Tateyamaria pelophila sp. nov., a facultatively anaerobic alphaproteobacterium isolated from tidal-flat sediment, and emended descriptions of the genus *Tateyamaria* and of *Tateyamaria omphalii*

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A Gram-negative motile rod, strain SAM4^T, was isolated from the highest positive dilution of a most probable number series inoculated with tidal-flat sediments from the German North Sea coast. The isolate grew at 4–35 °C and showed constant growth yields throughout almost the whole temperature range. Growth was observed between pH 6 and 9 and at salinities of 0.3–10.2 ‰. Strain SAM4^T required Na⁺ for growth, contained bacteriochlorophyll *a* and was catalase- and oxidase-positive. It was nutritionally versatile growing on a variety of carbon compounds including carbohydrates, amino acids and organic acids like lactate or succinate. It grew anaerobically on complex media such as marine broth, indicating fermentation, and by reducing trimethylammonium oxide. The dominant phospholipids were phosphatidylethanolamine and phosphatidylglycerol, whereas only traces of phosphatidylcholine and an unidentified lipid were found. The major fatty acid was *n*-C_{18:1ω7c}. The DNA G+C content was 56.4 mol%. The isolate was identified as a member of the *Roseobacter* clade within the class *Alphaproteobacteria*. However, based on phylogenetic, phenotypic and physiological data, it clearly differs from its closest relative *Tateyamaria omphalii*. Therefore, a novel species is proposed: *Tateyamaria pelophila* sp. nov., with strain SAM4^T (=DSM 17270^T=LMG 23018^T) as the type strain. Emended descriptions of the genus *Tateyamaria* and of *Tateyamaria omphalii* are also presented.

During the last two decades, molecular methods have helped to vastly expand our knowledge of microbial diversity and to identify the dominant bacterial phyla in various environments. Most of these new phyla are not represented in culture collections as no isolates are available so far (Hugenholtz *et al.*, 1998; Rappé & Giovannoni, 2003). A notable exception is the *Roseobacter* clade within the class *Alphaproteobacteria* (Selje *et al.*, 2004; Buchan *et al.*, 2005). Bacteria in the *Roseobacter* clade are globally distributed and form one of the

dominant groups of marine bacterioplankton (Mullins *et al.*, 1995; Gonzalez & Moran, 1997). Selje *et al.* (2004) found up to 2×10^5 *Roseobacter* clade cells ml⁻¹ in North Sea water, corresponding to approximately 20 % of the total DAPI cell count. Although *Roseobacter* clade bacteria are generally strict aerobes, they have also been detected repeatedly in marine sediments by molecular methods as well as by cultivation (Johnson & Hill, 2003; Süß *et al.*, 2004; Buchan *et al.*, 2005; Köpke *et al.*, 2005). Gonzalez *et al.* (1999) estimated that members of the *Roseobacter* clade may account for up to 11 % of the 16S rRNA gene copies in coastal sediment communities. Such a high abundance cannot be explained solely by the survival of strictly aerobic bacteria during burial or by oxic micro-layers around the burrows of macrozoobenthos. During a quantitative cultivation study performed with tidal-flat sediments taken close to the village of Neuuharlingersiel on the German North Sea coast in October 1998, strain SAM4^T was enriched and isolated from a most probable number (MPN) series prepared with sediments from 5 cm

Abbreviations: AQDS, anthraquinone-2,6-disulfonic acid; DPG, diphosphatidylglycerol; MPN, most probable number; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; TMAO, trimethylammonium oxide.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of *Tateyamaria pelophila* SAM4^T is AJ968651.

Plots showing the effect of temperature on growth of strain SAM4^T (supplementary Fig. S1) are available with the online version of this paper.

depth using oxic medium and with amino acids as electron donors (2 mM alanine and 1 mM each of cystine, arginine and asparagine). Strain SAM4^T was identified as a member of the *Roseobacter* clade and, although isolated under oxic conditions, was able to grow anaerobically.

