# *Thalassobaculum fulvum* sp. nov., isolated from deep seawater

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A novel Gram-stain-negative, rod-shaped (1.0–1.2×2.0–8.0 μm), non-motile without flagella strain, designated HSF7$^T$, was isolated from deep seawater. Strain HSF7$^T$ was able to grow at 20–40 °C (optimum 35 °C), pH 5.5–9.0 (optimum pH 6.5) and 0–10 % (w/v) NaCl (optimum 2 %). The G+C content of the genomic DNA was 69 mol%. Bacteriochlorophyll *a* and poly-β-hydroxybutyrate (PHB) granules were not found. The major fatty acids were $C_{18:1}\omega7c$ (69.3 %), $C_{16:0}$ (9.1 %) and $C_{19:0}$ cyclo $\omega8c$ (6.6 %). The polar lipids were phosphatidylglycerol, three unknown aminophospholipids, an unknown phospholipid, an unknown aminolipid and two unknown lipids. The only isoprenoid quinone was Q-10. 16S rRNA gene sequence analysis revealed that strain HSF7$^T$ was most closely related to *Thalassobaculum salexigens* DSM 19539$^T$, *Thalassobaculum litoreum* DSM 18839$^T$, *Nisaeadenitrificans* DSM 18348$^T$ and *Oceanibaculum indicum* MCCC 1A02083$^T$ with pairwise sequence similarities of 95.56 %, 95.21 %, 93.64 % and 92.65 %, respectively. On the basis of genotypic, phenotypic, phylogenetic and chemotaxonomic characteristics, strain HSF7$^T$ represents a novel species of the genus *Thalassobaculum*, or which the name *Thalassobaculum fulvum* sp. nov. is proposed. The type strain is HSF7$^T$(=KCTC 42651$^T$=MCCC 1K01158$^T$).

The genus *Thalassobaculum*, belonging to family *Rhodospirillaceae*, was first proposed by Zhang *et al.* (2008). At the time of writing, the genus *Thalassobaculum* comprises two species with validly published names according to LPSN (Euzeby, 1997; http://www.bacterio.net/index.html): *Thalassobaculum litoreum* (Zhang *et al.,* 2008)and *Thalassobaculum salexigens* (Urios *et al.,* 2010). Both these species were isolated from coastal seawater. Here we present a novel strain named HSF7$^T$, which was isolated from deep seawater in the South China Sea. Based on phylogenetic, genomic, chemotaxonomic and phenotypic characteristics, we propose that this strain represents a novel species of the genus *Thalassobaculum*.

A bottle of seawater was collected in October 2011 from the South China Sea (19°22′ N 115° 38′ E) at a depth of 2.5 km. The sample was stored at 4 °C in the lab until it was used. A standard dilution-plating method (Williama & Davies, 1965) on modified marine agar 2216 (MA) (Pan *et al.*, 2014) at 28 °C was used for isolation. Based on colony morphology (Kumar *et al.*, 2012), an opaque, regular-edged and yellowish-brown colony was picked and named strain HSF7$^T$. After purification, the strain was preserved as suspensions with 30 % (v/v) glycerol at −80 °C, and was also freeze-dried for long-term preservation.

The Gram-staining reaction was carried out according to Claus (1992). Cell morphology and the presence of flagella were observed by transmission electron microscopy (JEM-1230; JEOL) when cells were in the exponential phase of growth on the MA plate at 30 °C. Gliding motility was performed by the hanging-drop method (Suzuki, 2001). Poly-β-hydroxybutyrate (PHB) granules were assessed by staining with Sudan Black (Mesquita *et al.*, 2015). Bacteriochlorophyll *a* was exacted according to Zhang *et al.* (2008) and the absorption spectrum was measured at 300–800 nm (Kumar *et al.*, 2012) with ethanol as a blank. The temperature range for growth was determined in marine broth 2216 (MB; Difco) at 4, 10, 15, 20, 25, 28, 30, 35, 40, 45, 50 and 55 °C. The pH range for growth in MB was measured from pH 4.5 to pH 10.0 with an interval of 0.5 units, using

Abbreviations: PHB, poly-*B*-hydroxybutyrate.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain HSF7$^T$ is KP976094.

