Curr Microbiol
DOI 10.1007/s00284-017-1342-7

Aestuarium zhoushanense gen. nov., sp. nov., Isolated from the Tidal Flat

Xiao-Yun Yu[1] · Jing-Yu Zhai[2] · Ge-Yi Fu[3] · Xia Shen[1] · Zhe Zhao[1] · Min Wu[1] · Xin-Qi Zhang[4]

Received: 10 May 2017 / Accepted: 17 August 2017

Abstract A gram-stain-negative, aerobic, ovoid or short rod-shaped, and non-motile strain, designed G7^T was isolated from a tidal flat sample collected from the coast of East Sea in Zhoushan, China. Strain G7^T grew at 4–40 °C and pH 6.0–9.0 (optimum, 28 °C and pH 7.5) and with 0–7% (w/v) NaCl (optimum, 1%). The predominant respiratory quinone was Q-10 and the major fatty acids (>10%) identified were $C_{18:1}$ $\omega7c$, $C_{16:0}$ and summed feature 3 ($C_{16:1}$ $\omega7c$ and/or $C_{16:1}$ $\omega6c$). The polar lipids of strain G7^T consisted of phosphatidylglycerol, phosphatidylethanolamine, phosphatidylcholine, and four unidentified lipids. The genomic DNA G+C content was 56.7 mol%. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain G7^T formed a distinct lineage belonging to the *Roseobacter* clade of the family *Rhodobacteraceae*. On the basis of morphological, physiological, and chemotaxonomic characteristics, together with the results of phylogenetic analysis, strain G7^T is described as a novel species in a new genus, for which the name *Aestuarium zhoushanense* gen. nov., sp. nov. (type strain G7^T = MCCC 1K03229^T = KCTC 52584^T) is proposed.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequence and WGS whole-genome project of strain G7^T are KX890235 and CP021114-CP021117, respectively. The Digital Protologue database TaxonNumber for strain G7^T is TA00172.

Electronic supplementary material The online version of this article (doi:10.1007/s00284-017-1342-7) contains supplementary material, which is available to authorized users.

✉ Min Wu
wumin@zju.edu.cn

✉ Xin-Qi Zhang
xinqizhang@hotmail.com

1 College of Life Sciences, Zhejiang University, Hangzhou 310058, People's Republic of China

2 College of Geography and Environmental Sciences, Zhejiang Normal University, Jinhua 321004, People's Republic of China

3 Ocean College, Zhejiang University, Zhoushan 316000, People's Republic of China

4 School of Foresty and Biotechnology, Zhejiang Agriculture and Forestry University, Linan 311300, People's Republic of China

Introduction

The family *Rhodobacteraceae*, belonging to the order *Rhodobacterales* of the class *Alphaproteobacteria*, was established by Garrity et al. [8, 9]. At the time of writing, the family *Rhodobacteraceae* comprises 128 genera with 457 validly published species (http://www.bacterio.net/). The *Roseobacter* clade, which is the largest group within *Rhodobacteraceae*, including over 70 validly named genera and over 170 validly named species [21, 25]. Most members of the *Roseobacter* clade are aquatic bacteria that are frequently found in the marine environment, especially in coastal and polar oceans, where members of the *Roseobacter* clade comprises 15–25% of the total bacterioplankton communities [22]. Isolates of this clade display diverse physiologies, including such aerobic anoxygenic photosynthesis, oxidation or degradation of carbon monoxide, sulfite, methanesulfonic acid, dimethylsulfoniopropionate, methylamine, methyl bromide, lignin, aromatic compounds, production of allelopathic compounds and bioactive secondary metabolites, reduction of trace metals and indication of symbiotic and pathogenic

Published online: 12 September 2017

relationships [2, 20]. This paper describes a novel genus and species of the *Roseobacter* clade isolated from a coastal tidal flat sample, for which the name *Aestuarium zhoushanense* gen. nov., sp. nov. is proposed.

Materials and Methods

Strains and Culture Conditions

A tidal flat sample was collected from the coast of East Sea in Zhoushan, China (Depth of 0 m, 122.1035°E, 29.9522°N) in July, 2016. The isolate was obtained by the standard dilution plating technique at 20 °C on modified marine agar 2216 [19] and cultivated routinely on marine agar 2216 (MA; BD Difco^{TM}) or in marine broth 2216 (MB; Difco^{TM}) at 28 °C. Purified strain was maintained on MA at 4 °C for short-term storage and stored at −80 °C with 25% (v/v) glycerol and by lyophilization with 20% (w/v) skimmed milk for long-term preservation.