The closest relative of strain SAM4^T, *Tateyamaria omphalii* IAM 15108^T, was purchased from the Japan Collection of Microorganisms. Microbiological routine tests (Gram and flagella-staining, oxidase, catalase, enzyme activities) were performed with exponential phase cells according to standard procedures (Gerhardt *et al.*, 1994). Bacteriochlorophyll was determined spectrophotometrically (Cohen-Bazire *et al.*, 1957) with cells grown on marine broth 2216 (MB; Difco, Becton Dickinson) in the dark or under diurnal light/dark cycles. Absorption spectra were recorded from 400–900 nm on a UV/VIS spectrophotometer (Lambda2S; Perkin Elmer). The G + C content of the DNA was determined by HPLC analysis (Mesbah *et al.*, 1989) at the German Collection of Microorganisms and Cell Cultures (DSMZ, Braunschweig, Germany). For preparation of the MPN series, isolation and growth of pure cultures, a HEPES-buffered artificial oxic seawater medium was used (Süß *et al.*, 2004; Köpke *et al.*, 2005). For tests on anaerobic growth, the medium was cooled under an N₂/CO₂ (80:20, v/v) atmosphere and buffered by the addition of sodium bicarbonate (30 mmol l⁻¹ final concentration) from sterile stocks (Süß *et al.*, 2004). The anoxic basal medium contained resazurin (0.25 mg l⁻¹) as a redox indicator and was reduced by the addition of a few crystals of sterile sodium dithionite or sodium sulfide until the redox indicator turned colourless. For strain maintenance and some growth tests, an oxic dilute yeast extract-peptone-glucose medium (YPG) based on the oxic seawater medium described above was used (Süß *et al.*, 2004). Strain SAM4^T grew on MB, but cells often appeared very pleomorphic and growth was not always reproducible. Substrate utilization was tested in miniaturized assays in microtitre plates (Corning) using oxic mineral medium. For each substrate, an inoculum-free control was prepared and for each strain, a substrate-free blank was prepared (Süß *et al.*, 2008). Growth was checked by fluorimetry (Köpke *et al.*, 2005; Martens-Habbenha & Sass, 2006) and by subculturing. Fermentative growth was tested in anoxic sulfate-free medium with either glucose (5 mM) or Casamino acids (0.1%), or in anoxic sulfide-reduced MB or R2A medium (Becton Dickinson). Tests for anaerobic respiration were performed with sulfate-free anoxic mineral medium with either sodium acetate or lactate (10 mM each) in combination with the following electron acceptors (Köpke *et al.*, 2005; Süß *et al.*, 2008): nitrate (10 mM), nitrite (2 mM), thiosulfate (10 mM), sulfite (5 mM), ferrihydrite (20 mM), manganese oxides (10 mM), elemental sulfur, trimethylammonium oxide (TMAO; 5 mM), DMSO (5 mM), anthraquinone-2,6-disulfonic acid (AQDS; 0.5 mM), or humic acids (0.1 mg ml⁻¹; Sigma-Aldrich). Hydrogen consumption was tested in mineral medium under an N₂/CO₂ (80:20, v/v) gas phase containing

5% O₂ and 10% H₂. H₂ was analysed by GC (Webster *et al.*, 2009). Manganese oxidation was tested in the dark in mineral medium containing 10 mM MnCl₂. The Na⁺ requirement was checked in YPG medium with Na⁺ replaced by K⁺. Growth rates and yields were determined as described previously (Sass *et al.*, 2002). Susceptibility to antibiotics was tested by determination of the MIC in dilute YPG medium using a microtitre approach as outlined by Reeves *et al.* (1980) and recommended by the American Society for Microbiology (Murray *et al.*, 1995). Antibiotics were diluted in twofold steps from 256 to 0.00195 µg ml⁻¹ (chloramphenicol, erythromycin, gentamicin, kanamycin, streptomycin, tetracycline, trimethoprim) or 3200 to 0.0244 U (penicillin G) and cell titres were adjusted to 10⁶ cells ml⁻¹ in each well at the start of the experiment. Vitamin requirement was tested in mineral medium with 5 mM glucose and a mixture of the common 20 L-amino acids (each at 10 µM). Eight different assays lacking one of the following vitamins were prepared: *p*-aminobenzoate, biotin, folate, lipoate, nicotinate, pantothenate, pyridoxine and thiamine. Results were confirmed in at least three consecutive subcultures. *Tateyamaria omphalii* IAM 15108^T, *Roseobacter litoralis* DSM 6996^T and *Sulfitobacter pontiacus* DSM 10014^T were used as reference organisms. Whole-cell fatty acid patterns were analysed in cells grown aerobically with acetate as the sole electron and carbon source. Cells were harvested at the end of the exponential phase and washed with PBS buffer (130 mmol NaCl l⁻¹, 10 mmol sodium phosphate buffer l⁻¹, pH 7.2). Sequencing of the 16S rRNA gene and phylogenetic analysis were performed as described by Sass *et al.* (2002).