Four supplementary figures and a supplementary table are available with the online Supplementary Material.

40 mM of the following buffers to maintain pH: MES (for pH 4.5–6.0), PIPES (for pH 6.5–7.5), Tricine (for pH 8.0–8.5) and CAPSO (for pH 9.0–10.0). Tolerance of NaCl was at 0, 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, 18 and 20 % (w/v) NaCl. Growth in the modified MB with 2 % (w/v) NaCl as the sole salt was also determined.

Catalase and oxidase activity was tested by the method described by Wu *et al.* (2010) and Kovacs (1956), respectively. $H_2S$ production, indole production, methyl red (MR) and Voges–Proskauer (VP) tests, and hydrolysis of casein and gelatin were assayed according to Zhang *et al.* (2013). Tweens 20, 40, 60 and 80 were examined as described by Sun *et al.* (2015). Hydrolysis of tyrosine, CM-cellulose, xanthine and hypoxanthine were separately determined on MA with 0.5 % (w/v) tyrosine, 1 % (w/v) CM-cellulose, 0.4 % (w/v) xanthine and 0.4 % (w/v) hypoxanthine, respectively. Other biochemical properties and enzyme activities were tested using API ZYM and API 20NE strips (BioMérieux) following the manufacturer's instructions.

Different concentrations of yeast extract and trypticase peptone were tested to confirm the basal medium for the experiments on utilization of carbon sources, and yeast extract was found to be essential for growth, but not trypticase peptone. Basal medium was subsequently prepared for the substrate utilization experiments and comprised modified MB with 0.01 % (w/v) yeast extract and removal of trypticase peptone. The utilization of carbon sources was tested in the basal medium with 0.4 % carbon source, such as sugar, alcohol or organic acid. In the test, basal medium with substrates but without inoculation was a blank control and growth in the basal medium with inoculation but without substrates was the negative control. Growth was measured as absorbance at 600 nm. When the $OD_{600}$ measured in the test was equal to or less than the negative control, it was considered negative, and the thresholds of being weakly positive and positive were two-fold and more than two-fold of the negative control, respectively.

Anaerobic growth was tested in the modified MB with sodium thiosulfate (20 mM), sodium sulfite (5 mM), sodium sulfate (20 mM), sodium nitrite (5 mM), sodium nitrate (20 mM) and L-arginine (5 g $l^{-1}$) as electron acceptors, cysteine (1 g $l^{-1}$) as reductant and resazurin (1 mg $l^{-1}$) as oxygen indicator. Hungate tubes filled with $N_2$ were used for the test. For antimicrobial susceptibility tests, plates with exponential phase cells spread and incubated on MA at 30 °C for 6 h were used . The pretreated plate with antibiotics discs added was then incubated at 30 °C for 3 days. The antibiotic discs contained the following (μg per disc unless otherwise stated): ampicillin (10), gentamicin (10), erythromycin (15), penicillin (10 IU), polymyxin B (30 IU), streptomycin (10), chloramphenicol (30), nalidixic acid (30), furazolidone (30), co-trimoxazole (1.25), ciprofloxacin (5), ofloxacin (5), minocycline (30), piperacillin (100), ceftriaxone (30), doxycycline (30), cefoperazone (75), norfloxacin (10), cefalexin (30), cefazolin (30), neomycin (30), carbenicillin (100), amoxicillin (10), cefotaxime (30), cefalotin (30), cefalotin (30), cefradine (30), kanamycin (30) and amikacin (30).

