28 °C. The cells were saponified, methylated and extracted using the standard MIDI (Sherlock Microbial Identification System, version 6.0B) protocol. The fatty acids were analysed by gas chromatography (6850, Agilent Technologies) and identified using the TSBA6.0 database of the Microbial Identification System (Sasser, 1990). Analysis of the respiratory quinones and polar lipids was carried out by the Identification Service of the Zhejiang University and Jiangsu University, respectively. Both isoprenoid quinones and polar lipids were analysed according to a previously described method (Minnikin \textit{et al.}, 1984).

For comparison of the digital DNA–DNA hybridization (dDDH) and average nucleotide identity (ANI) values between strain s21-N3\textsuperscript{T} and the three reference strains, the genome sequence of strain s21-N3\textsuperscript{T} was sequenced using the Illumina HiSeq2000 platform by BGI (Shenzhen, China), generating 2 × 100 bp paired-end reads. Genome sequences of the three reference strains, E. marinus HWDM-33\textsuperscript{T}, ‘E. luteus’ KA37 and E. gangjinensis K7-2\textsuperscript{T}, were determined using the Illumina MiSeq platform by Shanghai Majorbio Pharm Technology (Shanghai, China) with a paired-end library. On the basis of the draft genome sequence, the DNA G+C content of strain s21-N3\textsuperscript{T} was determined. Meanwhile, dDDH values were analysed using the genome-to-genome distance calculator (GGDC2.0) (Auch \textit{et al.}, 2010). Additionally, ANI values were calculated using the algorithm of Goris \textit{et al.} (2007) using the EzGenome web service.

Cells of strain s21-N3\textsuperscript{T} were Gram-stain-negative rods that measured 0.5–2 μm in length and 0.3–0.5 μm in width. Strain s21-N3\textsuperscript{T} was motile (see Fig. S1 available in the online Supplementary Material). Colonies on MA
were yellow–brown, smooth, circular, opaque with entire margins and 0.8–1.0 mm in diameter after incubation at 28 °C for 3 days. Strain s21-N3T grew optimally at 28 °C and could grow at 4 °C and 37 °C, but not at 40 °C. The pH range and optimal pH for growth were pH 5–10 and 7–8, respectively. Strain s21-N3T grew optimally in the presence of 2–3 % (w/v) NaCl, but did not grow without NaCl or in the presence of >7 % NaCl. Strain s21-N3T showed facultatively anaerobic growth. Activities of catalase and oxidase, and aesculin hydrolysis of strain s21-N3T were positive. Hydrolysis of starch, casein, gelatin, and Tweens 20 and 40 of strain s21-N3T was negative. Strain s21-N3T had the ability for reduction of nitrate and denitrification. Sonicated cell extracts of strain s21-N3T showed an absorption peak at 450 nm, indicating the presence of carotenoids. Detailed phenotypic characteristics of strain s21-N3T are summarized in the species description. As shown in Table 1, several physiological and biochemical properties can be used to distinguish strain s21-N3T from the three closely related strains.

A complete 16S rRNA gene sequence (1493 bp) of strain s21-N3T was determined. As shown in Fig. 1, the neighbour-joining phylogenetic tree of 16S rRNA gene sequences showed that strain s21-N3T belonged to the genus *Erythrobacter* and formed a distinct branch, with a high bootstrap 99% value. The maximum-likelihood and minimum-evolution phylogenetic trees had nearly identical topology as the neighbour-joining tree. Strain s21-N3T shared high sequence similarities with the three closely related strains, *E. marinus* HWDM-33T (98.67 %), 'E. luteus' KA37 (97.80 %) and *E. gangjinensis* K7-2T (97.59 %). While the similarities between strain s21-N3T and other type strains of recognized species of the genus *Erythrobacter* ranged from 95.00 to 96.47 %.

The principal fatty acids of strain s21-N3T were summed feature 8 (C18:1ω7c/ω6c) and summed feature 3 (C16:1ω7c/ω6c). As listed in Table 2, although the kinds of fatty acids of strain s21-N3T were almost consistent with those of the three closely related strains *E. marinus* HWDM-33T (Jung et al., 2012), 'E. luteus' KA37 (Lei et al., 2015) and *E. gangjinensis* K7-2T (Lee et al., 2010), the proportions of them were different, such as summed feature 3 and C17:1ω6c. The respiratory lipoquinone of strain s21-N3T was identified as ubiquinone-10 (Q-10), which is in accordance with those of the three closely related strains and other type strains of recognized species of the genus *Erythrobacter* (Chimetta Tonon et al., 2014). The polar lipids of strain s21-N3T were identified as sphingoglycolipid, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylcholine, diphostatidylglycerol (DPG), unidentified phospholipids (PL) and several unknown lipids, which were similar to those of 'E. luteus' KA37 (Fig. S2). The distinct differences were the presence of DPG and PL in strain s21-N3T and 'E. luteus' KA37 and the absence of them in type strains *E. marinus* HWDM-33T and *E. gangjinensis* K7-2T.

