Terasakiispira papahanaumokuakeensis gen. nov., sp. nov., a gammaproteobacterium from Pearl and Hermes Atoll, Northwestern Hawaiian Islands

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A Gram-negative, helical bacterium designated PH27A^T was cultivated from an anchialine pool on Pearl and Hermes Atoll, Northwestern Hawaiian Islands. The obligately halophilic strain was motile by bipolar tufts of flagella and grew optimally at pH 7, and microaerobically or aerobically. Closest neighbours based on 16S rRNA gene nucleotide sequence identity are *Marinospirillum celere* v1c_Sn-red^T (93.31 %) and *M. alkaliphilum* Z4^T (92.10 %) in the family *Oceanospirillaceae*, class *Gammaproteobacteria*. PH27A^T is distinguished phenotypically from members of the genus *Marinospirillum* by its hydrolysis of gelatin, the absence of growth in media containing ≤1 % (w/v) NaCl and the ranges of temperature (12–40 °C) and pH (5–8) for growth. The major compound ubiquinone Q-9 distinguishes the quinone system of strain PH27A^T from those in members of the genus *Marinospirillum* and other members of the *Oceanospirillaceae*, in which the major quinone is Q-8. Major polar lipids in PH27A^T were phosphatidylethanolamine and phosphatidylglycerol, with moderate amounts of diphosphatidylglycerol and phosphatidylserine. Spermidine and cadaverine dominated the polyamine pattern; large proportions of cadaverine have not been reported in members of the genus *Marinospirillum*. Genotypic and chemotaxonomic data show that PH27A^T does not belong in the genus *Marinospirillum* or other genera of the family *Oceanospirillaceae* or the *Halomonadaceae*. We propose a new genus, *Terasakiispira* gen. nov., be created to accommodate *Terasakiispira papahanaumokuakeensis* gen. nov., sp. nov. as the type species, with PH27A^T (=ATCC BAA-995^T=DSM 16455^T=DSM 23961^T) as the type strain.

During a study of microbial diversity in lakes in the Hawaiian archipelago, bacteria were cultivated from a 0.3 m-deep anchialine pool on Southeast Island, Pearl and Hermes Atoll (27° 47′ 23″ N 175° 49′ 20″ W), Northwestern Hawaiian Islands, in October 2000 (Donachie *et al.*, 2004). Water salinity determined in the field with a hand-held refractometer was 26.9 ‰. One millilitre of water was transferred in the field to TA medium (Kurosawa *et al.*, 1998), pH 3.67, containing 2 % (w/v) NaCl, and incubated in darkness at 25 °C. After 1 month, 10 µl of the turbid medium was spread on marine agar 2216E (MA; Difco) and incubated at 30 °C. Strain PH27A^T grew after 48 h of incubation on MA as translucent amber, circular, raised and entire colonies of 2 mm diameter. The strain was purified through repeated transfers on MA. Culture purity was checked by consistency of colony characteristics, Gram staining and wet mounts. Working stocks were maintained on MA, and archived stocks were stored at −80 °C in marine broth 2216E (MB; Difco) containing 30 % (v/v) glycerol.

The tolerance of NaCl by PH27A^T was tested in 100 ml R-2 A broth (HiMedia Laboratories) in the range 0–15 % (w/v) NaCl, in 250 ml bottles in a shaker at 180 r.p.m., at 30 °C, over a period of 44 h. The inoculum for each was 100 µl of a 24 h culture in MB; growth was checked periodically over 72 h by measuring turbidity at 600 nm of each culture in a

†1954–2014.

Abbreviation: PHB, poly-β-hydroxybutyrate.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA and *gyrB* gene sequences of strain PH27A^T are AF513467 and KP743135, respectively. That of the *gyrB* gene nucleotide sequence of *M. celere* DSM 18438^T is KP743136.

Four supplementary figures are available with the online Supplementary Material.

Spectronic 20 Genesys spectrophotometer. The temperature range for growth was determined on MA incubated in the range 4–50 °C over 72 h, in 1 °C increments. The pH range for growth was determined in 30 ml modified TSSY (mTSSY) medium in 50 ml polypropylene tubes, on an orbital shaker at 30 °C, containing (per litre) Difco yeast extract (1 g), Difco Bacto-protone (2 g), Difco tryptone (17 g), Oxoid bacteriological peptone (3 g) and NaCl (30 g), in the range pH 4–9, with culture turbidity determined at 600 nm in a Beckman Coulter DU 700 Series UV/Vis spectrophotometer.

