Planktotalea frisia gen. nov., sp. nov., isolated from the southern North Sea

Sarah Hahnke,¹ Brian J. Tindall,² Peter Schumann,² Martin Sperling,^{1†} Thorsten Brinkhoff¹ and Meinhard Simon¹

Correspondence
Meinhard Simon
m.simon@icbm.de

¹Institute for Chemistry and Biology of the Marine Environment (ICBM), University of Oldenburg, D-26111 Oldenburg, Germany

²DSMZ – Deutsche Sammlung von Mikroorganismen und Zellkulturen, Inhoffenstraße 7B, D-38124 Braunschweig, Germany

A heterotrophic, aerobic bacterium, designated strain SH6-1^T, was obtained from a seawater sample collected from the open North Sea during a phytoplankton bloom. Strain SH6-1^T was isolated from a 10⁻⁶ dilution culture, which indicated a high abundance of this organism in the environmental sample. 16S rRNA gene sequence comparison revealed that strain SH6-1^T belonged to the marine *Roseobacter* clade (order *Rhodobacterales*) within the class *Alphaproteobacteria*. *Pelagicola litoralis* CL-ES2^T was the closest phylogenetic neighbour (96.4% 16S rRNA gene sequence similarity). Cells of strain SH6-1^T were small or elongated irregular rods. Optimal growth occurred between 20 and 25 °C and between pH 7.5 and 9.0 with peptone and yeast extract. On marine agar, the isolate formed non-pigmented, small, circular, convex colonies. For growth, cells required sodium ions and the vitamins pantothenic acid and nicotinic acid amide. The DNA G + C content was 53.8 mol%. The fatty acids (>1%) were C_{10:0} 3-OH, C_{16:0}, C_{12:1}, C_{12:1} 3-OH, C_{18:0}, C_{18:1} ω7c, C_{18:2} and 11-methyl C_{18:1} ω7c. The polar lipid pattern indicated the presence of phosphatidylcholine, phosphatidylglycerol, an unidentified aminolipid and one unidentified phospholipid. The major respiratory lipoquinone was ubiquinone Q-10. Strain SH6-1^T contained the genes *puLM*, which code for the bacterial photosynthesis reaction centre; however, no bacteriochlorophyll *a* could be detected. Physiological, genotypic and phenotypic differences from *P. litoralis* support the description of a novel genus and species, for which we suggest the name *Planktotalea frisia* gen. nov., sp. nov.; the type strain of the type species is SH6-1^T (=DSM 23709^T=LMG 25294^T).

Bacteria of the *Roseobacter* clade within the *Alphaproteobacteria* have been detected in a large variety of marine habitats, often in high abundance. Currently, more than 40 described genera are considered to be members of the *Roseobacter* clade (Brinkhoff *et al.*, 2008). They gain energy from a multitude of organic compounds and some members are capable of aerobic anoxygenic photosynthesis; however, they are all unable to grow autotrophically (for reviews, see Buchan *et al.* 2005; Wagner-Döbler & Biebl, 2006). In pelagic habitats of the North Sea, roseobacters were found to constitute 5–24% of DAPI cell counts (Eilers *et al.*, 2001; Alderkamp *et al.*, 2006).

Strain SH6-1^T was isolated from a water sample collected at 2 m depth on 12 May 2007 in the southern North Sea (54° 42' N 06° 48' E; 36 m total depth; salinity 34.17%) during a phytoplankton bloom. In order to enrich the abundant bacteria present in the water sample, serial dilution cultures were used. Prior to inoculation of the serial dilution, the seawater sample was filtered through a 1.2-µm filter to obtain single planktonic cells. Strain SH6-1^T was isolated from a 10⁻⁶ dilution in low-nutrient seawater medium (North Sea water filtered through 10 µm and amended with (l⁻¹) 5 mg yeast extract and 10 mg peptone; pH 8.0; sterilized by autoclaving). After incubation in the dark at 15 °C for 4 weeks, 100 µl of each dilution culture was spread on saltwater agar (SWM; containing 416 mM NaCl, 49.2 mM MgCl₂·6H₂O, 10 mM CaCl₂·2H₂O, 8.9 mM KCl, 28.2 mM Na₂SO₄, 10 mM HEPES, 840 µM KBr, 400 µM H₃BO₃, 150 µM SrCl₂, 400 µM NH₄Cl, 40 µM KH₂PO₄, 70 µM NaF; pH 8.0). The medium contained 30 mg yeast extract and 60 mg peptone (l⁻¹) as substrate and 20 g agar purified by washing five times in distilled

[†]Present address: GEOMAR Helmholtz Zentrum für Ozeanforschung, Düsternbrooker Weg 20, D24105 Kiel, Germany.