DNA–DNA hybridization has been considered to be the gold standard for bacterial species delineation, and therefore genome sequences of strain s21-N3T and three closely related strains were sequenced in this study. The complete genome sequence of strain s21-N3T and draft genome sequences for *E. marinus* HWDM-33T, 'E. luteus' KA37 and *E. gangjinensis* K7-2T were determined. On the basis of the genome sequences, the DNA G+C content of

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<td>DNA G+C content (mol%)†</td>
<td>58.18</td>
<td>59.09</td>
<td>67.16</td>
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†Data were different from those of previous studies.
strain s21-N₃ was 58.18 mol%, and the redetermined DNA G+C contents of strains *E. marinus* HWDM-33, *E. luteus* KA37 and *E. gangjinensis* K7-2 were 59.09, 67.16 and 62.71 mol%, respectively. The dDDH values between strain s21-N₃ and the three closely related strains were 18.60, 18.00 and 18.50%, respectively. Similarly, the ANI values between strain s21-N₃ and the three closely related strains were 74.24, 72.49 and 72.54%, respectively. The dDDH and ANI values are below the standard criteria for delineation of bacterial species (Wayne et al., 1987; Richter & Rossello-Mora, 2009), suggesting that strain s21-N₃ is a putative novel species of the genus *Erythrobacter*.

In conclusion, on the basis of phylogenetic evidence and several other physiological and biochemical characteristics, strain s21-N₃ is clearly a member of the genus *Erythrobacter*, but is distinguishable from all described species in this genus. Therefore, strain s21-N₃ is considered to represent a novel species of the genus *Erythrobacter*, for which the name *Erythrobacter atlanticus* sp. nov. is proposed.

**Description of *Erythrobacter atlanticus* sp. nov.**

*Erythrobacter atlanticus* (at.lan’ti.cus. N.L. masc. adj. atlanticus referring to the Atlantic Ocean, where the type strain was isolated).

Cells are Gram-stain-negative, rod-shaped, 0.5–2.0 × 0.3–0.5 μm, facultatively anaerobic and motile. Colonies on MA are yellow-brown, smooth, circular, opaque with entire margins and 0.8–1.0 mm in diameter after incubation at 28 °C for 3 days. Growth occurs at 4–37 °C (optimum 28 °C), at pH 5–10 (optimum pH 7–8) and with 1–7% NaCl (w/v) (optimum 2–3%). Positive for catalase and oxidase activities, but negative for hydrolysis of starch, casein, and Tweens 20 and 40. In the API ZYM strip, positive for alkaline phosphatase, leucine aminopeptidase, valine aminopeptidase, trypsin and acid phosphatase; weakly positive for esterase (C4) and naphthol-AS-BI-phosphoamidase; but negative for α-chymotrypsin, α-fucosidase, α-galactosidase, α-glucosidase, α-mannosidase, β-galactosidase, β-glucosidase, β-glucuronidase, cystine aminopeptidase, esterase lipase (C8), lipase (C14)
and N-acetyl-β-glucosaminidase. In the API 20E tests, negative for citrate utilization, H2S, indole and acetoin production, activities of β-galactosidase, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, urease, tryptophan deaminase and gelatinase, and acid production from D-glucose, mannitol, inositol, sorbitol, rhamnose, sucrose, melibiose, amygdalin and arabinose. In the API 20NE tests, positive for reduction of nitrate, denitrification, sucrose, melibiose, amygdalin and arabinose. In the API 20NE tests, positive for utilization of L-arabinose, D-mannose, potassium gluconate, adipic acid and malic acid; weakly positive for utilization of L-arabinose, D-mannose, N-acetylglucosamine and D-mannitol; negative for D-glucose fermentation indole production, gelatin hydrolysis, activities of arginine dihydrolase, β-galactosidase and urease, and utilization of capric acid, trisodium citrate and phenylacetlic acid. Carotenoid is detected. The principal fatty acids are summed feature 8 (C16:1ω7c/ω6c) and summed feature 3 (C16:1ω7c/ω6c). The respiratory lipoquinone is Q-10. The major polar lipids are sphingoglycolipid, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylglycerol and diphosphatidylglycerol. Table 1 shows the characteristics used to distinguish strain s21-N3T from other species of the genus.

The type strain, s21-N3T (=MCCC 1A00519T=KCTC 42697T) was isolated from deep-sea sediment of the Atlantic Ocean. The DNA G+C content of the type strain is 58.18 mol%.

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References