Cells from a 24 h, 30 °C culture on MA were observed for motility in sterile 2 % saline in a hanging drop under a ×100 objective with oil immersion. Flagella location was determined by negative staining and transmission electron microscopy (TEM) of cells from a 24 h, 30 °C shake-culture in mTSSY containing 3 % (w/v) NaCl. The presence of coccoid bodies was investigated by TEM and scanning electron microscopy of cells in the same medium shaken for 24 h and 7, 14 and 28 days at 30 °C.

Single colonies of PH27A^T on MA were tested for the presence of catalase and cytochrome oxidase *c* with 3 % (v/v) hydrogen peroxide (Sigma) and tetramethyl *p*-phenylenediamine (BBL), respectively. Intracellular poly-β-hydroxybutyrate (PHB) accumulation was assessed after Burdon (1946). Amylase activity was checked on starch medium (Difco) containing 3 % (w/v) NaCl by flooding the plates with iodine after 7 days of incubation at 30 °C (Donachie *et al.*, 2003). Constitutive enzyme activities were assessed in the API ZYM system. Responses in the eight conventional and 12 assimilation tests in API 20NE were also determined. Respiratory activity in the presence of sole carbon sources through reduction of tetrazolium chloride dye was tested in the Biolog GN system. All API ZYM, API 20NE and Biolog tests were inoculated with cells suspended in sterile 3 % (w/v) NaCl. The AUX medium in API 20NE was amended with 1 ml sterile 20 % (w/v) NaCl solution prior to suspending the cells. All API ZYM, API 20NE and Biolog tests were incubated at 30 °C for 24 h, at which point reactions were scored according to the manufacturers' instructions; controls in each were inoculated only with the cell-free diluent. Microaerophilic growth was investigated on MA in a candle jar at 30 °C (Gerhardt *et al.*, 1981), while anaerobic growth on MA was assessed in the BBL GasPak system with the BD GasPak EZ Anaerobe Container System sachet at 30 °C; the latter produces an atmosphere containing $<$1 % oxygen and $\geqslant$13 % CO_2 within 24 h.

Fatty acids in whole cells of PH27A^T were determined in the MIDI Sherlock Microbial Identification System (Sasser, 1997). Biomass for fatty acid analysis was grown on MA at 30 °C for 48 h. Biomass for analysis of polyamines, quinones and polar lipids was grown on 3.3× PYE (1 % peptone from casein, 1 % yeast extract, pH 7.2) supplemented with sea salts (3 %) used to prepare seawater for marine aquaria, and harvested late in the exponential growth phase (for polyamines) as recommended by Busse & Auling (1988), or in the stationary growth phase (for quinones and polar lipids). Polyamines were extracted after Busse & Auling (1988) and analysed as described by Busse *et al.* (1997). Quinones and polar lipids were extracted and analysed according to Tindall (1990a, b) and Altenburger *et al.* (1996). HPLC analyses were carried out using the equipment described by Stolz *et al.* (2007).

Fragments of the 16S rRNA and *gyrB* genes in PH27A^T were amplified by colony PCR with *Pfu* DNA polymerase and primers 27F and 1492R, and UP1 and UP2r, respectively (Lane, 1991; Yamamoto & Harayama, 1995; Kuo *et al.*, 2013). *Marinospirillum celere* DSM 18438^T was obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ); a fragment of the *gyrB* gene of this culture was amplified as described above. PCR products purified in the MoBio PCR Purification kit were sequenced at a core facility at the University of Hawai'i. Consensus, manually edited 16S rRNA gene and *gyrB* sequences were compared with nucleotide sequences in public databases through BLASTN searches (Altschul *et al.*, 1997); the 16S rRNA gene sequence was also compared to those in the EzTaxon database (Kim *et al.*, 2012). The 16S rRNA gene and *gyrB* sequences for PH27A^T were aligned in MAFFT with those of type strains of species of the genus *Marinospirillum* and other genera (Katoh *et al.*, 2002), trimmed with trimAl (Capella-Gutiérrez *et al.*, 2009) and visualized in FigTree (http://tree.bio.ed.ac.uk/software/figtree/). Phylogenetic relationships between the strains were inferred with the RAxML tool using the maximum-likelihood method (Stamatakis, 2006). The G+C content of genomic DNA in PH27A^T was determined after Mesbah *et al.* (1989) by the Identification Service of the Leibniz Institute DSMZ.