Abbreviation: Bchl, bacteriochlorophyll.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain SH6-1^T is FJ882052.

A supplementary figure is available with the online version of this paper.

water. After autoclaving, SWM was supplemented with (l^{-1}) 1 ml filter-sterilized trace element solution SL 10 (Tschech & Pfennig, 1984), 1 ml filter-sterilized 5-fold concentrated multivitamin solution (Balch *et al.*, 1979) and 15 ml of autoclaved 1 M $NaHCO_3$. Plates were incubated at 15 °C in the dark. Single colonies were restreaked five times for purification. Unless otherwise stated, further cultivation and phenotypic tests were performed using SWM with 0.1 g yeast extract and 0.2 g peptone l^{-1} .

Production of bacteriochlorophyll *a* (Bchl *a*) was determined by spectrophotometric analysis. Cells grown in the dark were harvested by centrifugation at 10 000 r.p.m. for 10 min, and pigments were extracted with acetone/methanol (7:2, v/v) as described by Clayton (1966). The extract was examined for light absorbency (350–900 nm) with a Beckmann DU 520 general purpose UV/VIS spectrophotometer. *Dinoroseobacter shibae* DFL 12^T (=DSM 16493^T) was used as a positive control.

The isolate was tested for growth at 4, 10, 17, 20, 25, 28, 30, 32, 35, 37, 42 and 45 °C and at pH 5–11 (in increments of 0.5 pH units) with the pH adjusted with sterile NaOH and HCl solutions (Wagner-Döbler *et al.*, 2004). It is known that the pHs of solutions pH >9.0 do not remain stable because CO_2 , either in the air or from metabolic activity, dissolves in the medium and causes the pH to drop. Therefore, only growth during the first 48 h of incubation was considered. Clear differences could be detected and any growth after the first 48 h was scored as negative. Further growth experiments were performed in artificial seawater medium (ASW) according to Zech *et al.* (2009) with addition after autoclaving of (l^{-1}) 15 ml autoclaved 1 M $NaHCO_3$ and 1 ml multivitamin solution, as above.

Growth with 0, 12.5, 25, 37.5, 50, 65, 80 and 100 g salts l^{-1} was determined in test tubes. The test medium was made from a concentrated stock salt solution (l^{-1} : 80 g NaCl, 16 g Na_2SO_4 , 1 g NH_4Cl , 12 g $MgCl_2 \cdot 6H_2O$, 2 g KCl, 0.6 g $CaCl_2 \cdot 2H_2O$, 0.76 g $NaHCO_3$; autoclaved separately) and supplemented with (l^{-1}) 0.2 g KH_2PO_4 , 0.25 g NH_4Cl , 0.05 g yeast extract, 0.05 g peptone, 1 ml multivitamin solution, as above, and 1 ml trace element solution (Zech *et al.*, 2009). Before inoculation, cells were precultured in SWM and washed once with the lowest concentration of salt solution. Osmotolerance was studied using NaCl only, at 0.01, 0.05, 0.1, 0.25, 0.37, 0.5, 0.7, 1.0, 1.5 and 2.0 M NaCl, in a minimal medium (l^{-1} : 1 g magnesium acetate, 40 mg yeast extract, 7 g $MgSO_4 \cdot 7H_2O$, 0.2 g KH_2PO_4 , 0.25 g NH_4Cl , 3 g $MgCl_2 \cdot 6H_2O$, 0.5 g KCl, 0.15 g $CaCl_2 \cdot 2H_2O$, 0.3 g $KHCO_3$; autoclaved separately), with vitamins and trace elements as mentioned above for growth with salts.