Genomic DNA of the strain HSF7$^T$ was extracted by a Quick Bacteria Genomic DNA Extraction kit (DongSheng Biotech). The 16S rRNA gene was amplified by PCR using universal primer pair 27F (5′-GAGAGTTTGATCMTGGCTCAG-3′) and 1492R (5′-TACGGYTACCTTGTTACGAC-3′). The purified PCR products were cloned into the vector pMD19-T (TaKaRa) and then sequenced. Multiple sequences alignment was performed with the CLUSTAL X program of the MEGA5 software package (Tamura *et al.*, 2011). Phylogenetic trees were reconstructed using the following three methods: neighbour-joining (Saitou & Nei, 1987), maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Fitch, 1987), within the MEGA5 package. Bootstrap analysis was based on 1000 replications. The G+C content of the genomic DNA of strain HSF7$^T$ was determined by HPLC according to the method of Mesbah *et al.* (1989).

After incubation in MB at 30 °C for 2 days, cells in exponential phase were freeze-dried and used for fatty acid methyl esters (FAMEs), polar lipid and isoprenoid quinone analyses. FAMEs were extracted as described by Kuykendall *et al.* (1998) and analysed by the Sherlock Microbial Identification System (MIDI). Polar lipids were extracted using 80 ml chloroform/methanol/water (1 : 2 : 1, by vol,), separated by two-dimensional TLC on silica gel 60 $F_{254}$ plates (Merck) and then analysed as described by Xu *et al.* (2011). Isoprenoid quinones were analysed as described by Minnikin *et al.* (1984).

Cells of strain HSF7$^T$ were Gram-stain-negative, rod-shaped, non-motile without flagella and strictly aerobic (Fig. S1, available in the online Supplementary Material). Colonies were sticky after strain HSF7$^T$ grew on MA at 30 °C for 4 days. Strain HSF7$^T$ grew at 20–40 °C (optimum 35 °C), pH 5.5–9.0 (optimum pH 6.5) and with 0–10 % NaCl (optimum 2 %). Strain HSF7$^T$ was susceptible to ampicillin (10 μg), penicillin G (10 IU), streptomycin (10 μg), chloramphenicol (30 μg), ciprofloxacin (5 μg), ofloxacin (5 μg), ceftriaxone (30 μg), cefoperazone (75 μg), norfloxacin (10 μg), clindamycin (2 μg), oxacillin (1 μg), medemycin (30 μg), cefalexin (30 μg), cefazolin (30 μg), carbenicillin (100 μg), amoxicillin (10 μg), cefotaxime (30 μg), cefalotin (30 μg) and cefalotin (30 μg), but resistant to gentamicin (10 μg), erythromycin (15 μg), polymyxin B (30 IU), nalidixic acid (30 μg), furazolidone (30 μg), co-trimoxazole (1.25 μg), minocycline (30 μg), piperacillin (100 μg), doxycycline (30 μg), clindamycin(2 μg), oxacillin (1 μg), medemycin (30 μg), cefazolin (30 μg), neomycin (30 μg), cefradine (30μg), kanamycin (30 μg) and amikacin (30 μg). Detailed physiological and biochemical properties are displayed in Table 1 and the species description.

On the basis of 16S rRNA gene sequence similarity, strain HSF7$^T$ was affiliated with the family *Rhodospirillaceae* and shared high sequence similarities with genera

**Table 1.** Comparison of the phenotypic characteristics of strain HSF7$^T$ and related reference strains

Strains: 1, HSF7$^T$; 2, *T. litoreum* DSM 18839$^T$; 3, *T. salexigens* DSM 19539$^T$ (Urios *et al.,* 2010); 4, *N. denitrificans* DSM 18348$^T$; 5, *O. indicum* MCCC 1A02083$^T$. All data from this study except where otherwise indicated. All strains were positive for oxidase and catalase. All strains, except *T. salexigens* DSM 19539$^T$ (no data available), were negative for hydrolysis of casein, CM-cellulose, xanthine, hypoxanthine and Tweens 40, 60 and 80, indole production, and methyl red and Voges–Proskaur tests. +, Positive; w, weakly positive; −, negative, MP, monopolar; ND, no data available.