PH27A^T formed colonies overnight on MA incubated microaerophilically or aerobically at 30 °C. The strain did not grow anaerobically. Colonies were translucent beige to amber, flat to slightly raised, entire, smooth, shiny and circular, 2 mm in diameter. Cells swarmed over the surface of MA. Gram-stained cells appeared as curved rods, occasionally as slight helices, usually singly, and stained Gram-negative. Unstained cells in wet mounts were helical, with 4–5 turns per cell, and were motile by bipolar tufts of flagella (Fig. 1). Coccoid bodies of the type described in members of the genus *Marinospirillum* were not observed by light microscopy, TEM or scanning electron microscopy of cells of strain PH27A^T grown in mTSSY for 28 days (Satomi *et al.*, 1998, 2004; Zhang *et al.*, 2002), although one or both poles of some cells showed some expansion (Fig. 1). Rare, thin-walled, round bodies of 500 nm diameter did not appear to be budding from vegetative cells, nor was there any indication that they were derived from the vegetative cell being 'absorbed', as reported for *M. celere* (Namsaraev *et al.*, 2009) (Fig. 2). Larger bodies of the type reported in *Marinospirillum megaterium* (Satomi *et al.*, 1998) were also not observed.

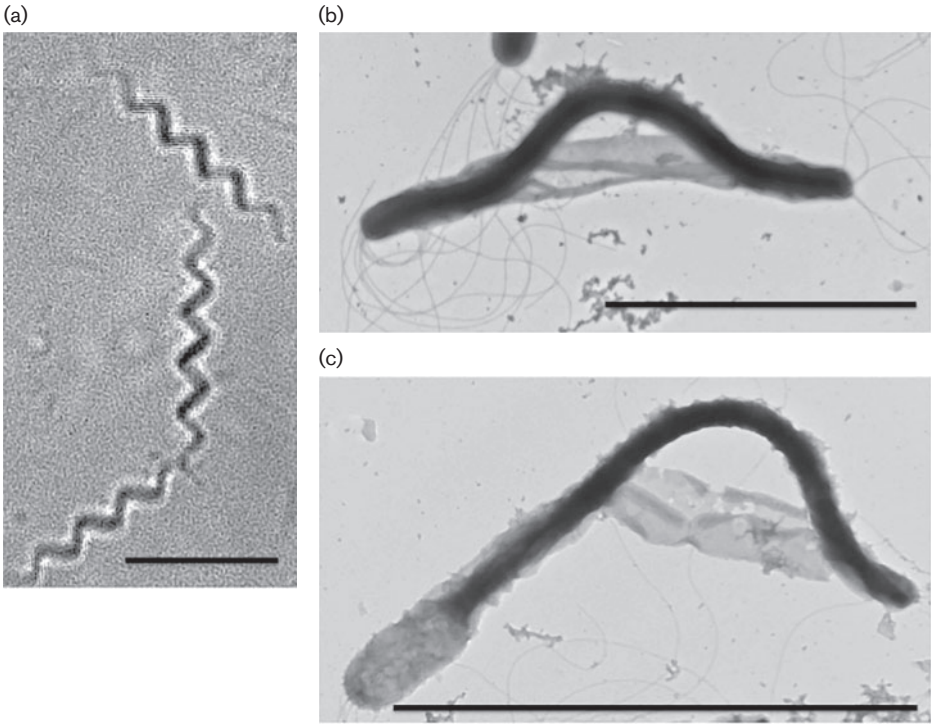

Fig. 1. Cells of PH27A^T are helical (a) (light micrograph) with bipolar tufts of flagella (b) (negative stain) and occasional enlargement of the pole(s) in mTSSY broth cultures >28 days old (c) (negative stain). Bars, 5 μm.

PH27A^T was catalase-positive and oxidase-positive. Growth did not occur in R-2 A broth containing ≤1 % (w/v) NaCl, but the strain did grow in this medium in the presence of 2–15 % (w/v) NaCl; by 27 h of incubation, comparable turbidities were attained in all R2-A broth cultures with 2–10 % (w/v) NaCl, inclusive (Fig. S1, available in the online Supplementary Material). Growth on MA occurred between 12 and 40 °C. There was negligible growth in mTSSY at pH 4 and none at pH 9, both after 5 days. The strain grew well at pH 5–8, with highest turbidity attained at pH 7 (Fig. S2). PHB accumulated in the cells. Starch was not hydrolysed. Responses in API ZYM and Biolog GN are given in the species description. In API 20NE, the strain reduced nitrate to nitrite, hydrolysed gelatin and assimilated D-glucose, L-arabinose, capric acid, malic acid and trisodium citrate.