To determine the maximal doubling time, growth in SWM under optimal conditions (25 °C, pH 7.5, 3.75 % salt) was done in triplicate. Every 1–2 h, the test tubes were vigorously shaken and growth was monitored by measuring the OD_{600} (Spectronic 70) as a proxy for cell growth. Growth rates (μ) and doubling time ($t_d = \ln 2 / \mu$) were

determined by linear regression of semi-logarithmic plots of cell density against time.

Requirement for single vitamins was tested in ASW supplemented with all 20 amino acids (each 1 mM) as the carbon source. Cells were precultured in SWM and washed with ASW without substrate prior to inoculation. All amino acids were added since strain SH6-1^T did not grow in minimal medium with single amino acids. The vitamins nicotinic acid amide, thiamine, pantothenic acid, pyridoxal hydrochloride, cyanocobalamin, riboflavin and biotin (all at 0.05 mg l^{-1}) were tested in medium from which one was omitted.

Tests for growth on single substrates (1 g l^{-1}) were performed in ASW as described by Martens *et al.* (2006). Additionally, the algal osmolyte dimethylsulfoniopropionate (100 μ M) was tested. Utilization of amino acids was tested at a final concentration of 5 mM except for tyrosine, which was added to 1 mM because of precipitation at 5 mM. The pH was adjusted to 7.5. All of the test substrates are listed in the species description. Cells were precultured in SWM to exponential phase and washed twice with ASW without substrate in 2-ml reaction vessels. For each substrate, 5 ml medium was inoculated in duplicate with 10 μ l washed cells. Since no or only very weak growth was observed on single substrates, assays were supplemented with 0.1 g yeast extract l^{-1} , similarly to Wagner-Döbler *et al.* (2004). Cell cultures with 0.1 g yeast extract l^{-1} were used as a negative control. Growth was determined after incubation at 20 °C for at least 1 week by an increase in OD_{600} ; growth was scored as negative when equal to or less than that in the negative control.

Gram staining was performed using the Hucker staining method, as described by Murray *et al.* (1994). To test for cytochrome oxidase, a single colony was streaked onto a cellulose strip saturated with test reagent (l^{-1} : 1 g ascorbic acid, 10 g tetramethyl-*p*-phenylenediamine-HCl). Blue coloration showed oxidase activity (positive control *Micrococcus luteus*). To assay catalase reaction, a drop of 5 % H_2O_2 was added to a dense cell culture. *Escherichia coli* was used as a positive control. Exoenzyme activities (hydrolysis of gelatin, starch and Tween 80) were analysed on SWM solidified with 9 % (w/v) gelatin or 1.5 % (w/v) agarose and supplemented with 0.2 % (w/v) starch or 1 % (v/v) Tween 80 (Smibert & Krieg, 1994). Reduction of nitrate was tested in anoxic ASW containing 0.5 g resazurin l^{-1} . After autoclaving, the medium was reduced by addition of ~1 mg sterile sodium dithionite (causing discoloration of the resazurin) and nitrate was added as 1 g sodium nitrate l^{-1} . The medium was placed into test tubes containing a small inverted glass tube, the headspace was flushed with N_2/CO_2 (80:20, v/v) and the tubes were sealed. Substrates (l^{-1} : 0.2 g glucose and 0.1 g yeast extract) and sodium nitrate were injected from anoxic stock solutions. Cells were precultured in SWM to exponential phase under N_2 before inoculation. Accumulation of bubbles in the inverted glass tube indicated N_2 formation by nitrate reduction. *Roseobacter denitrificans* DSM 7001^T was used as a positive

control. Antibiotic susceptibility was tested by agar diffusion using penicillin G, streptomycin sulfate, chloramphenicol and kanamycin sulfate, as described in Brinkhoff *et al.* (2004). *Phaeobacter gallaeciensis* BS107^T and *Rhodobacter sphaeroides* Van Niel's ATH 2.4.1^T were used as controls. For transmission electron microscopy (TEM), cells were grown in SWM to exponential phase, washed once in distilled water, air dried on 200-mesh copper grids (Plano) and examined using a 902A electron microscope (Zeiss), as described previously (Chávez *et al.*, 2004).