Cell biomass of the strain for DNA extraction and for the analyses of isoprenoid quinones and polar lipids was obtained from cultures grown for 2 days in MB at 28 °C. For cellular fatty acids analysis, cell mass of the isolate was harvested from MA plates after incubation for 5 days at 28 °C. Strain G7^T has been deposited at the MCCC (Marine Culture Collection of China) and the KCTC (Korean Collection for Type Cultures).

Determination of 16S rRNA Gene Sequence and Phylogenetic Analysis

The 16S rRNA gene of G7^T was amplified using universal primers 27F/1492R [13]. PCR products were cloned into pMD-19T vector (TaKaRa) for sequencing [33]. The nearly complete 16S rRNA sequence (1428 bp) was performed pairwise sequence alignment by the EzTaxon-e server [12]. Phylogenetic trees were constructed using the neighbor-joining [24], maximum-parsimony [7] and maximum-likelihood [6] methods with the MEGA version 5.0 software package [28]. All bootstrap analyses were based on 1000 replications. Genome DNA of strain G7^T was extracted using CTAB methods as described by Clarke et al. [3]. The complete genome was sequenced by Majorbio Genome Institute (Shanghai) with PacBio platform and assembled by HGAP [1]. Average nucleotide identity was calculated with OrthoANI [17]. DNA G+C mol% was calculated from the genomic sequences.

Phenotypic Characterization

Cell morphological characteristics were observed by optical microscopy (BX40; Olympus) after gram staining and by transmission electron microscopy (JEM-1230; JEOL) after uranyl acetate staining. Motility was determined by microscopic observation and inoculation in semi-solid agar medium. Cultures incubated for 2 days were used to determine the optimal growth while those incubated for 14 days were used to determine the growth limits. The temperature range for growth was determined at 4–50 °C (4, 10, 15, 20, 25, 28, 30, 35, 37, 40, 42, 45 and 50 °C). The pH range for growth was measured from 5.0 to 10.0 (at 0.5 pH unit intervals) by supplementing 50 mM buffering agents, including MES (pH 5.0–6.0), PIPES (pH 6.5–7.0), Tricine (pH 7.5–8.5) and CAPSO (pH 9.0–10.0). Tolerance of NaCl (0–15.0%, w/v, at intervals of 1%) was investigated in NaCl-free MB [36].

Antibiotic susceptibility was tested on MA plates with discs containing the following antibiotics (μg per disc unless stated otherwise): kanamycin (30), streptomycin (10), erythromycin (15), rifampicin (5), chloramphenicol (30), gentamycin (10), tetracycline (30), cephradine (30), neomycin (30), ciprofloxacin (5) and nalidixic acid (30).

Catalase and oxidase activities were individually determined using 3% (v/v) hydrogen peroxide solution and oxidase reagent (bioMérieux). Anaerobic growth was examined in an anaerobic jar (MGC) with AnaeroPack (MGC) on modified MA supplemented with potassium nitrate (0.1%, w/v) as a potential electron acceptor for 30 days. The pigment absorption spectrum analysis was performed using a DU 800 Spectrophotometer (Beckman; absorption spectrum from 300 to 1000 nm) based on the method described by Wu et al. [31]. Tests for nitrate reduction, hydrolysis of starch, gelatin, skim milk and urea were performed according to Dong and Cai [4]. Tweens 20, 40, 60 and 80 were examined as described by Sun et al. [27]. Indole production test was assayed according to Zhang et al. [37]. Hydrolysis of hypoxanthine and xanthine were tested as described [10]. Degradation of L-tyrosine was performed according to Zhang et al. [38]. The utilization of carbon sources was tested in the basal medium with 0.4% (w/v) of each carbon source as described by Su et al. [26]. Additional enzyme activities and physiological characteristics were tested by API 20NE and API ZYM kits (bioMérieux) according to the manufacturer's instructions.

Determination of Isoprenoid Quinones, Fatty Acids and Polar Lipids

For chemotaxonomic studies, cell mass of strain G7^T and three reference strains were cultured under the same conditions unless otherwise stated. Isoprenoid quinones were extracted as described by Komagata and Suzuki [14] and analysed by LC–MS (Agilent) [29]. Cellular fatty acids methyl esters were extracted as described by Kuykendall et al. [15] and analyzed according to the instructions of the

microbial identification system (MIDI; Microbial ID). The polar lipids were extracted and separated by two-dimensional TLC (silica-gel plates 60, Merck 5554) and identified using standard procedures [5, 18].