For whole cell fatty acid patterns, freeze-dried cells were subjected to alkaline hydrolysis and subsequent acidification and extraction as described by Sass *et al.* (2002). In contrast to the MIDI procedure (Sasser, 1997), fatty acids were converted to their trimethylsilyl (TMS) ester derivatives using N-methyl-N-trimethylsilyltrifluoroacetamide (CS Chromatographie Service, Langerwehe, Germany). The esterified fatty acids were analysed by GC-FID. Double bond positions of monounsaturated fatty acids were confirmed by preparation of dimethyldisulfide adducts and GC-MS analysis (Rütters *et al.*, 2002; Sass *et al.*, 2002). Extraction and fractionation of lipids and analysis of polar lipids (including phospholipids) were performed as described previously (Rütters *et al.*, 2001, 2002).

Strain SAM4^T was isolated from an MPN series [6 × 10⁷ cells (g sediment)⁻¹] using a standard dilution plating technique. Older colonies became pinkish on nutrient-rich MB, but remained beige on YPG agar. Cells were Gram-negative rods (0.7–1.4 µm wide and 1.5–3.0 µm long) and were motile by means of a single flagellum. Endospores were not observed. Strain SAM4^T exhibited catalase and oxidase activities. Like its closest relative, *T. omphalii* IAM 15108^T, strain SAM4^T did not hydrolyse agar, alginate, or gelatin, whereas weak cellulase and chitinase activities were found. No acid production from carbohydrates was found

in strain SAM4^T, whereas *T. omphalii* IAM 15108^T produced acid from D-mannose and D-glucose. Further biochemical parameters of strain SAM4^T and *T. omphalii* IAM 15108^T are given in Table 1 and the species descriptions. Bacteriochlorophyll *a* was detected only in older cultures of strain SAM4^T grown in the dark or under diurnal light/dark cycles. Spectra revealed characteristic peaks at 768–770 nm and 477–481 nm, and a smaller one at 594–595 nm. A very similar spectrum was obtained with *Roseobacter denitrificans* DSM 7001^T as a reference strain. The DNA G+C content of strain SAM4^T was 56.4 mol%.

Strain SAM4^T and *T. omphalii* IAM 15108^T required Na⁺ and grew at salinities of 0.3–10.2% and 0.2–6.0%, respectively. The pH ranges for growth were 6.0–9.0 (strain SAM4^T) and 5.4–8.9 (*T. omphalii* IAM 15108^T). Strain SAM4^T grew at temperatures between 4 and 35 °C, with optimum growth at 25 °C [growth rate (μ) on YPG was 1.72 d⁻¹] and showed more or less constant growth yields with values around 140 mg dry weight per l YPG medium at temperatures between 4 and 30 °C (see Supplementary Fig. S1, available in IJSEM Online). However, plotting log(μ) against the inverse temperature in an Arrhenius plot revealed that a linear increase was restricted to temperatures between 4 and 20 °C. At temperatures above 20 °C, log(μ) hardly increased, indicating harmful temperature effects (data not shown). Due to its temperature range for growth and growth yield to temperature relationship, strain SAM4^T can be characterized as psychrotolerant (Isaksen & Jørgensen, 1996).