| Characteristic | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| Colony colour | Yellowish-brown | Cream–yellow*[a] | Cream | Cream[b] | Grey[c] |
| Cell size (width×length; μm) | 1.0–1.2×2.0-8.0 | 0.3−0.56×1.3−1.5[a] | 0.7±0.2×1.6±0.3 | 0.9±0.2×2.5±0.6[b] | 0.6−1.5×2.3−2.5[c] |
| Flagella | − | MP[a] | MP | MP[b] | MP[c] |
| Temperature range for growth (optimum) (°C) | 20–40 (35) | 10–35 (30–35)[a] | 15–37 (30) | 15–44 (30)[b] | 10–42 (25–37)[c] |
| pH range for growth (optimum) | 5.5–9 (6.5) | 7–9 (8)[a] | 5–10 (8) | 5.0–9.0 (6.0)[b] | 6–11 (7–9)[c] |
| Salt tolerance (optimum) (%, w/v) | 0–10 (2) | 1–10 (2.0–4.0)[a] | 0.7–5.4 (3.4–4.0) | 0–6.0 (2.0)[b] | 0–9.0 (0.5–7.0)[c] |
| Growth with NaCl as the sole salt | − | − | ND | − | + |
| Hydrolysis of: | | | | | |
| Gelatin | + | + | ND | − | − |
| Tyrosine | + | − | ND | − | − |
| $H_2S$ production | − | − | ND | + | + |
| Reduction of nitrate to nitrite | + | + | − | − | + |
| Utiliazation of: | | | | | |
| D-Ribose | − | + | − | − | − |
| D-Fructose | − | − | + | + | + |
| D-Glucose | + | − | + | + | + |
| Trehalose | + | − | − | + | − |
| D-Galactose | + | − | − | − | + |
| Lactose | − | − | + | + | + |
| Sucrose | − | + | − | − | − |
| Acetate | − | − | − | + | − |
| Raffinose | − | − | − | + | − |
| Inositol | − | − | − | − | + |
| Xylitol | + | − | − | − | − |
| Succinate | w | − | − | − | + |
| API ZYM tests | | | | | |
| Lipase (C14) | + | − | − | − | − |
| Valine arylamidase | + | − | + | + | + |
| Cystine arylamidase | + | − | − | w | − |
| α-Chymotrypsin | + | − | − | − | − |
| API 20NE tests | | | | | |
| Urease | + | + | ND | − | − |
| DNA G+C content (mol %) | 69 | 68[a] | 65 | 60[b] | 65[c] |
| Polar lipids† | PG, PN2, PL1, L1, AL3, PN3, PN4, L2 | PG, PN2, PN1, L1, L3, PN4,PL2, L4, L2 | ND | PG, PN2, PN1, PN3, PN4, PN5 | PG, PN2, AL3, PL3, PL4, PL5 |

*Data from: *a*, Zhang *et al.*, 2008 *b*, Urios *et al.*, 2008 *c*, Lai *et al.*, 2009a.
†PG, phosphatidylglycerol; PN, unidentified aminophospholipid; PL, unidentified phospholipid; AL, unidentified aminolipid; L, unidentified lipid.

*Thalassobaculum*, *Nisaea* and *Oceanibaculum*, of which type species were *Thalassobaculum litoreum* DSM 18839$^T$(95.21), *Nisaea denitrificans* DSM 18348$^T$ (93.64 %) and *Oceanibaculum indicum* MCCC 1A02083$^T$(92.65 %), respectively, sharing low sequence similarities (<92 %) with other species of the family *Rhodospirillaceae*. Though the three genera above formed an independent cluster on all the three types of phylogenetic trees (Figs 1, S3 and S4), strain HSF7$^T$ solidly clustered with the genus *Thalassobaculum* in the phylogenetic trees, supporting that strain HSF7$^T$ is a novel species of the genus *Thalassobaculum*.