Fatty acids in whole cells of PH27A^T comprised hexadecanoic acid (28.08 %), *cis*-11-octadecenoic acid (26.89 %), *cis*-9-hexadecenoic acid (25.31 %), 3-hydroxydodecanoic acid (10.74 %), dodecanoic acid (3.24 %), tetradecanoic acid (2.57 %), 3-hydroxydecanoic acid (2.28 %) and 2-hydroxydodecanoic acid (0.88 %); 99.99 % of the fatty acids were named. The strain is similar in terms of fatty acid composition to those members of the genus *Marinospirillum* for which such data have been reported, i.e. major components are hexadecanoic, *cis*-11-octadecenoic and *cis*-9-hexadecenoic acids (Satomi *et al.*, 2004), although it contained substantially less *cis*-11-octadecenoic acid than the 48.52 % reported in *M. celere* v1c_Sn-red^T (Namsaraev *et al.*, 2009). Such profiles distinguish species in a genus, but they do not differentiate genera of the family *Oceanospirillaceae* (Sakane & Yokota, 1994; Arahal *et al.*, 2007a; Namsaraev *et al.*, 2009).

The quinone system of strain PH27A^T comprised ubiquinones Q-9 (86.4 %) and Q-8 (13.6 %), clearly distinguishing the strain from members of the genus *Marinospirillum*, in which the major ubiquinone is Q-8 (Satomi *et al.*, 1998). However, the quinone systems of members of the genera *Halomonas* and *Cobetia* are also dominated by ubiquinone Q-9 (Franzmann & Tindall, 1990; Arahal *et al.*, 2007b; Guzmán *et al.*, 2010; Poli *et al.*, 2007; Yumoto *et al.*, 2004). Major polar lipids were phosphatidylethanolamine and phosphatidylglycerol, molecules also reported in members of the genera *Halomonas* and *Cobetia* (Franzmann & Tindall, 1990; Jiang *et al.*, 2014; Wang *et al.*, 2012; Romanenko *et al.*, 2013). Diphosphatidylglycerol and phosphatidylserine were present in moderate amounts, and minor amounts of four lipids lacking a phosphate residue, an amino group or a sugar moiety were also detected (Fig. S3). The polyamine pattern in PH27A^T contained the predominant compounds spermidine [7.7 μmol (g dry wt)$^{-1}$] and cadaverine [5.2 μmol (g dry wt)$^{-1}$], and minor to trace amounts of putrescine [0.7 μmol (g dry wt)$^{-1}$], 1,3-diaminopropane [0.2 μmol (g dry wt)$^{-1}$] and spermine