Amplification and purification of the 16S rRNA gene were carried out as described by Brinkhoff & Muyzer (1997) using the primer pair 27F (GM3F) and 1492R (GM4R). Sequencing was performed by GATC Biotech (Konstanz, Germany). Phylogenetic analysis was performed using the ARB software package (<http://www.arb-home.de>; Ludwig *et al.*, 2004). Detection of genes encoding the subunits of the photosynthetic reaction centre complex (*pufL* and *pufM*) was performed by PCR using the primers *pufL* forward (5'-CTKTTGACTTCTGGGTSGG-3') and *pufM* reverse (5'-CCATSGTCCAGCGCCAGAA-3') (Béjà *et al.*, 2002). Reactions were performed in an Eppendorf thermocycler with the following programme: 94 °C for 3 min, 30 cycles at 94 °C for 1 min, 60 °C for 1 min and 72 °C for 1 min and a final extension step at 72 °C for 10 min. Amplification products were purified using the peqGOLD MicroSpin Cycle-Pure kit (PEQLAB Biotechnologie, Erlangen, Germany) and sequenced at GATC Biotech.

For the following analyses, cell biomass of strain SH6-1^T and *Pelagicola litoralis* CL-ES2^T was obtained from cells cultivated in SWM to exponential phase at 20 °C. Respiratory lipoquinones and polar lipids were extracted from 100 mg freeze-dried cells using the method described by Tindall (1990a, b). Respiratory lipoquinones were separated into their structural classes by TLC. Bands were eluted and further separated and identified by HPLC using an RP₁₈ column (Tindall, 1996). Polar lipids were separated by two-dimensional chromatography and identified on the basis of their *R_F* values in combination with their reaction with specific staining reagents (Tindall, 1990a, b). Fatty acid methyl esters were released from 10 mg freeze-dried cells using methodologies that release only ester-linked fatty acids or ester- and amide-linked fatty acids (Labrenz *et al.*, 1998; Strömpl *et al.*, 1999). Initially, the samples were analysed using the MIDI Sherlock system version 6 and the TSBA40 peak naming tables. Peaks that were not identified or were not unambiguously identified by the MIDI Sherlock system were further characterized by GC-MS using a Varian CP-3800 GC coupled to a Varian 320-MS single quadrupole MS in electron impact mode. The DNA G+C content of strain SH6-1^T was determined by extracting genomic DNA according to Cashion *et al.* (1977) and subsequently determining the deoxyribonucleosides by HPLC (Mesbah *et al.*, 1989; Tamaoka & Komagata, 1984).

After 7 days on SWM at 20 °C, colonies were circular, convex and whitish with a shiny surface and up to 0.5 mm

in diameter. Single cells grown in liquid medium were short rods to irregular elongated rods with a length of 0.5–4.0 µm and a width of 0.5–1.0 µm (Fig. S1, available in IJSEM Online). Cells were Gram-negative. Flagella were not detected by microscopy and the cells did not show clear motility; however, the cells exhibited slight wobbling.

Strain SH6-1^T grew at 4–32 °C (optimum 20–25 °C), at pH 6.0–9.5 (optimum pH 7.5–9.0) and with 1.25–8.0 % salt (optimum 3.75 % salt). The isolate had an absolute requirement for Na⁺ and its osmotolerance ranged from 0.25 to 0.7 M NaCl. Under optimum conditions in SWM, the doubling time was 3.7 h. Strain SH6-1^T showed no or only very weak growth on minimal medium; however, growth was stimulated with the addition of 0.1 g yeast extract l⁻¹. The following carbon sources supported cell growth: L-alanine, L-arginine, L-aspartic acid, L-proline, L-serine, L-tryptophan, L-tyrosine, (+)-D-xylose, (+)-D-glucose, (+)-D-mannose, (+)-D-galactose, (–)-D-fructose, (–)-D-ribose, (–)-D-mannitol, sucrose, maltose, cellobiose, trehalose, lactose, sodium acetate, sodium pyruvate, sodium malate, citric acid, disodium succinate, sodium lactate, glycerol and Tween 80. No growth was observed in the absence of vitamins. Strain SH6-1^T was dependent on pantothenic acid and nicotinic acid amide. Cells were susceptible to penicillin G, streptomycin sulfate and chloramphenicol, but not to kanamycin sulfate. The isolate exhibited oxidase, but not catalase activity.