The only isoprenoid quinone in strain HSF7$^T$ was Q-10, which was the same as *T. litoreum* DSM 18839$^T$, *N. denitrificans* DSM 18348$^T$ and *O. indicum* MCCC 1A02083$^T$. Strain HSF7$^T$ had a similar DNA G+C content (69 mol%) as *T. litoreum* DSM 18839$^T$(68 mol%) (Zhang *et al.*, 2008), while *N. denitrificans* DSM 18348$^T$ and *O. indicum* MCCC 1A02083$^T$ were 60 mol% (Urios *et al.*, 2008) and 65 mol% (Lai *et al.*, 2009a), respectively.

The fatty acid profiles of strain HSF7$^T$ and the reference strains are shown in Table S1. The major fatty acids (≥5 %) of strain HSF7$^T$, $C_{18:1}\omega7c$ (69.3 %), $C_{16:0}$ (9.1 %) and $C_{19:0}$ cyclo $\omega8c$ (6.6 %), were similar to *T. litoreum* DSM 18839$^T$, though proportional differences existed. The major fatty acid $C_{19:0}$ cyclo $\omega8c$ was found in strain HSF7$^T$, but not in *N. denitrificans* DSM 18348$^T$. Strain HSF7$^T$ showed differences from *O. indicum* MCCC 1A02083$^T$, such as with $C_{16:0}$ 3-OH (trace v. 1.9, respectively), summed feature 2 ($C_{16:1}$ iso I and/or $C_{14:0}$ 3-OH) (trace v. 3.3 %, respectively) and $C_{19:0}$ cyclo $\omega8c$ (6.6 % vs 18.3 %, respectively). The polar lipids of strain HSF7$^T$and the reference strains are shown in Fig. S2. Strain HSF7$^T$ had a similar polar lipid profile to *T. litoreum* DSM 18839$^T$, such as the major polar lipids phosphatidylglycerol (PG), unknown aminophospholipid PN2 and unknown lipid L1, and the minor polar lipids PN4 and L2. The differences were that the minor polar lipids L4 (unknown lipid), PL2 (unknown phospholipid) and PN3 (unknown aminophospholipid) were absent in strain HSF7$^T$.