Results and Discussion

Genotypic Characterization and Taxonomic Conclusion

On the basis of 16S rRNA gene sequence alignment, strain G7^T exhibited the highest similarity value (95.5%) with *Aestuariihabitans beolgyonensis* KCTC 32324^T and to species of genera *Donghicola* (≤95.4%) and *Octadecabacter* (≤95.2%). As shown in Fig. 1, phylogenetic trees constructed using the neighbor-joining, maximum-likelihood and maximum-parsimony algorithms indicated that strain G7^T formed an independent lineage within the *Roseobacter* clade of the family *Rhodobacteraceae*, and formed a robust cluster with *Ketogulonicigenium vulgare* DSM 4025^T, *K. robustum* X6L^T, *Oceanicola granulosus* KCTC 12145^T, and *Roseisalinus antarcticus* DSM 11466^T. The type species *K. vulgare* DSM 4025^T was not deposited at any collection institutes. Therefore, *A. beolgyonensis* KCTC 32324^T [35], *O. granulosus* KCTC 12145^T [2] and *R. antarcticus* DSM 11466^T [16] were selected as references for physiological and chemotaxonomic analysis. Strain *A. beolgyonensis* KCTC 32324^T and *O. granulosus* KCTC 12145^T were purchased from Korean Collection for Type Cultures (KCTC). *R. antarcticus* DSM 11466^T was obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ).

The genomic G+C content of strain G7^T was determined to be 56.7 mol%, which was different from the related type strains, such as *A. beolgyonensis* KCTC 32324^T (62.7%), *O. granulosus* KCTC 12145^T (71.5%), *K. vulgare* DSM 4025^T (54.0%) [30], *R. antarcticus* DSM 11466^T (67.0%) and the type genus of the family *Rhodobacteraceae* (>60 mol%) [34]. The OrthoANI value between strain G7^T and *K. vulgare* DSM 4025^T was calculated to be 69.8%, which was lower than the threshold value of 95% ANI relatedness for species demarcation [11, 23]. The WGS whole-genome accession numbers of strain G7^T were CP021114-CP021117. The genotypic and phylogenetic analyses indicated that strain G7^T represented a novel species of a novel genus of the family *Rhodobacteraceae* within the class *Alphaproteobacteria*.

Phenotypic Characterization

Cells of strain G7^T was observed to be gram-stain negative, aerobic, non-motile and ovoid or short rod-shaped (0.6–0.8 μm in width and 1.0–2.5 μm in length). Ultrathin sections revealed that strain G7^T possessed cytoplasmic membrane and an outer membrane and accumulated PHB as described in many members of *Roseobacter* clade (Fig. 2). Flagellum was not observed. Colonies were 0.5–1.0 mm in diameter, circular, slightly convex and greyish yellow with entire margins after incubation on MA

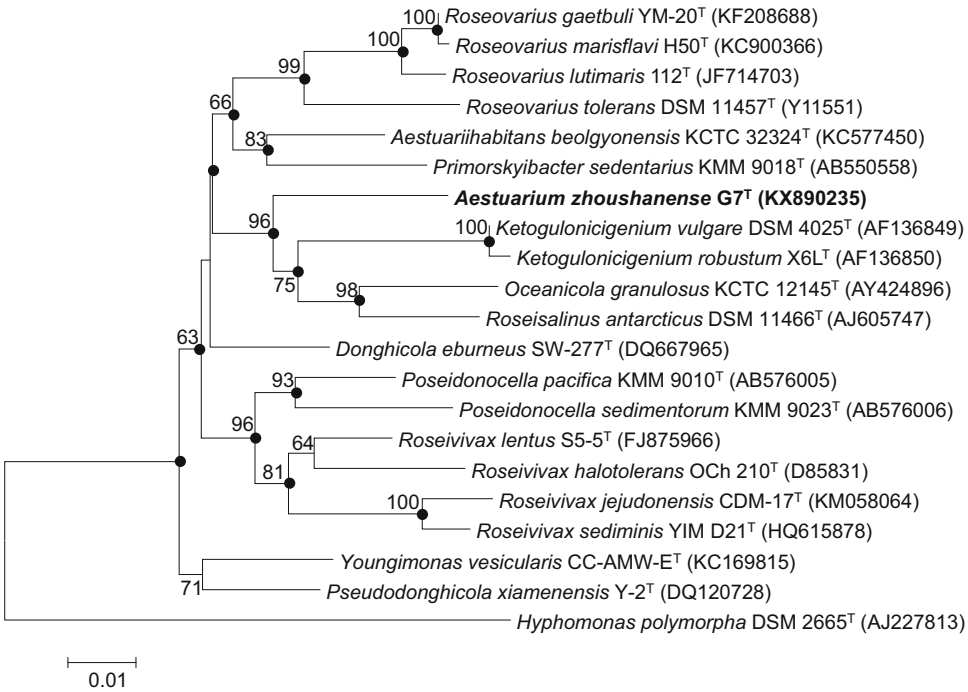

Fig. 1 Neighbour-joining phylogenetic tree of strain G7^T and some closely related taxa, based on 16S rRNA gene sequences (1428 bp). Numbers at branching points represent bootstrap values (%) from 1000 replicates; only values ≥50% are shown. *Solid circles* indicate that the corresponding nodes were also recovered in maximum-likelihood and maximum-parsimony trees. *Hyphomonas polymorpha* DSM 2665^T (AJ227813) was used as an outgroup. *Bar*, 0.01 substitutions per nucleotide position