Analysis of the 16S rRNA gene sequence indicated that strain SH6-1^T shared 96.4 % sequence similarity with *P. litoralis* CL-ES2^T, its nearest described relative (Kim *et al.*, 2008; Fig. 1). The DNA G+C content of strain SH6-1^T was 53.8 mol%. Cells contained the genes *pufLM* for the bacterial photosynthesis reaction centre; however, in laboratory cultures, no Bchl *a* or any other pigments could be detected.

The predominant respiratory lipoquinone of strain SH6-1^T was ubiquinone Q-10. The fatty acid composition was dominated by C_{18:1}ω7c; other fatty acids were C_{10:0} 3-OH, C_{16:0}, C_{12:1}, C_{12:1} 3-OH, C_{16:0}, C_{18:0}, C_{18:2}, 11-methyl C_{18:1}ω7c and summed feature 7 (comprising C_{19:1}ω6c and/or unknown ECL 18.846) (Table 1). The fatty acid compositions of strain SH6-1^T and *P. litoralis* CL-ES2^T showed some differences in fatty acids that were present in low concentrations. *P. litoralis* CL-ES2^T contained C_{16:0} 2-OH, which was not present in strain SH6-1^T, and much larger amounts of C_{14:1} 3-OH than strain SH6-1^T. Strain SH6-1^T, however, contained C_{12:1} 3-OH, which was not present in *P. litoralis* CL-ES2^T.

The polar lipids of strain SH6-1^T were phosphatidylcholine, phosphatidylglycerol, one unidentified aminolipid and one unidentified phospholipid. Comparison of the polar lipid patterns of the isolate with the reference strain revealed clear differences (Fig. 2). Further differential characteristics of strain SH6-1^T and *P. litoralis* CL-ES2^T are summarized in Table 2.

On the basis of the distinctive features presented here, we propose that strain SH6-1^T be assigned to a new genus as a

Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequences showing the phylogenetic relationships between strain SH6-1^T and representatives of the family *Rhodobacteraceae* within the class *Alphaproteobacteria*. Bootstrap values based on 1000 replicates are shown at branch nodes. Filled circles indicate that the corresponding nodes were also recovered in trees generated with the maximum-likelihood method. Selected members of the class *Gammaproteobacteria* were used as an outgroup (not shown). Bar, 5% sequence divergence.

representative of a novel species, for which the name *Planktotalea frisia* gen. nov., sp. nov. is proposed.

Description of *Planktotalea* gen. nov.

Planktotalea (Plank.to.ta'le.a. Gr. adj. *planktos* roaming, wandering; L. fem. n. *talea* a slender staff, a rod; N.L. fem. n. *Planktotalea* a planktonic/roaming rod).

Gram-negative, aerobic, oxidase-positive and catalase-negative. Cells are small to irregular rods (0.5–4.0 µm long and 0.4–1.0 µm wide). Contains *pufLM*, the genes that encode subunits of the bacterial photosynthetic reaction centre complex. Growth in minimal medium on single substrates is poor. Cells require vitamins as well as sodium ions. The major respiratory lipoquinone is Q-10. The polar lipids are phosphatidylcholine, phosphatidylglycerol, an unidentified aminolipid and an unidentified phospholipid. The major fatty acids (>1%) are C_{10:0} 3-OH, C_{12:1}, C_{12:1} 3-OH,

C_{16:0}, C_{18:0}, C_{18:1}ω7c, C_{18:2}, 11-methyl C_{18:1}ω7c and summed feature 7 (comprising C_{19:1}ω6c and/or unknown ECL 18.846); traces of C_{14:1} 3-OH are also found. Whereas most of the fatty acids were ester-linked, a small amount of C_{14:1} 3-OH was amide-linked. The genus is a member of the class *Alphaproteobacteria*, order *Rhodobacterales*. The type, and only, species is *Planktotalea frisia*.

Description of *Planktotalea frisia* sp. nov.

Planktotalea frisia (fri'si.a. L. fem. adj. *frisia* Frisian, pertaining to Frisia, a coastal region along the south-eastern corner of the North Sea, i.e. the German Bight, from where the organism was obtained).

Has the following characteristics in addition to those given in the genus description. On SWM, colonies are small, circular, convex and whitish with a shiny surface and up to

Table 1. Fatty acid compositions of strain SH6-1^T and *Pelagicola litoralis* CL-ES2^T, using methodologies which release only ester-linked (M1) or ester- and amide-linked (M2) fatty acids

–, Not detected.

