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Phaeobacter leonis sp. nov., an alphaproteobacterium from Mediterranean Sea sediments

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Abstract:

A novel Gram-stain-negative, strictly aerobic, heterotrophic bacterium, designated 306^T, was isolated from near-surface (109 cm below the sea floor) sediments of the Gulf of Lions, in the Mediterranean Sea. Strain 306^T grew at temperatures between 4 and 32 °C (optimum 17–22 °C), from pH 6.5 to 9.0 (optimum 8.0–9.0) and between 0.5 and 6.0% (w/v) NaCl (optimum 2.0%). Its DNA G+C content was 58.8 mol%. On the basis of 16S rRNA gene sequence similarity, the novel isolate belongs to the class *Alphaproteobacteria* and is related to the genus *Phaeobacter*. It shares 98.7% 16S rRNA sequence identity with *Phaeobacter arcticus,* its closest phylogenetic relative. It contained Q-10 as the only respiratory quinone, $C_{18:1}\omega7c$ and $C_{16:0}$ as major fatty acids (>5%) and phosphatidylethanolamine, phosphatidylglycerol, phosphatidylcholine, diphosphatidylglycerol, two unidentified lipids and an aminolipid as polar lipids. The chemotaxonomic data are consistent with the affiliation of strain 306^T to the genus *Phaeobacter*. Results of physiological experiments, biochemical tests and DNA–DNA hybridizations (with *P. arcticus*) indicate that strain 306^T is genetically and phenotypically distinct from the five species of the genus *Phaeobacter* with validly published names. Strain 306^T therefore represents a novel species, for which the name *Phaeobacter leonis* sp. nov. is proposed. The type strain is 306^T (=DSM 25627^T=CIP 110369^T=UBOCC 3187^T).

Main text

The genus *Phaeobacter* was introduced by Martens *et al.* (2006), after reclassification of *Roseobacter* species as members of the genus *Phaeobacter* on the basis of common genetic and phenotypic characteristics. The genus *Phaeobacter* incorporates Gram-stain-negative, aerobic, chemoorganoheterotrophic, ovoid or rod-shaped, motile strains containing Q-10 as the main respiratory quinone. In November 2012, this genus encompassed five species: *Phaeobacter gallaeciensis* was isolated from cultures of a bivalve mollusc (Martens *et al.*, 2006; Ruiz-Ponte *et al.*, 1998), *Phaeobacter inhibens* from the German Wadden Sea (Martens *et al.*, 2006), *Phaeobacter arcticus* from marine sediments of the Arctic Ocean (Zhang *et al.*, 2008), *Phaeobacter daeponensis* from tidal flat sediment of the Yellow Sea (Yoon *et al.*, 2007) and *Phaeobacter caeruleus* from a marine electroactive biofilm (Vandecandelaere *et al.*, 2009).

The present study describes a novel bacterial isolate, strain 306^T, isolated from marine sediments of the Mediterranean Sea.

In October 2008, a sediment core was recovered in the Gulf of Lions (42° 41.596′ N, 03° 50.493′ E; Water depth: 291 m), in the Western Mediterranean Sea and subsampled for microbiological analyses as described elsewhere (Ciobanu *et al.*, 2012). An anoxic sediment sample from 109 cm below the sea floor was spread on an agar plate composed

of artificial seawater (Ciobanu *et al.*, 2012) and glucose (10 mM) and then incubated at 20°C. After 10 days of incubation, a beige colony was picked, purified by repeated streaking on marine agar 2216 (MA; Difco) plates and referenced as strain 306^T. Stock cultures were stored at –80°C, in marine broth 2216 (MB, Difco) supplemented with 5% (v/v) DMSO, until characterization.