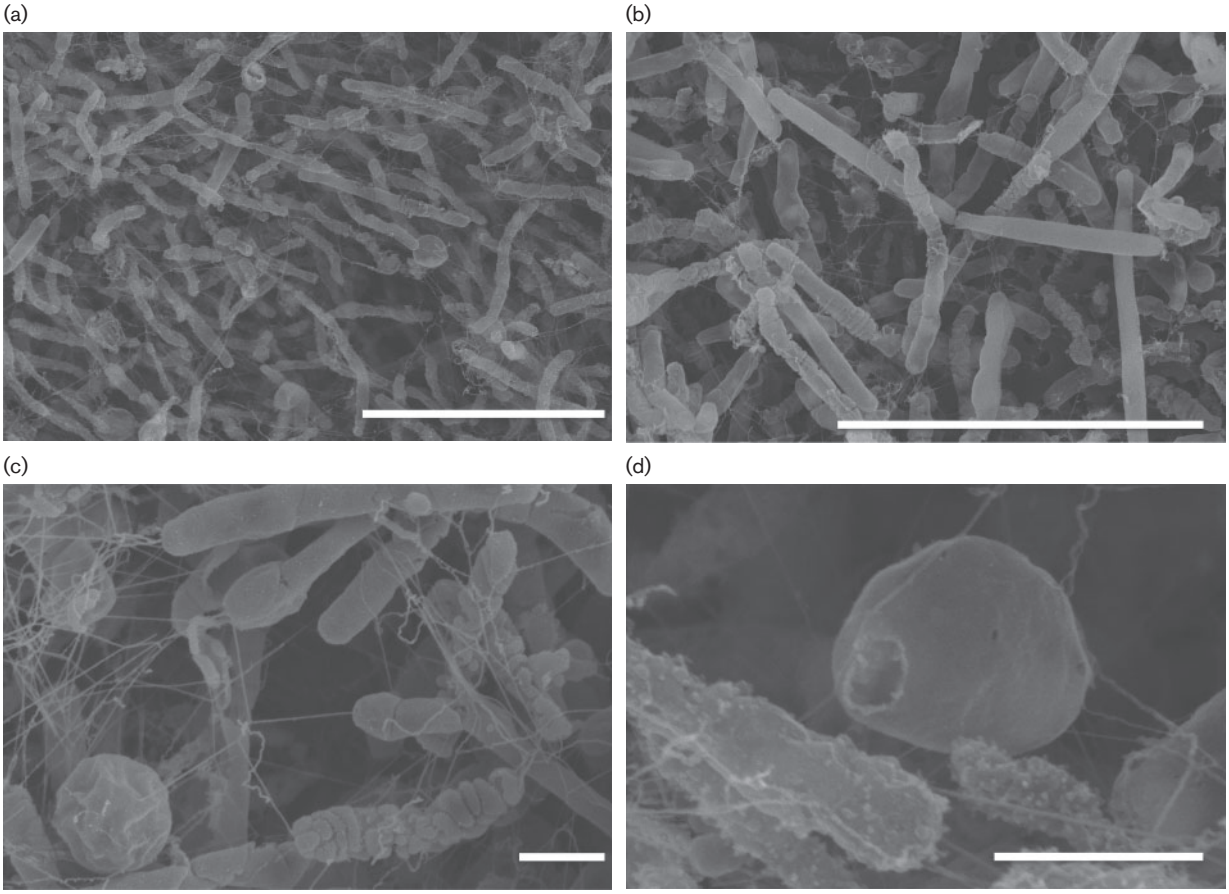

Fig. 2. Cells of a 28 day culture of PH27A^T in mTSSY broth. (a, b) Note absence of 2.5 μm-diameter coccoid bodies widely reported in members of the genus *Marinospirillum*. (c, d) Occasional round bodies of 500 nm diameter. Bars, 5 μm (a, b) and 500 nm (c, d).

[<0.1 μmol (g dry wt)$^{-1}$]. A large proportion of cadaverine in the polyamine pattern has not been reported in members of the genera *Marinospirillum* and *Halomonas* (Hamana *et al.*, 2000, 2006).

Comparisons of the 16S rRNA gene nucleotide sequence of strain PH27A^T to those of type strains in public databases placed the strain in the class *Gammaproteobacteria*. The closest such neighbours in BLAST searches were *Marinospirillum alkaliphilum* Z4^T (1317/1430, 92.10 %) and *M. minutulum* NBRC 15450^T (1319/1436, 91.85 %) (Altschul *et al.*, 1997). The closest neighbour in the EzTaxon database was *M. celere* v1c_Sn-red^T (93.78 %) (Kim *et al.*, 2012); a BLAST alignment of the 16S rRNA gene sequences of PH27A^T and *M. celere* v1c_Sn-red^T showed that they share 93.31 % identity. Members of the genus *Marinospirillum* share >95 % 16S rRNA gene sequence identity with at least one other member of the genus (Table 1); PH27A^T does not. This is pertinent, since bacteria sharing less than 95 or even 96 % nucleotide sequence identity in their 16S rRNA genes are generally assigned to different genera (Ludwig *et al.*, 1998: Everett *et al.*, 1999; Schloss & Handelsman, 2004; León *et al.*, 2014). Such assignments cannot rest solely upon nucleotide sequences, however, as Stackebrandt (2006) notes: '[A new genus should] ... be described when a strain ... branches outside the radiation of a validly described genus and the isolated phylogenetic position is accompanied by distinct phenotypic properties not found among the neighboring genera'. With respect to the nucleotide sequence of the 16S rRNA gene, PH27A^T occupies the basal position in a monophyletic group with only the members of the genus *Marinospirillum* (Fig. 3). The tree based on *gyrB* sequences also shows PH27A^T in the basal position in a monophyletic group with all extant type strains of species of the genus *Marinospirillum*, plus *Oceanospirillum japonicum* ATCC 19191^T (Fig. S4). The strain is distinguished phenotypically from members of the genus *Marinospirillum* and other members of the family *Oceanospirillaceae* with helical cells and bipolar tufts of flagella through its hydrolysis of gelatin, a trait absent from *Marinospirillum* but reported as negative, weak or differing among strains of the genera *Oceanospirillum* and *Pseudospirillum* (Satomi *et al.*, 2002;

Table 1. 16S rRNA gene nucleotide sequence identities among members of the genus *Marinospirillum* and PH27A^T determined in BLAST

Identities >95 % are highlighted in bold.