Fatty acid	SH6-1 ^T		<i>P. litoralis</i> CL-ES2 ^T	
	M1	M2	M1	M2
C _{16:0}	2.44	6.44	2.91	3.26
C _{18:0}	2.57	1.75	0.75	0.70
C _{12:1} *	1.68	2.56	2.24	2.46
C _{18:1} ω7c	75.36	70.97	75.91	71.31
C _{18:2} *	10.34	11.45	10.09	9.54
C _{10:0} 3-OH	1.54	1.36	4.01	3.37
C _{12:1} 3-OH	2.53	1.82	–	–
C _{14:1} 3-OH*	–	0.18	–	2.67
C _{16:0} 2-OH	–	–	1.13	1.11
11-Methyl C _{18:1} ω7c	2.29	2.74	2.96	2.73
Summed feature 7†	1.18	0.34	–	0.14

*The fatty acid is not listed in the MIDI Sherlock TSBA40 peak naming table and its identity was confirmed by GC-MS.

†Summed features represent two or three fatty acids that cannot be separated by the Microbial Identification System. Summed feature 7 consisted of C_{19:1}ω6c and/or unknown ECL 18.846.

Fig. 2. Polar lipid profiles of strain SH6-1^T (a) and *Pelagicola litoralis* CL-ES2^T (b). AL, Unidentified aminolipid; DPG, diphosphatidylglycerol; L, lipid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PL, phospholipid.

1 mm in diameter. Grows at 4–32 °C (optimum 20–25 °C) and at pH 6.0–9.5 (optimum pH 7.5–9.0). Grows with 1.25–8.00 % salt (optimum 3.75 % salt). Has an absolute requirement for Na⁺; osmotolerance ranges from 0.25 to 0.70 M NaCl. Does not grow without the vitamins pantothenic acid and nicotinic acid amide. Does not reduce nitrate to N₂. Does not hydrolyse gelatin or starch, but is able to hydrolyse Tween 80. In ASW with 0.1 g yeast extract l⁻¹, utilizes L-alanine, L-arginine, L-aspartic acid, L-proline, L-serine, L-tryptophan, L-tyrosine, (+)-D-xylose, (+)-D-glucose, (+)-D-mannose, (+)-D-galactose, (–)-D-fructose, (–)-D-ribose, (–)-D-mannitol, sucrose, maltose, cellobiose, trehalose, lactose, sodium acetate, sodium pyruvate, sodium malate, citric acid, disodium succinate, sodium lactate, glycerol and Tween 80, but not L-asparagine, L-cysteine, L-glutamine, L-glutamic acid, glycine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-threonine, L-valine, (+)-L-arabinose, (+)-L-rhamnose, (–)-L-fucose, (–)-D-sorbitol,

Table 2. Differential characteristics of strain SH6-1^T and *Pelagicola litoralis* CL-ES2^T

Data were taken from this study and (Kim *et al.*, 2008). Both strains were rods and formed circular and convex colonies. +, Positive; w, weakly positive (OD₆₀₀ ≤ 0.2); –, negative; ND, no data available.

Characteristic	SH6-1 ^T	<i>P. litoralis</i> CL-ES2 ^T
Colony colour	Whitish	Creamy
Cell shape	Irregular	Club-shaped
Cell size (µm)	0.5–4 × 0.5–1.0	1.1–7.0 × 0.5–1.4
Motility	Slight	–
<i>pufLM</i>	+	ND
Catalase	–	+
Growth at 4 °C	+	–
DNA G + C content (mol%)	53.8	47
Salinity (%)		
Range	1.25–8.00	2–6
Optimum	3.75	3–4
Osmotolerance (NaCl; M)	0.25–0.70	ND
Growth temperature (°C)		
Range	4–32	20–30
Optimum	20–25	25
Growth pH		
Range	6.0–9.5	6.0–8.0
Optimum	7.5–9.0	7.0
Utilization of:		
Proline	w	+
Serine	w	+
Citrate	w	–
Sucrose	w	–
Trehalose	w	–
Succinate	w	–
Pyruvate	w	–
Glycerol	w	–
Hydrolysis of:		
Starch	–	+
Tween 80	w	+

(+)-D-glucosamine, laminarin, starch, inulin, xylan, sodium formate, sodium propionate or dimethylsulfoniopropionate. In laboratory culture, does not express Bchl *a* or any other pigments.