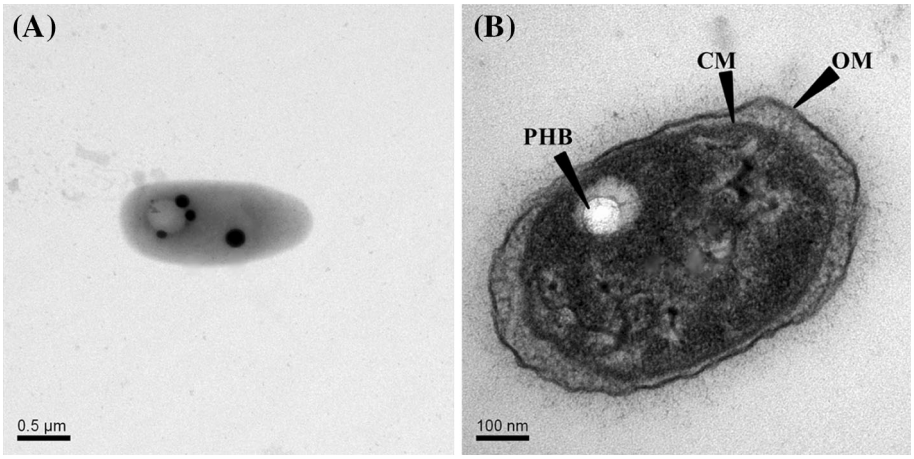

Fig. 2 Transmission electron micrographs showing the cell morphology and ultrastructure of strain G7^T. *Bars* 0.5 μm (**a**), 100 nm (**b**). *CM* cytoplasmic membrane, *OM* outer membrane, *PHB* poly-*β*-hydroxybutyrate

at 28 °C for 3 days. Under the optimal growth conditions, cells reached the logarithmic phase in MB at the third day and on MA at the fifth day. Extracts of bacteriochlorophyll α (Bchl α) did not show absorption maxima in the visible and in the infrared range (Supplementary Fig. S1); extracts of the reference strain *R. antarcticus* DSM 11466^T showed a larger absorption peak at 870 nm and smaller peaks at 800–801 and 590–592 nm [16]. Optimum growth occurred at 1% (w/v) NaCl, 28 °C and pH 7.5. Strain G7^T grew well without NaCl, which differed from the reference strains *A. beolgyonensis* KCTC 32324^T, *O. granulosus* KCTC 12145^T, and *R. antarcticus* DSM 11466^T that could not grow without NaCl (Table 1). The isolate was susceptible to kanamycin, streptomycin, erythromycin, rifampicin, chloramphenicol, gentamycin, cephradine, neomycin and ciprofloxacin, and was resistant to tetracycline and nalidixic acid. Other physiological and biochemical characteristics were given in Table 1 and in the species description.

Isoprenoid Quinones, Fatty Acids and Polar Lipids Characterization

The predominant respiratory quinone detected in strain G7^T was Q-10 (>99%), which was typical of the vast majority of the class *Alphaproteobacteria* [32]. The cellular fatty acid profiles of strain G7^T and the reference strains *A. beolgyonensis* KCTC 32324^T, *O. granulosus* KCTC 12145^T and *R. antarcticus* DSM 11466^T are compared in Table 2. As listed in Table 2, $C_{18:1}$ *ω7c* (51.6%), $C_{16:0}$ (20.3%) and summed feature 3 ($C_{16:1}$ *ω7c* and/or $C_{16:1}$ *ω6c*) (12.6%) were the major fatty acids (>10%) detected in strain G7^T. These data were similar with other three reference strains, and all the four strains possessed $C_{18:1}$ *ω7c* as the most abundant fatty acid. However, the profiles of strain G7^T were distinguishable from the three reference strains by differences in the proportions of some fatty acids. For example, $C_{16:0}$ and summed feature 3 were the major fatty acids of strains G7^T, but they were strikingly lower detected in reference strains *A. beolgyonensis* KCTC 32324^T (5.3 and 0.7%, respectively), *O. granulosus* KCTC 12145^T(11.8 and 1.2%, respectively) and *R. antarcticus* DSM 11466^T(14.5% and not detected, respectively). The polar lipids detected in strain G7^T were phosphatidylglycerol (PG), phosphatidylethanolamine (PE), phosphatidylcholine (PC) and four unidentified lipids (L1-4) (Supplementary Fig. S2). Two polar lipids PG and PC were also presented in *A. beolgyonensis* KCTC 32324^T, *O. granulosus* KCTC 12145^T and *R. antarcticus* DSM 11466^T as major polar lipids, but the moderate component PE and two minor amounts (L1 and L4) were not found in other three reference strains (Supplementary Fig. S2).