Strain SAM4^T was nutritionally versatile, utilizing a wide range of substrates such as peptone, carbohydrates, amino acids and organic acids such as lactate or acetate, whereas primary alcohols or aromatic compounds were not utilized (see Table 1 and species description). No growth was observed using the C₁-compounds formate, methanol, methylamine, dimethyl sulfide or DMSO. *T. omphalii* IAM 15108^T utilized similar substrates including carbohydrates, and amino, dicarboxylic and fatty acids (see Table 1 and species description), but in contrast to strain SAM4^T also grew with some aliphatic alcohols. Strain SAM4^T oxidized sulfite, thiosulfate and elemental hydrogen, but not elemental sulfur, whereas *T. omphalii* IAM 15108^T oxidized neither hydrogen nor sulfur compounds. Hydrogen consumption was also found in *R. litoralis* DSM 6996^T, but not in *S. pontiacus* DSM 10014^T.

Strain SAM4^T grew anaerobically with acetate as electron donor and TMAO as electron acceptor, but not with DMSO, nitrate, nitrite, sulfite, elemental sulfur, thiosulfate, soluble ferric citrate, iron hydroxide, manganese oxides, AQDS or humic acids. *T. omphalii* IAM 15108^T reduced nitrate (Kurahashi & Yokota, 2007) and TMAO, but none of the other electron acceptors tested. However, growth in assays with TMAO was very weak and cells were generally elongated by up to ten times the normal length indicating unfavourable conditions. TMAO reduction was also found in *R. denitrificans*, but occurred only in the light (Shiba,

1991). *S. pontiacus* DSM 10014^T reduced DMSO, but no anaerobic respiration was found with *R. litoralis* DSM 6996^T. Strain SAM4^T grew fermentatively on anoxic sulfide-reduced media. Generally, anaerobic growth on mineral medium with glucose ceased after one to three transfers, but persisted longer on anaerobic MB. As indicated by the colourless redox indicator resazurin, the positive assays were indeed oxygen-free. However, although visible colonies were formed under clearly anoxic conditions, these generally became smaller with each transfer until subculturing under anoxic conditions failed. At the moment, we can only speculate about the reason for this growth behaviour, but it may indicate the failure of an assimilatory pathway under anoxic conditions that can at least partly be compensated for by the use of complex media like MB. Reproducible anaerobic growth on complex MB or R2A (Becton Dickinson) was also found with *T. omphalii* IAM 15108^T and *S. pontiacus* DSM 10014^T. Although liquid anoxic cultures generally did not become turbid, they were dominated by dividing cells and cell numbers reached up to 10⁷ ml⁻¹.

The antibiotic MICs determined for strain SAM4^T, *T. omphalii* IAM 15108^T, *R. litoralis* DSM 6996^T and *S. pontiacus* DSM 10014^T are given in Table 2. Strain SAM4^T required biotin, pantothenate and thiamine for growth, whereas *T. omphalii* IAM 15108^T required only pantothenate and biotin. In our tests, *R. litoralis* DSM 6996^T and *S. pontiacus* DSM 10014^T required biotin, nicotinate and thiamine, thus confirming previous reports (Shiba, 1991; Sorokin, 1995).

The major phospholipids of strain SAM4^T were phosphatidylglycerol (PG; 72%) and phosphatidylethanolamine (PE; 28%). Phosphatidylcholine (PC) and an unidentified lipid were present in trace amounts. The dominant fatty acid was *n*-C_{18:1}ω7c (90.6%). Other fatty acids present in lower amounts included *n*-C_{16:0} (4.9%), *n*-C_{16:1}ω7c (1.2%), *n*-C_{18:0} (0.9%), *n*-C_{14:0} (0.8%), 11-methyl-C_{18:1} (0.7%) and *n*-C_{18:1}ω9c (0.4%), whereas only traces (<0.2%) of *n*-C_{14:1}ω5c, ai-C_{15:0}, i-C_{16:0} and *n*-C_{16:1}ω9c were detected.

Phylogenetic analysis based on 16S rRNA gene sequences revealed that strain SAM4^T was most closely related to *T. omphalii* IAM 15108^T (97.1% similarity). High similarities were also found to *R. litoralis* DSM 6996^T (96.5%), *R. denitrificans* DSM 7001^T (96.2%) and *Sulfitobacter mediterraneus* DSM 12244^T (96.1%). The maximum-likelihood tree shown in Fig. 1 is typical of all trees obtained showing the position of strain SAM4^T and *Tateyamaria omphalii* IAM 15108^T on a separate branch close to members of the genera *Roseobacter* and *Sulfitobacter*.