The type strain, SH6-1^T (=DSM 23709^T =LMG 25294^T), was isolated from water from a North Sea sample taken at a depth of 2 m. The DNA G + C content of the type strain is 53.8 mol%. The type strain is susceptible to penicillin G, streptomycin sulfate and chloramphenicol, but resistant to kanamycin sulfate.

Acknowledgements

We thank Heike Oetting for technical assistance with the transmission electron microscopy and Thomas Langer for carrying out growth experiments with different vitamin mixtures. This work was supported

by the Lower Saxonian Volkswagen Foundation (VW-Vorab) within the Lower Saxonian–Israeli joint program.

References

- Alderkamp, A. C., Sintes, E. & Herndl, G. J. (2006). Abundance and activity of major groups of prokaryotic plankton in the coastal North Sea during spring and summer. *Aquat Microb Ecol* **45**, 237–246.
- Balch, W. E., Fox, G. E., Magrum, L. J., Woese, C. R. & Wolfe, R. S. (1979). Methanogens: reevaluation of a unique biological group. *Microbiol Rev* **43**, 260–296.
- Béjà, O., Suzuki, M. T., Heidelberg, J. F., Nelson, W. C., Preston, C. M., Hamada, T., Eisen, J. A., Fraser, C. M. & DeLong, E. F. (2002). Unsuspected diversity among marine aerobic anoxygenic phototrophs. *Nature* **415**, 630–633.
- Brinkhoff, T. & Muyzer, G. (1997). Increased species diversity and extended habitat range of sulfur-oxidizing *Thiomicrospira* spp. *Appl Environ Microbiol* **63**, 3789–3796.
- Brinkhoff, T., Bach, G., Heidorn, T., Liang, L., Schlingloff, A. & Simon, M. (2004). Antibiotic production by a *Roseobacter* clade-affiliated species from the German Wadden Sea and its antagonistic effects on indigenous isolates. *Appl Environ Microbiol* **70**, 2560–2565.
- Brinkhoff, T., Giebel, H.-A. & Simon, M. (2008). Diversity, ecology, and genomics of the *Roseobacter* clade: a short overview. *Arch Microbiol* **189**, 531–539.
- Buchan, A., González, J. M. & Moran, M. A. (2005). Overview of the marine *Roseobacter* lineage. *Appl Environ Microbiol* **71**, 5665–5677.
- Cashion, P., Holder-Franklin, M. A., McCully, J. & Franklin, M. (1977). A rapid method for the base ratio determination of bacterial DNA. *Anal Biochem* **81**, 461–466.
- Chávez, F. P., Lünsdorf, H. & Jerez, C. A. (2004). Growth of polychlorinated-biphenyl-degrading bacteria in the presence of biphenyl and chlorobiphenyls generates oxidative stress and massive accumulation of inorganic polyphosphate. *Appl Environ Microbiol* **70**, 3064–3072.
- Clayton, R. K. (1966). Spectroscopic analysis of bacteriochlorophylls *in vitro* and *in vivo*. *Photochem Photobiol* **5**, 669–677.
- Eilers, H., Pernthaler, J., Peplies, J., Glöckner, F. O., Gerdt, G. & Amann, R. (2001). Isolation of novel pelagic bacteria from the German Bight and their seasonal contributions to surface picoplankton. *Appl Environ Microbiol* **67**, 5134–5142.
- Kim, Y.-G., Hwang, C. Y. & Cho, B. C. (2008). *Pelagicola litoralis* gen. nov., sp. nov., isolated from coastal water in Korea. *Int J Syst Evol Microbiol* **58**, 2102–2106.
- Labrenz, M., Collins, M. D., Lawson, P. A., Tindall, B. J., Braker, G. & Hirsch, P. (1998). *Antarctobacter heliothermus* gen. nov., sp. nov., a budding bacterium from hypersaline and heliothermal Ekho Lake. *Int J Syst Bacteriol* **48**, 1363–1372.