On the basis of the phylogenetic, physiological and chemotaxonomic distinctiveness and other differential properties, strain G7^T is considered to represent a novel species of a novel genus within the family *Rhodobacteraceae*, for which the name *Aestuarium zhoushanense* gen. nov., sp. nov., is proposed.

Description of *Aestuarium* gen. nov

Aestuarium (Aes.tu.a.ri'um. L. n. *aestuarium*, an estuary, tidal flat).

Cells are Gram-stain negative, aerobic, ovoid or short rod-shaped and non-motile. Catalase- and oxidase-positive. The major isoprenoid quinone is Q-10. The principal cellular fatty acids (>10%) are $C_{18:1}$ *ω7c* and summed feature 3 ($C_{16:1}$ *ω7c* and/or $C_{16:1}$ *ω6c*). The major polar lipids include phosphatidylglycerol, phosphatidylethanolamine, phosphatidylcholine and four unidentified lipids. The G+C content of the genomic DNA of the type strain of the type species is 56.7 mol% (by genome). The phylogenetic

Table 1 Differential phenotypic characteristics of strain G7^T and representatives of some phylogenetically related taxa

Characteristic	1	2	3	4
Source	Tidal flat	Tidal flat[a]	Sea water[b]	Lake water[c]
Flagella	Not found	Not found[a]	Not found[b]	Not found[c]
Motility	−	−	−	+[c]
Colony colour	Greyish yellow	Greyish yellow	Faint yellow	Red
Type of metabolism	Aerobic	Aerobic[a]	Aerobic to microaerophilic[b]	Aerobic to microaerophilic[c]
Optimum temperature (°C)	28	30[a]	28[b]	16–26[c]
Growth at 4 °C	+	+[a]	+[b]	+[c]
Growth at 37 °C	+	−[a]	+[b]	−[c]
Growth at 0% NaCl (w/v)	+	−[a]	−[b]	−[c]
Bchl α production	−	ND[a]	ND[b]	+[c]
PHB Accumulation	+	ND[a]	+[b]	+[c]
Hydrolysis of				
Gelatine	+	+	−	−[c]
Tween 40	+	+	−	+[c]
Tween 60	+	+	W	ND[c]
Tween 80	−	−	−	±[c]
L-Tyrosine	−	−	+	ND[c]
Nitrate reduction	−	−	−	W[c]
Utilization of				
Acetate	+	−	−	+
D-Fructose	+	−	−	+
D-Galactose	+	−	+	+
D-Glucose	+	−	+	+
Succinic acid	−	−	+	+
API ZYM				
Esterase (C4)	+	−	+	+
Valine arylamidase	W	+	+	W
Cysteine arylamidase	−	−	W	W
Acid phosphatase	+	W	W	+
Napthol-AS-Bi-phosphopydrase	+	W	W	W
α-galactosidase	−	−	−	+
β-galactosidase	−	−	+	+
α-glucosidase	+	−	W	+
β-glucosidase	+	−	+	+
N-acetyl-β-glucosami-nidase	−	−	+	+
α-mannosidase	−	−	−	W
β-fucosidase	−	−	+	W
API 20NE				
Hydrolysis of				
Urea	+	−	+	−
Esculin	+	−	+	+
β-galactosidase	W	−	+	+

Table 1 continued

Characteristic	1	2	3	4
DNA G + C content (mol%)	56.7	62.7[a]	70.9–71.5[b]	67.0[c]

Strains: 1, G7^T; 2, *Aestuariihabitans beolgyonensis* KCTC 32324^T; 3, *Oceanicola granulosus* KCTC 12145^T; 4, *Roseisalinus antarcticus* DSM 11466^T. All data are obtained from this study unless indicated

+ positive

− negative

W weakly positive

ND no data available

[a] Data from Yoon et al. [35]

[b] Data from Cho and Giovannoni [2]

[c] Data from Labrenz et al. [16]

Table 2 Cellular fatty acid profile (%) of strain G7^T and the type strains of related species