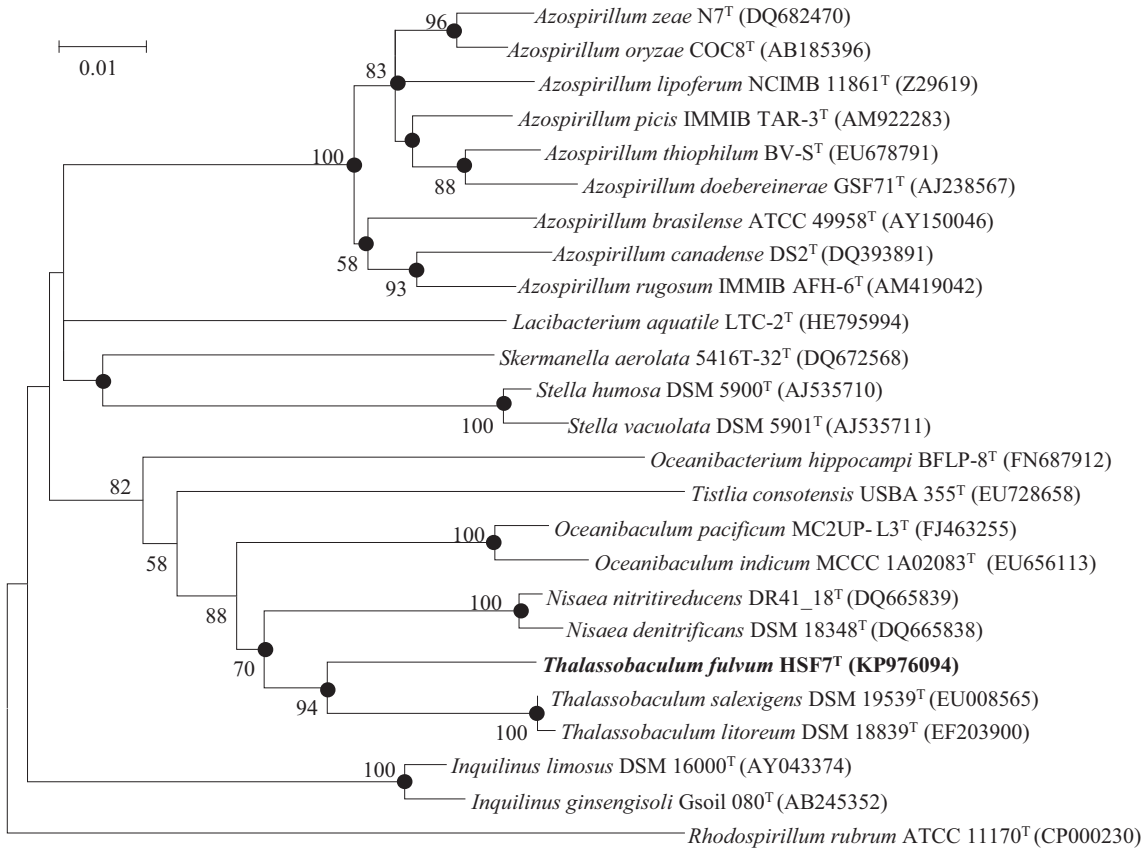


**Fig. 1.** Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences, showing the relationship of strain HSF7$^T$ with related taxa. *Rhodospirillum rubrum* ATCC 11170$^T$ was used as an outgroup. Numbers at nodes are bootstrap values based on 1000 replications; only values >50 % are shown. Filled circles indicate nodes also obtained in both maximum-likelihood and maximum-parsimony trees. Bar, 0.01 substitutions per nucleotide position.

Not only data from the phylogenetic, genomic and chemotaxonomic analyses above but also phenotypic characteristics clearly indicated that strain HSF7$^T$ belonged to the genus *Thalassobaculum*. For example, both strain HSF7$^T$ and *T. litoreum* DSM 18839$^T$ could not grow when NaCl was used as the sole salt while *O. indicum* MCCC 1A02083$^T$ could. Urease and hydrolysis of gelatin were positive in both strain HSF7$^T$ and *T. litoreum* DSM 18839$^T$, while negative in both *N. denitrificans* DSM 18348$^T$ and *O. indicum* MCCC 1A02083$^T$. On the contrary, $H_2S$ production and the utilization of D-fructose and lactose were negative in both strain HSF7$^T$ and *T. litoreum* DSM 18839$^T$, but positive in both *N. denitrificans* DSM 18348$^T$ and *O. indicum* MCCC 1A02083$^T$. However, some phenotypic differences could be easily found between strain HSF7$^T$ and *T. litoreum* DSM 18839$^T$. For example, cells of strain HSF7$^T$ had no flagella, but *T. litoreum* DSM 18839$^T$had a polar flagellum; the optimal pH of strain HSF7$^T$ (pH 6.5) was lower than that of *T. litoreum* DSM 18839$^T$ (pH 8); and strain HSF7$^T$ could grow at 40 °C, but *T. litoreum* DSM 18839$^T$ could not. Furthermore, hydrolysis of tyrosine and utilization of D-ribose, D-glucose, trehalose, D-galactose and xylitol were positive in strain HSF7$^T$, but negative in *T. litoreum* DSM 18839$^T$. Compared with *T. salexigens* DSM 19539$^T$, the other species of the genus *Thalassobaculum*, strain HSF7$^T$ had the same isoprenoid quinone and similar fatty acid profiles (Urios *et al.*, 2010). Nevertheless, strain HSF7$^T$ was different from *T. salexigens* DSM 19539$^T$ in some phenotypic characteristics as follows. Strain HSF7$^T$ could reduce nitrate to nitrite, but *T. salexigens* DSM 19539$^T$ could not. In the API ZYM system, strain HSF7$^T$ was positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), cysteine arylamidase, $\alpha$-chymotrypsin and naphthol-AS-BI- phosphoamidase activities, while *T. salexigens* DSM 19539$^T$ was negative (Urios *et al.*, 2010). Strain HSF7$^T$ could not utilize D-fructose and mannitol, while *T. salexigens* DSM 19539$^T$ could in Biolog GN2 MicroPlates (Urios *et al.*, 2010). More detailed differences are shown in Table 1.