- Ludwig, W., Strunk, O., Westram, R., Richter, L., Meier, H., Yadukumar, Buchner, A., Lai, T., Steppi, S. & other authors (2004). ARB: a software environment for sequence data. *Nucleic Acids Res* **32**, 1363–1371.
- Martens, T., Heidorn, T., Pukall, R., Simon, M., Tindall, B. J. & Brinkhoff, T. (2006). Reclassification of *Roseobacter gallaeciensis* Ruiz-Ponte *et al.* 1998 as *Phaeobacter gallaeciensis* gen. nov., comb. nov., description of *Phaeobacter inhibens* sp. nov., reclassification of *Ruegeria algicola* (Lafay *et al.* 1995) Uchino *et al.* 1999 as *Marinovum algicola* gen. nov., comb. nov., and emended descriptions of the genera *Roseobacter*, *Ruegeria* and *Leisingera*. *Int J Syst Evol Microbiol* **56**, 1293–1304.
- Mesbah, M., Premachandran, U. & Whitman, W. B. (1989). Precise measurement of the G+C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int J Syst Bacteriol* **39**, 159–167.
- Murray, R. G. E., Doetsch, R. N. & Robinow, C. F. (1994). Determinative and cytological light microscopy. In *Methods for General and Molecular Bacteriology*, pp. 21–41. Edited by P. Gerhardt, R. G. E. Murray, W. A. Wood & N. R. Krieg. Washington, DC: American Society for Microbiology.
- Smibert, R. M. & Krieg, N. R. (1994). Phenotypic characterization. In *Methods for General and Molecular Bacteriology*, pp. 607–654. Edited by P. Gerhardt, R. G. E. Murray, W. A. Wood & N. R. Krieg. Washington, DC: American Society for Microbiology.
- Strömpl, C., Tindall, B. J., Jarvis, G. N., Lünsdorf, H., Moore, E. R. B. & Hippe, H. (1999). A re-evaluation of the taxonomy of the genus *Anaerovibrio*, with the reclassification of *Anaerovibrio glycerini* as *Anaerobaculum glycerini* gen. nov., comb. nov., and *Anaerovibrio burkinabensis* as *Anaerobaculum burkinensis* [corrig.] gen. nov., comb. nov. *Int J Syst Bacteriol* **49**, 1861–1872.
- Tamaoka, J. & Komagata, K. (1984). Determination of DNA base composition by reversed-phase high-performance liquid chromatography. *FEMS Microbiol Lett* **25**, 125–128.
- Tindall, B. J. (1990a). A comparative study of the lipid composition of *Halobacterium saccharovororum* from various sources. *Syst Appl Microbiol* **13**, 128–130.
- Tindall, B. J. (1990b). Lipid composition of *Halobacterium lacusprofundi*. *FEMS Microbiol Lett* **66**, 199–202.
- Tindall, B. J. (1996). Respiratory lipoquinones as biomarkers. In *Molecular Microbial Ecology Manual* (section 4.1.5, suppl. 1). Edited by A. Akkermans, F. de Bruijn & D. van Elsas. Dordrecht: Kluwer.
- Tschech, A. & Pfennig, N. (1984). Growth yield increase linked to caffeate reduction in *Acetobacterium woodii*. *Arch Microbiol* **137**, 163–167.
- Wagner-Döbler, I. & Biebl, H. (2006). Environmental biology of the marine *Roseobacter* lineage. *Annu Rev Microbiol* **60**, 255–280.
- Wagner-Döbler, I., Rheims, H., Felske, A., El-Ghezal, A., Flade-Schröder, D., Laatsch, H., Lang, S., Pukall, R. & Tindall, B. J. (2004). *Oceanibulbus indolifex* gen. nov., sp. nov., a North Sea alphaproteobacterium that produces bioactive metabolites. *Int J Syst Evol Microbiol* **54**, 1177–1184.
- Zech, H., Thole, S., Schreiber, K., Kalhöfer, D., Voget, S., Brinkhoff, T., Simon, M., Schomburg, D. & Rabus, R. (2009). Growth phase-dependent global protein and metabolite profiles of *Phaeobacter gallaeciensis* strain DSM 17395, a member of the marine *Roseobacter*-clade. *Proteomics* **9**, 3677–3697.