Fatty acid	1	2	3	4
Straight-chain				
$C_{16:0}$	20.3	5.3	11.8	14.5
$C_{17:0}$	tr	18.4	tr	–
$C_{18:0}$	1.1	1.1	1.0	1.6
Unsaturated				
$C_{16:1}$ $\omega 11c$	–	–	–	4.5
$C_{17:1}$ $\omega 8c$	–	1.7	tr	–
$C_{18:1}$ $\omega 7c$	51.6	43.6	66.8	66.3
11 methyl $C_{18:1}$ $\omega 7c$	4.8	3.8	2.1	6.5
$C_{19:0}$ cyclo $\omega 8c$	–	tr	8.8	–
Hydroxy				
$C_{10:0}$ 3OH	7.7	3.2	1.8	2.4
$C_{11:0}$ 3OH	–	1.4	tr	–
$C_{12:0}$ 3OH	–	–	1.8	–
$C_{12:1}$ 3OH	–	–	–	1.7
$C_{15:0}$ 2OH	–	7.8	–	–
$C_{16:0}$ 2OH	–	4.6	–	–
$C_{17:0}$ 2OH	–	2.0	–	–
Summed features[a]				
1	–	1.9	–	–
3	12.6	tr	1.2	–
7	tr	–	2.6	1.2

Strains: 1, G7^T; 2, *A. beolgyonensis* KCTC 32324^T; 3, *O. granulosus* KCTC 12145^T; 4, *R. antarcticus* DSM 11466^T. Fatty acids that represented <1.0% in all strains were not shown. All data are from this study (cellular fatty acids of *R. antarcticus* DSM 11466^T were extracted after incubation on MA at 20 °C for 10 days)

Summed feature 1 comprises iso-$C_{15:1}$ H and/or $C_{13:0}$ 3OH

Summed feature 3 comprises $C_{16:1}$ $\omega 7c$ and/or $C_{16:1}$ $\omega 6c$

Summed feature 7 comprises $C_{19:1}$ $\omega 6c$ and/or $\omega 7c$ and/or $C_{19:0}$ cyclo

tr, trace (<1.0%); –, not detected

[a] Summed features represent one or more fatty acids that cannot be separated by GLC with the MIDI system

analysis based on 16S rRNA gene sequence indicates that the novel taxon forms a distinct lineage belonging to the *Roseobacter* clade of the family *Rhodobacteraceae*. The type species is *Aestuarium zhoushanense*.

Description of *Aestuarium zhoushanense* sp. nov

Aestuarium zhoushanense (zhou.shan.en'se. N.L. neut. adj. *zhoushanense* referring to the Zhoushan city in China, from which the type strain was isolated).

Cells are gram-stain-negative, aerobic, non-motile and ovoid or short rod-shaped, approximately 0.6–0.8 μm in width and 1.0–2.5 μm in length without flagella. Accumulate PHB granules. Colonies are 0.5–1.0 mm in diameter, circular, slightly convex and greyish yellow with entire margins after incubation on MA at 28 °C for 3 days. Growth occurs at 4–40 °C (optimum, 28 °C), pH 6.0–9.0 (optimum, pH 7.5) and with 0–7% (w/v) NaCl (optimum, 1%). NaCl is not necessary for growth. Does not produce Bchl α. Positive results in tests for catalase and oxidase activities, hydrolysis of Tween 40, Tween 60, urea and gelatin. Negative for nitrate and nitrite reduction, indole production, and hydrolysis of Tween 20, Tween 80, starch, skim milk, L-tyrosine, hypoxanthine and xanthine. Acetate, D-galactose, D-fructose and D-glucose are utilized as sole carbon and energy sources, but succinic acid is not utilized. In API ZYM tests, production of alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, acid phosphatase, napthol-AS-BI-phosphohydrase, α-glucosidase and *β*-glucosidase are positive; production of valine arylamidase is weakly positive. In API 20NE tests, fermentation of glucose and hydrolysis of urea, esculin and gelatin are positive; weakly positive for *β*-galactosidase. All negative properties tested using API ZYM and API 20NE strips are presented in Supplementary Table 1.The predominant respiratory quinone is Q-10. Principal cellular fatty acids (>10%) are $C_{18:1}$ $\omega 7c$, $C_{16:0}$ and summed feature 3 ($C_{16:1}$ $\omega 7c$ and/or $C_{16:1}$ $\omega 6c$). The polar lipids comprise phosphatidylglycerol, phosphatidylethanolamine, phosphatidylcholine and four unidentified lipids. The G+C content of genomic DNA of the type strain is 56.7 mol%.

The type strain, $G7^T$ (=MCCC 1K03229^T = KCTC 52584^T), was isolated from a tidal flat sample collected from the coast of East Sea in Zhoushan, China. The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequence and WGS whole-genome project of strain $G7^T$ are KX890235 and CP021114-CP021117, respectively. The DPD TaxonNumber for strain $G7^T$ is TA00172.

Acknowledgements This work was supported by the National Natural Science Foundation of China (Grant no. 31470005) and the Talent Startup Project of the Research Development Fund of Zhejiang Agricultural and Forestry University (2014FR074).

Compliance with Ethical Standards

Conflict of interest Authors declare that there is no conflict of interest.