Both strands of the almost complete 16S rRNA gene of strain 306^T were amplified from a single colony using the universal primers 8F, 1492R (DeLong *et al*., 1992) and Eubint (5'-GCG CCA GCA GCC GCG GTA A-3'), and then sequenced with the Bid Dye technology (Beckman Coulter Genomics, Essex, UK). Contiging was performed from 5 overlapping sequence fragments. The sequence obtained was a continuous stretch of 1355 bp. Phylogenetic analyses were done with SeaView4 using the Muscle Multiple Alignment option to align sequences. Sequences of the nearest neighbors used to perform the alignment were imported from the Ribosomal Database Project (RDP) website. Phylogenetic trees were constructed using the SeaView4 software, on the basis of Neighbour Joining and PhyML (GTR model) algorithms. The robustness of the inferred topologies was assessed by bootstrap analyses based on 1000 replications. Percentages of sequence identity were calculated under BioEdit with the Sequence Identity Matrix calculation option. The 16S rRNA gene-based analysis located the strain 306^T within the class *Alphaproteobacteria*, in the bacterial domain. The results of different phylogenetic reconstructions performed with two treeing algorithms located the novel isolate within the genus *Phaeobacter*, order *Rhodobacterales* (Fig. 1). Within this genus, the novel isolate clustered with *Phaeobacter arcticus* (Zhang *et al*., 2008), sharing 98.7% 16S rRNA gene

sequence similarity with the type strain of this species, suggesting that the novel isolate may represent a novel species of the genus *Phaeobacter* (Stackebrandt & Ebers, 2006). However, with the data set used for the phylogenetic reconstruction, the genus *Phaeobacter* does not form a monophyletic group (Fig. 1). This inconsistency between tree topology and taxonomy has already been observed in previous phylogenetic reconstructions including *Phaeobacter* and *Leisingera* species (Yoon *et al*., 2007). This may highlight the need for further taxonomic revision and the existence of a complex *Leisingera*/*Phaeobacter* group. Considering that the genus *Leisingera* encompasses only two species and displays a short phylogenetic distance with the presently described members of the genus *Phaeobacter*, it is also possible that this topology is a phylogenetic artefact. DNA-DNA hybridization experiments were performed to further elucidate the taxonomic position of strain 306^T and to determine its DNA-DNA relatedness value with respect to *P. arcticus* DSM 23566^T. They were performed by the Identification Service of the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ, Braunschweig, Germany), as described by De Ley *et al*. (1970), with the modifications described by Huss *et al*. (1983), using a Cary 100 Bio UV/VIS-spectrophotometer. Level of DNA-DNA relatedness between strain 306^T and *P. arcticus* was far below the threshold value of 70% (9.8 %), suggesting that strain 306^T represents a novel species.

The DNA G+C content was determined by the Identification service of the DSMZ, by HPLC analysis, from the ratio of deoxyguanosine (dG) and thymidine (dT) according to the method of Mesbah *et al*. (1989). The DNA G+C content of strain 306^T was 58.8 mol%.

112 Colonies of the novel isolate on MA were smaller than 1 mm in diameter after 3 days
113 incubation, beige-pigmented, regularly circular, convex, creamy, smooth with an entire edge.
114 Morphological characteristics of the cells were determined by light microscopy (Olympus
115 BX60), by scanning electron microscopy (FEI Quanta 200) and by transmission electron
116 microscopy (Jeol JEM 100 CX II). SEM observations were done after a standard HMDS-based
117 (HexaMethylDiSilasane) preparation and TEM observations after negative staining with uranyl
118 acetate (2% v/v). Briefly, cells of strain 306^T were short thickset rods or oval-shaped cells of 0.7-
119 2 μm in length (mean 1.5 ± 0.37, n=30) and 0.4-1 μm in width (mean 0.58 ± 0.07, n=30) in the
120 mid-exponential phase of growth (see supplementary Fig. S1a and S1b in IJSEM Online). Cells
121 could occur singly but formed often chains of about 5 cells or were grouped in aggregates of up
122 to 30 cells (Fig. S1a). They divided by constriction (Fig. S1b). Cells were flagellated and highly
123 motile. Gram staining was performed as described by Gerhardt *et al*. (1994), except that the
124 discoloration step was done with ethanol 70% (v/v); Cells stained Gram-negative. Formation of
125 spores was never observed.
126
127 Chemotaxonomic analyses were performed on mid to late-exponential phase of growth
128 cultures grown for 4-5 days in MB, at 20°C with shaking (90 rpm). The following
129 procedures were used: the determination of whole-cell fatty acid composition was done by the
130 standard protocol of the Sherlock Microbial Identification System (MIDI Inc, Newark, USA)
131 (Kuykendall *et al.,* 1988), the analysis of respiratory quinones was carried out by thin layer
132 chromatography and then by HPLC, as described elsewhere (Tindall, 1990a, 1990b), and
133 separation of polar lipids was performed by two dimensional silica gel thin layer chromatography