Strain	1	2	3	4	5	6
1. *M. alkaliphilum* Z4^T	(100)					
2. *M. celere* v1c_Sn-red^T	**95.19**	(100)				
3. *M. insulare* K^T	93.59	92.61	(100)			
4. *M. megaterium* H7^T	93.41	92.56	**98.39**	(100)		
5. *M. minutulum* ATCC 19193^T	94.51	93.25	**98.54**	**98.13**	(100)	
6. PH27A^T	92.10	93.31	91.07	90.80	91.85	(100)

González & Whitman, 2006). PH27A^T also grows microaerophilically and aerobically, but not in media containing ≤1 % NaCl (w/v), or below 12 °C; it is also moderately acidophilic to acidotolerant (Table 2). Of note, too, is that coccoid body production is reported in all members of the genus *Marinospirillum*, but none were observed in PH27A^T through different incubation periods. Coccoid body production is also a defining characteristic of the core group of *Oceanospirillum*, and in others no longer part of the core group, but is not characteristic of all genera of the family *Oceanospirillaceae* (Satomi *et al.*, 2002; González & Whitman, 2006).

One might posit that PH27A^T belongs in the genus *Halomonas* or *Cobetia*, given the shared abundance of ubiquinone Q-9. However, the chemotaxonomic value of such data is limited at the species level because the highly conserved nature of the character means that identical quinone systems can occur in closely related genera (Arahal & Ventosa, 2006). Furthermore, the polyamine pattern with the major compounds spermidine and cadaverine clearly distinguishes PH27A^T from members of the genera *Cobetia* and *Halomonas* (Auling *et al.*, 1991; Hamana, 1997; Hamana *et al.*, 2006), which were reported to contain only spermidine as the major polyamine. Moreover,

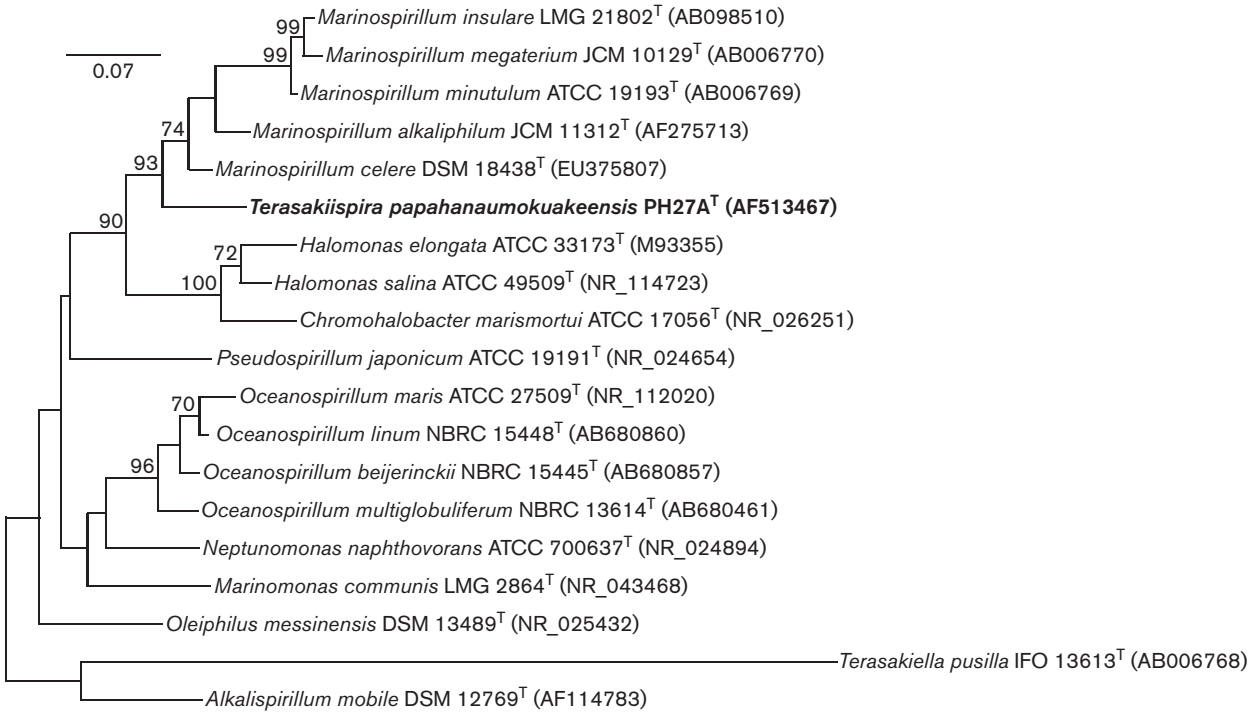

Fig. 3. Phylogenetic tree showing the relationship of PH27A^T with type strains of species in related genera in the *Gammaproteobacteria* on the basis of 1461 nt of the 16S rRNA gene. See text for alignment details. The *Gammaproteobacteria Alkalispirillum mobile* DSM 12769^T and *Terasakiella pusilla* ATCC 33338^T were used as the outgroup. Bootstrap values for 1000 replicates are shown as percentages. Bar, 0.07 nt substitutions per site.