The phylogenetic grouping of strain SAM4^T and *T. omphalii* IAM 15108^T into a single genus is supported by the results of phenotypic investigations. Both strains exhibit almost identical enzyme patterns (except for ornithine deaminase) and utilize similar substrates. They can be distinguished from species of the genera *Roseobacter*

Table 1. Differential phenotypic characteristics of strain SAM4^T and members of the genera *Tateyamaria*, *Roseobacter*, *Sulfitobacter* and *Oceanibulbus*

Taxa: 1, strain SAM4^T (data from this study); 2, *T. omphalii* IAM 15108^T (Kurahashi & Yokota, 2007); 3, *R. litoralis* DSM 6996^T (Shiba, 1991); 4, *R. denitrificans* DSM 7001^T (Shiba, 1991); 5, *S. pontiacus* DSM 10014^T (Sorokin, 1995); 6, *S. mediterraneus* DSM 12244^T (Pukall *et al.*, 1999); 7, *S. donghicola* JCM 14565^T (Yoon *et al.*, 2007); 8, *S. guttiformis* DSM 11458^T (Labrenz *et al.*, 2000); 9, *S. brevis* DSM 11443^T (Labrenz *et al.*, 2000); 10, *O. indolifex* DSM 14862^T (Wagner-Döbler *et al.*, 2004). +, Positive; –, negative; (+), weakly positive; tr, trace; ND, not determined or not described. All of the taxa shown are positive for catalase and oxidase, and require Na⁺ for growth.

Characteristic	1	2	3	4	5	6	7	8	9	10
Cell shape	Rod	Coccoid-rod	Ovoid-rod	Ovoid-rod	Rod	Rod	Coccoid-rod	Rod	Rod	Irregular rods
Motility	+	–	+	+	+	+	–	+	+	–
Rosettes	–	–	–	–	+	+	ND	+	+	ND
Bacteriochlorophyll <i>a</i>	+	–	+	+	–*	–	–	+	–	–
DNA G+C content (mol%)	56.4	61.6	59.6	56.3–58	62.1	59	56.9	55–56	58	60.1
Growth at:										
4 °C	+	+	+	+	+	+	–	+	+	–
37 °C	–	+	–	–	–	–	–	–	–	–
pH range	6–9	5.4–8.9†	7.0–9.5	7.0–9.5	6.5–8.5	6.5–8.5	6–9	5.5–9.5	5.5–9.5	7–9
Hydrolysis of:										
Gelatin	–	–	+	+	–‡	+	–	–	–	–
Starch	–	+†	–	–	+*	+*	–	–	–	–
Tween 80	–	–†	+	+	+*	+*	(+)	+	+	–
Utilization of:										
Cellobiose	+	+†	–‡	–‡	–†	ND	–	–	(+)	–
Maltose	+	+†	+‡	+‡	–†	–	–	–	+	–
Trehalose	+	+†	+‡	+‡	–†	ND	–	(+)	+	–
D-Mannose	+	+†	+‡	+‡	–†	–	–	(+)	+	–
L-Arabinose	–	–†	+/-‡	+‡	–†	–	–	–	+	–
D-Gluconate	+	+†	+‡	+‡	+	+	ND	–	(+)	–
DL-Lactate	+	+†	–‡	+	+	+	ND	–	–	+
Acetate	+	+†	+	+	+	+	–	+	+	–
Butyrate	–	+†	–	–	+	+	–	–	+	–
L-Ornithine	+	+†	–‡	–‡	+	+	ND	–	–	+
Nitrate reduction	–	+	–	+	+§	–	–	–	–	–
Oxidation of sulfite	+	–†	–†	ND	+	+	ND	–	–	ND
Vitamin requirement:										
Biotin	+	+†	+	+	+†	–	ND	(+)	(+)	ND
Nicotinate	–	–†	+	+	+†	–	ND	(+)	(+)	ND
Pantothenate	+	+†	–	–	–†	–	ND	+	+	ND
Thiamine	+	–†	+	+	+	–	ND	(+)	+	ND
Susceptible to (µg ml ⁻¹):										
Kanamycin (10)	–	–†	+†	ND	+*	+*	+	ND	+*	ND
Streptomycin (20)	+	–†	+†	+	–†	ND	+	+	+	ND
Tetracycline (30)	+	–†	–†	+	+*	+*	+	+	+	ND
Presence of:										
<i>n</i> -C _{18:2}	–	–	+‡	–‡	–	–	–	+	+	+
<i>n</i> -C _{18:0}	+	–	+‡	+‡	–	+	+	+	+	–
11-methyl-C _{18:1}	+	+	–‡	–‡	–	+	+	–	+	–
cyclo-C ₁₉ FA	–	+	–‡	–‡	–	–	–	–	–	–
PG	+	ND	+¶	+¶	+¶	ND	+	+	+	+
PE	+	ND	–¶	–¶	+¶	ND	+	+	+	+
PC	tr	ND	+¶	–¶	–¶	ND	+	+	+	+
DPG	–	ND	+¶	+¶	+¶	ND	(+)	–	+	+
Unidentified aminolipid	–	ND	+¶	+¶	–¶	ND	–	+	+	+