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

References

1. Chin CS, Alexander DH, Marks P, Klammer AA, Drake J, Heiner C, Clum A, Copeland A, Huddleston J, Eichler EE, Turner SW, Korlach J (2013) Nonhybrid, finished microbial genome assemblies from long-read SMRT sequencing data. Nat Methods 10:563–569
2. Cho JC, Giovannoni SJ (2004) *Oceanicola granulosus* gen. nov., sp. nov. and *Oceanicola batsensis* sp. nov., poly-*β*-hydroxybutyrate-producing marine bacteria in the order '*Rhodobacterales*'. Int J Syst Evol Microbiol 54:1129–1136
3. Clarke JD (2009) Cetyltrimethyl ammonium bromide (CTAB) DNA miniprep for plant DNA isolation. Cold Spring Harbor Protoc 2009:5177–5178
4. Dong X, Cai M (2001) Determinative manual for routine bacteriology. Scientific Press, Beijing
5. Fang MX, Zhang WW, Zhang YZ, Tan HQ, Zhang XQ, Wu M, Zhu XF (2012) *Brassicibacter mesophilus* gen. nov., sp nov., a strictly anaerobic bacterium isolated from food industry wastewater. Int J Syst Evol Microbiol 62:3018–3023
6. Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783–791
7. Fitch WM (1971) Toward defining the course of evolution: minimum change for a specific tree topology. Syst Zool 20:406–416
8. Garrity GM, Bell JA, Lilburn T (2005) Order III Rhodobacterales ord. nov. In: Brenner DJ, Krieg NR, Staley JT, Garrity GM (eds) Bergey's manual of systematic bacteriology, 2nd edn. Springer, New York, p 161
9. Garrity GM, Bell JA, Lilburn T (2006) Order III. *Rhodobacterales* ord. nov. In list of new names and new combinations previously effectively, but not validly, published, validation list no. 107. Int J Syst Evol Microbiol 56:1–6
10. Gordon RE, Mihm JM (1957) A comparative study of some strains received as nocardiae. J Bacteriol 73:15–27
11. Goris J, Konstantinidis KT, Klappenbach JA, Coenye T, Vandamme P, James MT (2007) DNA–DNA hybridization values and their relationship to whole-genome sequence similarities. Int J Syst Evol Microbiol 57:81–91
12. Kim OS, Cho YJ, Lee K, Yoon SH, Kim M, Na H, Park SC, Jeon YS, Lee JH, Yi H (2012) Introducing EzTaxon-e: a prokaryotic 16S rRNA gene sequence database with phylotypes that represent uncultured species. Int J Syst Evol Microbiol 62:716–721
13. Kim SB, Falconer C, Williams E, Goodfellow M (1998) *Streptomyces thermocarboxydovorans* sp. nov. and *Streptomyces thermocarboxydus* sp. nov., two moderately thermophilic carboxydotrophic species from soil. Int J Syst Bacteriol 48:59–68
14. Komagata K, Suzuki KI (1987) Lipid and cell-wall analysis in bacterial systematics. Methods Microbiol 19:161–207
15. Kuykendall LD, Roy MA, O'Neill JJ, Devine TE (1998) Fatty acids, antibiotic resistance, and deoxyribonucleic acid homology groups of *Bradyrhizobiurn japonicum*. Int J Syst Bacteriol 38:358–361