Table 2. Phenotypic and genotypic data for PH27A^T and related type strains

Strains: 1, PH27A^T; 2, *M. celere* v1c_Sn-red^T; 3, *M. alkaliphilum* Z4^T; 4, *M. minutulum* ATCC 19193^T; 5, *M. insulare* K^T; 6, *M. megaterium* H7^T; 7, *O. linum* ATCC 11336^T; 8, *O. maris* ATCC 27509^T; 9, *O. beijerinckii* ATCC 12754^T; 10, *O. multiglobuliferum* IFO 13614^T; 11, *H. pacifica* ATCC 27122^T; 12, *C. amphilecti* 46-2^T. Characteristics of value in differentiating strain PH27A^T from members of the genus *Marinospirillum* and the core group of *Oceanospirillum* are their major ubiquinone, with Q-9 in PH27A^T and Q-8 in the others, the hydrolysis of gelatin and absence of coccoid body formation in PH27A^T, and ranges of salinity, pH and temperature for growth. All strains accumulate PHB. +, Growth/positive/present; −, no growth/negative/absent; W, weak; V, variable; ND, no data available/not reported. Data shown are from this work, Hylemon *et al.* (1973), Terasaki (1979), Baumann *et al.* (1983), Dobson & Franzmann (1996), Satomi *et al.* (1998, 2004), Zhang *et al.* (2002), Mata *et al.* (2002), Namsaraev *et al.* (2009) and Romanenko *et al.* (2013).

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12
Cell shape*	H	H	H	H	H	H	H	H	H	H	R	R
Cell length (μm)	5–10	1.4–15	2.7–4.0	2.0–2.8	2.5–7.5	5–15	7.0–30.0	7.0–15.0	7.0–15.5	2.0–10.0	1.5–3.0	1.1–1.3
Cell diameter (μm)	0.3	0.2–0.4	0.2–0.3	0.3–0.4	0.1–0.2	0.8–1.2	0.4–0.6	0.8–0.9	0.7–1.0	0.5–0.9	0.8–1.1	0.8–0.9
Flagella†	BT	BT	BT	BT	BT	BT	BT	BT	BT	BT	P	SP, ML
Coccoid bodies	−	+	+	+	+	+	+	+	+	+	ND	ND
Oxygen requirement‡	Mi/Ae	Ae	Ae	Ae	Ae	Mi	Ae	Ae	Ae	Ae	Ae	Ae
Catalase	+	+	+	+	+	(or W	W	+	W	+	+	+
Gelatinase	+	−	−	−	−	−	−	−	−	−	−	−
Reduction of nitrate	+	−	+	+	+	−	−	−	−	−	−	−
Urease	−	−	+	−	−	−	−	−	−	−	+	−
Lipase (Tween 80)	+	−	−	+	V	ND	ND	ND	ND	ND	+	−
(Alkaline) phosphatase	W	−	ND	−	+	−	+	−	+	+	+	+
Growth temperature (°C)	12–40	13–55	8–49	4–30	4–37	4–25	11–38	2–33	7–41	6–37	4–45	4–42
NaCl concentration for growth (%)	2–15	0.5–12.0	0.2–5.0	0.2–10.0	0.5–10.0	0.9–9.0	0.5–9.75	0.5–8.0	0.5–9.75	0.5–4.0	0–20	0–20
pH range for growth	5–8	8–10.5	7.0–11.0	7.0–10.5	6.5–10.0	7.5–9.0	ND	ND	ND	5.5–9.0	5–10	4.5–10.5
pH optimum	7	9.5	9.5	9.0	8.0	8.0	ND	ND	ND	ND	ND	6.5–8.5
Major ubiquinone	Q-9	Q-8	Q-8	Q-8	Q-8	Q-8	Q-8	Q-8	Q-8	Q-8	Q-9	ND
DNA G+C content (mol%)	52.4	52.3	46.8	42.5	42.1	44.4	48	46	47	46.1	67–68	63.4

*H, Helical; R, rod.

†BT, Bipolar tufts; ML, multiple, lateral; P, peritrichous; SP, single polar.