Table 1. cont.*Data from Yoon *et al.* (2007).

†Data from this study.

‡Data from Labrenz *et al.* (1999).§Data from Ivanova *et al.* (2004).||Data from Pukall *et al.* (1999).¶Data from Labrenz *et al.* (1998).#Data from Labrenz *et al.* (2000).

and *Sulfitobacter* by their vitamin requirement (requirement for pantothenate, but not for nicotinate) and their physiological capacities (absence of casein and Tween 80 hydrolysis) and their insensitivity to kanamycin. In contrast to species of the genus *Roseobacter*, strain SAM4^T and *T. omphalii* IAM 15108^T utilize L-ornithine and cellobiose, but not L-arabinose, whereas species of the genus *Sulfitobacter* generally do not utilize the disaccharides cellobiose, maltose and trehalose or D-mannose. Like *T. omphalii* IAM 15108^T, strain SAM4^T contains the branched 11-methyl-C_{18:1} fatty acid, which is also found in some species of the genus *Sulfitobacter*, but is absent in species of the genus *Roseobacter* and in *Oceanibulbus indolifex* DSM 14862^T (Table 1); and both lack the *n*-C_{18:2} fatty acid which is widespread among species of the genera *Roseobacter*, *Sulfitobacter* and *Oceanibulbus*. However, it may be that some of these differences are due to variations in the analytical procedures applied. Strain SAM4^T differs from members of the genus *Roseobacter* with respect to its phospholipids as it contains almost exclusively PE and PG, whereas in species of the genus *Roseobacter* PE is absent, but diphosphatidylglycerol (DPG) is a major lipid (Labrenz *et al.*, 1998). DPG is also present in *O. indolifex* DSM 14862^T (Wagner-Döbler *et al.*, 2004) and in most species of the genus *Sulfitobacter* (e.g. Labrenz *et al.*, 2000; Ivanova *et al.*, 2004). The only reported exception is *Sulfitobacter guttiformis* DSM 11458^T (formerly *Staleyia guttiformis*),

which, like strain SAM4^T, contains PG, PE and PC and produces bacteriochlorophyll *a* (Labrenz *et al.*, 2000).

In conclusion, phylogenetic evidence based on 16S rRNA gene sequences, physiological and lipid data suggest that strain SAM4^T represents a novel species within the genus *Tateyamaria*, for which the name *Tateyamaria pelophila* is proposed.

Emended description of the genus *Tateyamaria* Kurahashi and Yokota 2007

The description is based on that given by Kurahashi & Yokota (2007) with the following amendments and changes. Cells may be motile by polar flagella and may produce bacteriochlorophyll *a*. Cells are positive for catalase, oxidase and phosphatase. The type species is *Tateyamaria omphalii*.

Description of *Tateyamaria pelophila* sp. nov.

Tateyamaria pelophila (pe.lo'phi.la. Gr. n. *pelos* mud; Gr. adj. *philos* -ê -on loving; N.L. fem. adj. *pelophila* mud-loving).