16. Labrenz M, Lawson PA, Tindall BJ, Collins MD, Hirsch P (2005) *Roseisalinus antarcticus* gen. nov., sp. nov., a novel aerobic bacteriochlorophyll *a*-producing α-proteobacterium isolated from hypersaline Ekho Lake, Antarctica. Int J Syst Evol Microbiol 55:41–47
17. Lee I, Kim YO, Park SC, Chun J (2016) OrthoANI: an improved algorithm and software for calculating average nucleotide identity. Int J Syst Evol Microbiol 66:1100–1103
18. Minnikin DE, Odonnell AG, Goodfellow M, Alderson G, Athalye M, Schaal A, Parlett JH (1984) An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. J Microbiol Methods 2:233–241
19. Pan J, Sun C, Zhang XQ, Huo YY, Zhu XF, Wu M (2014) *Paracoccus sediminis* sp. nov., isolated from Pacific Ocean marine sediment. Int J Syst Evol Microbiol 64:2512–2516
20. Piekarski T, Buchholz I, Drepper T, Schobert M, Wagner-Doebler I, Tielen P, Jahn D (2009) Genetic tools for the investigation of *Roseobacter* clade bacteria. BMC Microbiol 9:265
21. Pujalte MJ, Lucena T, Ruvira MA, Arahal DR, Macián MC (2014) The family *Rhodobacteraceae*. In: Rosenberg E, DeLong EF, Lory S, Stackebrandt E, Thompson F (eds) The Prokaryotes-Alphaproteobacteria and Betaproteobacteria, 4th edn. Springer, Berlin, pp 545–577
22. Qu L, Tian XX, Wang C, Sun C, Chen J (2017) *Salinovum rubellum* gen. nov., sp. nov., isolated from sediment of saltern. Int J Syst Evol Microbiol 67:1623–1628
23. Richter M, Rosselló-Móra R (2009) Shifting the genomic gold standard for the prokaryotic species definition. Proc Natl Acad Sci 106:19126–19131
24. Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406–425
25. Simon M, Scheuner C, Meier-Kolthoff JP, Brinkhoff T, Wagner-Döbler I, Ulbrich M, Klenk H-P, Schomburg D, Petersen J, Göker M (2017) Phylogenomics of *Rhodobacteraceae* reveals evolutionary adaptation to marine and non-marine habitats. ISME J 11(6):1–17
26. Su Y, Wang R, Sun C, Han S, Hu J, Wu D, Ma Z, Chen J, Wu M (2016) *Thalassobaculum fulvum* sp. nov., isolated from deep seawater. Int J Syst Evol Microbiol 66:2186–2191
27. Sun C, Pan J, Zhang XQ, Su Y, Wu M (2015) *Pseudoroseovarius zhejiangensis* gen. nov., sp nov., a novel alpha-proteobacterium isolated from the chemical wastewater, and reclassification of *Roseovarius crassostreae* as *Pseudoroseovarius crassostreae* comb. nov., *Roseovarius sediminilitoris* as *Pseudoroseovarius sediminilitoris* comb. nov. and *Roseovarius halocynthiae* as *Pseudoroseovarius halocynthiae* comb. nov. Antonie van Leeuwenhoek 108:291–299
28. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol 28:2731–2739
29. Tindall BJ, Sikorski J, Smibert RA, Krieg NR (2007) Phenotypic characterization and the principles of comparative systematics. In: Reddy TJBCA, Breznak JA, Marzluf G, Schmidt TM, Snyder LR (eds) In methods for general and molecular microbiology, 3rd edn. ASM Press, Washington DC, pp 330–393
30. Urbance JW, Bratina BJ, Stoddard SF, Schmidt TM (2001) Taxonomic characterization of *Ketogulonigenium vulgare* gen. nov., sp. nov. and *Ketogulonigenium robustum* sp. nov., which oxidize L-sorbose to 2-keto-L-gulonic acid. Int J Syst Evol Microbiol 51:1059–1070
31. Wu YH, Meng FX, Xu L, Zhang XQ, Wang CS, Oren A, Wu M, Xu XW (2013) *Roseivivax pacificus* sp. nov., isolated from deepsea sediment. Int J Syst Evol Microbiol 63:4574–4579
32. Xie CH, Yokota A (2005) *Pleomorphomonas oryzae* gen. nov., sp nov., a nitrogen-fixing bacterium isolated from paddy soil of *Oryza sativa*. Int J Syst Evol Microbiol 55:1233–1237
33. Xu XW, Wu YH, Zhou Z, Wang CS, Zhou YG, Zhang HB, Wang Y, Wu M (2007) *Halomonas saccharevitans* sp. nov., *Halomonas arcis* sp. nov. and *Halomonas subterranea* sp. nov., halophilic bacteria isolated from hypersaline environments of China. Int J Syst Evol Microbiol 57:1619–1624
34. Yang LQ, Tang L, Liu L, Salam N, Li WJ, Liu X, Jin G, Jiao N, Zhang Y (2017) *Rubricella aquisinus* gen. nov., sp. nov., a novel member of the family *Rhodobacteraceae*. Antonie van Leeuwenhoek 110:331–337
35. Yoon JH, Park S, Jung YT (2013) *Aestuariihabitans beolgyonensis* gen. nov., sp. nov., a novel alphaproteobacterium isolated from tidal flat sediment. Antonie van Leeuwenhoek 104:217–224
36. Yu XY, Tao TY, Fu GY, Su Y, Han SB, Wang RJ, Wu M, Sun C (2016) *Marinibaculum pumilum* gen. nov., sp. nov., isolated from seawater. Int J Syst Evol Microbiol 66:4844–4849
37. Zhang WY, Huo YY, Zhang XQ, Zhu XF, Wu M (2013) *Halolamina salifodinae* sp. nov. and *Halolamina salina* sp. nov., two extremely halophilic archaea isolated from a salt mine. Int J Syst Evol Microbiol 63:4380–4385
38. Zhang XQ, Wu YH, Zhou X, Zhang X, Xu XW, Wu M (2016) *Parvularcula flava* sp. nov., an alphaproteobacterium isolated from surface seawater of the South China Sea. Int J Syst Evol Microbiol 66:3498–3502