On the basis of the phylogenetic, genomic, chemotaxonomic and phenotypic characteristics, we propose that strain HSF7$^T$ represents as a novel species of the genus *Thalassobaculum*, named *Thalassobaculum fulvum* sp. nov.

## Description of *Thalassobaculum fulvum* sp. nov.

*Thalassobaculum fulvum* (ful'vum. L. neut. adj. *fulvum* yellowish-brown).

Colonies on MA after incubation for 4 days at 30 °C are circular, opaque, regular-edged, yellowish-brown, shiny, convex and 1.0–2.0 mm in diameter. Cells are approximately 1.0–1.2 μm wide and 2.0–8.0 μm long. Growth occurs at 20–40 °C (optimum, 35 °C), pH 5.5–9.0 (optimum pH 6.5) and with 0–10 % NaCl (optimum 2 %). No growth occurs in media containing only NaCl as the sole salt. Bacteriochlorophyll *a* and PHB granules are not detected. No growth is observed under anaerobic conditions in modified MB. Nitrate can be reduced to nitrite, but not to $N_2$. Hydrolysis of casein, CM-cellulose, xanthine and hypoxanthine are negative, as well as indole production, $H_2S$ production, methyl red and Voges–Proskaur tests. Oxidase, catalase and gelatinase activities are positive. Hydrolyses Tween 20 and tyrosine, but not Tween 40, 60, 80. In API ZYM tests, positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), leucine arylamidase, valine arylamidase, cysteine arylamidase, $\alpha$-chymotrypsin, acid phosphatase, naphthol-AS-BI- phosphoamidase, but negative for trypsin, $\alpha$-galactosidase, $\beta$-galactosidase, $\beta$-glucuronidase, $\alpha$-glucosidase, $\beta$-glucosidase, *N*-acetyl-$\beta$-glucosaminidase, $\alpha$-mannosidase and $\alpha$-fucosidase. In API 20NE tests, positive for reduction of nitrate, urease and hydrolysis of gelatin, but negative for production of indole, fermentation of arginine dihydrolase, D-glucose, $\beta$-galactosidase, hydrolysis of aesculin and assimilation of glucose, arabinose, mannose, mannitol, maltose, phenylacetate, caprate, adipate, *N*-acetylglucosamine, potassium gluconate, malate and citrate. In carbon source utilization tests, positive for D-glucose, trehalose, D-galactose, erythritol and xylitol, weakly positive for succinate and pyruvate, and negative for D-ribose, D-fructose, lactose, sucrose, acetate, raffinose, inositol, $\alpha$-ketoglutaric acid, xylose, mannitol, citrate, L-rhamnose, salicin and oxalate. The isoprenoid quinone is Q-10. Dominant cellular fatty acids are $C_{18:1}\omega7c$, $C_{16:0}$ and $C_{19:0}$ cyclo $\omega8c$. The polar lipids are phosphatidylglycerol, three unknown aminophospholipids (PN2, PN3, PN4), an unknown phospholipid (PL1), an unknown aminolipid (AL3) and two unknown lipids (L1, L2).

The type strain, HSF7$^T$ (=KCTC 42651$^T$=MCCC 1K01158$^T$) was isolated from deep seawater in the South China Sea. The G+C content of genomic DNA of the type strain is 69 mol%.

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