Gram-negative rods, 0.7–1.4 µm wide and 1.5–3.0 µm long. Motile by means of a polar flagellum. Colonies on MB agar plates are beige to pinkish. Temperature range for growth is 4–35 °C, with fastest growth at 25 °C. Growth is observed between pH 6 and 9 and at salinities from 0.3 to 10.2%. Requires Na⁺. Substrates used aerobically are: peptone, Casamino acids, yeast extract, starch, chitin, xylan, laminarin, cellobiose, maltose, sucrose, trehalose, D-fructose, D-glucose, D-mannose, D-xylose, D-mannitol, D-sorbitol, D-gluconate, D-glucosamine, L-rhamnose, acetate, propionate, valerate, capronate, caprylate, glycolate, DL-lactate, pyruvate, succinate, DL-malate, citrate, glycerol, L-alanine, L-arginine, L-asparagine, L-aspartate, L-glutamine, L-glutamate, L-leucine, L-lysine, L-methionine, L-ornithine and L-valine. No growth is observed with chitin, cellulose, L-arabinose, D-ribose, formate, butyrate, crotonate, malonate, DL-tartrate, 2-ketoglutarate, L-cysteine, L-glycine, L-histidine, L-isoleucine, L-leucine, L-phenylalanine, L-serine, L-threonine, L-tryptophan, L-tyrosine, methanol, ethanol, *n*-propanol, *n*-butanol, betaine, methylamine, ethanolamine, DMSO, dimethylsulfide, benzoate, salicylate or nicotinate. Grows anaerobically for a few transfers with glucose as substrate or on MB. Under anoxic conditions,

Table 2. MICs determined for strain SAM4^T and the type strains of *T. omphalii*, *R. litoralis* and *S. pontiacus*

Strains: 1, SAM4^T; 2, *T. omphalii* IAM 15108^T; 3, *R. litoralis* DSM 6996^T; 4, *S. pontiacus* DSM 10014^T. Concentrations are given in µg ml⁻¹, except for penicillin G (U).

Antibiotic	1	2	3	4
Chloramphenicol	0.25	4	0.5	0.5
Erythromycin	0.25	0.063	8	16
Gentamicin	2	512	8	16
Kanamycin	256	>512	32	64
Penicillin G	<0.002	0.016	<0.002	0.5
Streptomycin	4	128	64	128
Tetracycline	4	512	128	64
Trimethoprim	256	2	256	>256

Fig. 1. Maximum-likelihood tree based on 16S rRNA sequences showing the phylogenetic position of strain SAM4^T within the *Roseobacter* clade of the class *Alphaproteobacteria*. *Jannaschia helgolandensis* Hel 10^T (GenBank accession no. AJ438157) served as outgroup (not shown). Bootstrap values of 50 % or more are shown at nodes. Accession numbers are given in parentheses. Bar, 1 % sequence divergence.

TMAO serves as an electron acceptor, whereas DMSO, nitrate, nitrite, sulfite, elemental sulfur, thiosulfate, ferric citrate, iron hydroxide, manganese oxides, AQDS and humic acids are not reduced. Sulfite, thiosulfate and elemental hydrogen are oxidized lithoheterotrophically, but not elemental sulfur or carbon monoxide. Weak manganese-oxidizing activity. Cells exhibit oxidase and catalase activity and contain bacteriochlorophyll *a*. Sulfide is not produced from L-cysteine, L-methionine or thiosulfate. Agar, agarose, alginate, casein, gelatin, starch, Tweens 20 and 80, tyrosine and xylan are not hydrolysed. Positive for arginine deaminase, tryptophan deaminase, leucine arylamidase, valine arylamidase and phosphatase activities. Weak β -glucosidase and *N*-acetylglucosaminidase activity is present. Negative for arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, lipase, urease, cysteine arylamidase and ornithine deaminase. Indole is not produced. Phenylacetate is utilized. Does not produce acid from L-arabinose, D-xylose, D-ribose, D-fructose, D-glucose, D-mannose, D-galactose, cellobiose, maltose, trehalose, sucrose, lactose, L-rhamnose, D-mannitol, D-sorbitol or glycerol. Requires biotin, pantothenate and thiamine for growth, but not *p*-aminobenzoate, folate, lipoate, nicotinate, pyridoxine, cyanocobalamin or riboflavin. Cells are susceptible to chloramphenicol, erythromycin, gentamicin, penicillin G, streptomycin and tetracycline, but are resistant to kanamycin and trimethoprim. The major phospholipids are PE and PG. PC and an