‡Ae, Aerobic; Mi, microaerophilic.

genotypic data here distinguish PH27A^T from members of the genera *Halomonas* and *Cobetia*, with the nearest species in each on the basis of 16S rRNA gene sequences being *Halomonas pacifica* NBRC 102220^T (91.40 %) and *Cobetia amphilecti* 46-2^T (90.97 %) (see Stackebrandt & Ebers, 2006). Furthermore, the genomic DNA G+C content in PH27A^T is 52.4 mol%, or 5.6 to ~10 mol% higher than in all members of the genus *Marinospirillum* except *M. celere* v1c_Sn-red^T, in which it is almost identical (52.3 mol%) (Namsaraev *et al.*, 2009); these values are at the bottom of the range (52–68 mol%) reported for the genus *Halomonas* (Arahal *et al.*, 2007b). In contrast, the genomic DNA G+C content of type strains in the core group of the genus *Oceanospirillum* is 46–48 mol% (Hylemon *et al.*, 1973; Terasaki, 1979).

On the basis of phenotypic, chemotaxonomic and phylogenetic characteristics, strain PH27A^T does not belong in the genus *Marinospirillum* or *Oceanospirillum* or other extant genera. We therefore propose that the strain represents the type species of a new genus, *Terasakiispira* gen. nov., and that PH27A^T is the type strain of the type species of this genus, with the name *Terasakiispira papahanaumokuakeensis* gen. nov., sp. nov.

Description of *Terasakiispira* gen. nov.

Terasakiispira (Te.ra.sa.ki.i.spi′ra. N.L. fem. n. *Terasakiispira* named to honour Yasuke Terasaki, a Japanese microbiologist, for his contributions to the study of spiral-shaped bacteria; L. fem. n. *spira* a spiral; N.L. fem. n. *Terasakiispira* Terasaki's spiral).

Cells are helical, stain Gram-negative and are motile by bipolar tufts of flagella. Aerobic and obligately halophilic. Fatty acids include hexadecanoic acid, *cis*-11-octadecenoic acid and *cis*-9-hexadecenoic acid. Ubiquinone Q-9 is the predominant quinone. The polyamine pattern contains spermidine and cadaverine. Comparative analysis of the nucleotide sequence of the 16S rRNA and *gyrB* gene nucleotide sequences indicates that the genus belongs in the class *Gammaproteobacteria*. The type species is *Terasakiispira papahanaumokuakeensis*.

Description of *Terasakiispira papahanaumokuakeensis* sp. nov.

Terasakiispira papahanaumokuakeensis (pa.pa.ha.nau.mo′ku′a.ke.en′sis. N.L. fem. adj. *papahanaumokuakeensis* derived from Papahānaumokuākea, the Marine National Monument within which the type strain was isolated).

Exhibits the following properties in addition to those given in the genus description. Circular, translucent amber to beige colonies, flat to slightly raised, entire, smooth, shiny, 2 mm in diameter after 24 h at 30 °C on MA. Cells are 0.3 μm wide by 5–10 μm long. Coccoid bodies are not formed during 28 days of incubation in mTSSY. Catalase- and oxidase-positive. Does not grow in media containing ≤1 % (w/v) NaCl. Grows in R-2 A broth containing 2–15 % (w/v) NaCl. Grows aerobically on MA between 12 and 40 °C. Grows microaerophilically on MA. Nitrate is reduced to nitrite. Tests positive in Biolog GN for utilization of Tweens 40 and 80, L-fucose, pyruvic acid methyl ester, *cis*-aconitic acid, citric acid, D-gluconic acid, β-hydroxybutyric acid, *p*-hydroxyphenylacetic acid, DL-lactic acid, malonic acid, propionic acid, D-saccharic acid, succinic acid, bromosuccinic acid, L-alanine, L-asparagine, L-aspartic acid, L-glutamic acid, L-histidine, hydroxy-L-proline, L-proline, L-pyroglutamic acid and γ-aminobutyric acid. Esterase (C_4), esterase lipase (C_8) and naphthol-AS-BI-phosphohydrolase are expressed in API ZYM; results for alkaline phosphatase and leucine arylamidase activities are weak to negative. Gelatin is hydrolysed. The type strain shares the chemotaxonomic characteristics listed in the genus description. In addition to Q-9, minor amounts of Q-8 are present. Major polar lipids are phosphatidylethanolamine and phosphatidylglycerol; moderate amounts of diphosphatidylglycerol and phosphatidylserine are present.

The type strain, PH27A^T (=ATCC BAA-995^T=DSM 16455^T=DSM 23961^T), was isolated from an anchialine pond on Southeast Island, Pearl and Hermes Atoll, Northwestern Hawaiian Islands. The genomic DNA G+C content of the type strain is 52.4 mol%.

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