unidentified lipid were detected only in trace amounts. The major cellular fatty acid is *n*-C_{18:1} ω 7c. The following fatty acids are present at lower concentrations: *n*-C_{16:0}, *n*-C_{14:1} ω 5c, *n*-C_{14:0}, ai-C_{15:0}, i-C_{16:0}, *n*-C_{16:1} ω 7c, *n*-C_{16:1} ω 9c, *n*-C_{18:1} ω 9c, *n*-C_{18:0} and 11-methyl-C_{18:1}.

The type strain is SAM4^T (=DSM 17270^T=LMG 23018^T), isolated from tidal-flat sediment on the German North Sea coast. The DNA G+C content is 56.4 mol%.

Emended description of *Tateyamaria omphalii* Kurahashi and Yokota 2007

Tateyamaria omphalii (om.phal'i.i. N.L. gen. n. *omphalii* of *Omphalius*, the genus of the top shell, from which the strain was isolated).

The description of *Tateyamaria omphalii* is as given by Kurahashi & Yokota (2007) with the following additions. Growth is observed between pH 5.4 and 8.9 and at salinities from 0.2 to 6.0 ‰. Substrates used aerobically are: peptone, Casamino acids, yeast extract, starch, cellobiose, maltose, sucrose, trehalose, D-fructose, D-mannose, D-xylose, D-mannitol, D-gluconate, D-glucosamine, acetate, propionate, butyrate, valerate, DL-lactate, malonate, succinate, fumarate, DL-malate, 2-ketoglutarate, ethanol, *n*-propanol, ethylene glycol, glycerol, L-asparagine, L-aspartate, L-glutamine, L-histidine, L-glutamate, L-lysine, L-ornithine, L-proline, L-serine, L-threonine, L-tryptophan,

L-valine and benzoate. Grows anaerobically for a few transfers on complex media such as R2A or MB. Under anoxic conditions, nitrate and TMAO can serve as electron acceptors, whereas DMSO, nitrite, sulfite, elemental sulfur, thiosulfate, ferric citrate, iron hydroxide, manganese oxides, AQDS or humic acids are not reduced. No growth is observed with chitin, laminarin, cellulose, lactose, L-arabinose, D-galactose, D-ribose, D-sorbitol, DL-tartrate, formate, caproate, caprylate, crotonate, glycolate, DL-alanine, L-arginine, L-cysteine, L-glycine, L-isoleucine, L-leucine, L-methionine, L-phenylalanine, L-tyrosine, methanol, *n*-butanol, betaine, methylamine, ethanolamine, DMSO, dimethylsulfide, salicylate or nicotinate. Elemental hydrogen, thiosulfate, sulfite, elemental sulfur and Mn^{2+} are not oxidized. Agar, agarose, alginate, casein, Tween 20, Tween 80, tyrosine and xylan are not hydrolysed. Arginine deaminase, tryptophan deaminase, leucine arylamidase, valine arylamidase, ornithine deaminase and phosphatase are present. Negative for β -glucosidase, *N*-acetylglucosaminidase, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, lipase and cysteine arylamidase. Phenylacetate is not utilized. Produces acid from D-mannose, but not from D-fructose, cellobiose, D-xylose or trehalose. Requires biotin and pantothenate for growth, but not *p*-aminobenzoate, folate, lipoate, nicotinate, pyridoxine, thiamine, cyanocobalamin or riboflavin. Cells are susceptible to chloramphenicol, erythromycin, penicillin G and trimethoprim, but are resistant to gentamicin, kanamycin, streptomycin and tetracycline.

The type strain is MKT107^T (=IAM 15108^T=KCTC 12333^T), which was isolated from the gastrointestinal tract of the molluscan top shell *Omphalius pfeifferi pfeifferi* at Chiba, Japan.

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