followed by a revelation of total lipids and specific functional groups, as described elwewhere (Bligh and Dyer, 1959; Tindall *et al.,* 2007). Strain 306^T contained Q-10 as the unique respiratory quinone. The main polar lipids included phosphatidylethanolamine, phosphatidylglycerol, phosphatidylcholine, diphosphatidylglycerol, an unidentified aminolipid and two unidentified lipids (Fig.S3). The cellular fatty acids (>1%) in strain 306^T comprised $C_{18:1}\omega7c$, $C_{16:0}$, 11-methyl $C_{18:1}$ $\omega7c$, $C_{10:0}$ 3-OH, $C_{18:0}$ and an unidentified cellular fatty acid with an equivalent chain length (ECL) of 11.799 (Table S1). The presence of $C_{18:1}$ $\omega7c$ together with Q-10 is typical of the vast majority of taxa within the *Alphaproteobacteria*. *Phaeobacter arcticus* and strain 306^T appear to be the only members of the genus *Phaeobacter* that do not produce $C_{12:0}$ 3-OH. These chemotaxonomic characteristics, and especially the quinone content and polar lipid pattern, support the affiliation of strain 306^T to the genus *Phaeobacter*.

Unless stated otherwise, physiological characterization was carried out aerobically in MB medium in triplicate and the cell suspension was incubated with shaking in the dark. The determination of temperature, pH and NaCl ranges for growth were done in 10 ml aerobic tubes of MB. Growth was monitored routinely by measuring the increase in optical density at 600 nm using a Spectronic 401 spectrophotometer. Cell numbers were determined by direct cell counting using a modified Thoma chamber (depth 10 μm). Growth rates were calculated using linear regression analysis of five to nine points along the linear portions of the growth curves logarithmically-transformed. The determination of the temperature range for growth was tested over the range 4-37°C (i.e. 4, 10, 17, 22, 30, 32, 34, 36, 37°C). The strain was found to be mesophilic (Fig.S2), growing at 4-32°C (optimally 17-22°C), but no growth occurred at 34°C. Salt tolerance was tested at 20°C in MB prepared with various concentrations of NaCl (0, 0.5, 2,

156 3, 4, 6, 8, 15 and 30% w/v). The NaCl concentrations allowing growth ranged from 0.5% (w/v)
157 to 6% NaCl, with an optimum at 2%. No growth was observed at 8% (w/v) NaCl or without
158 NaCl. The pH range for growth was tested from initial pH 3.0 to initial pH 11.0, at ambiant
159 temperature, in basal MB medium buffered and adjusted to the required pH (initial pH at 20 °C),
160 as described elsewhere (Alain *et al.*, 2002). Growth was observed from pH 6.5 to pH 9.0, the
161 optimum being around pH 8.0-9.0. No growth was observed at pH 6.0 and below, neither at
162 pH10.
163
164 Biochemical characteristics of strain 306^T were determined, in duplicates, at 25°C by using
165 Api20E and Api20NE microplates (Biomerieux) according to the manufacturer's instructions.
166 These galleries were inoculated with cells grown on MB plates and suspended in a 3% (w/v)
167 NaCl solution. These tests indicated that strain 306^T was weakly positive for trisodium citrate
168 assimilation, phenylacetic acid assimilation (Api20NE), capric acid oxidation and positive for
169 phenylacetic acid oxidation. To investigate the capacity of the strain to catabolize different
170 substrates as sole carbon and energy sources with oxygen as a terminal electron acceptor, the
171 strain was grown in the dark, on the mineral basis of MB medium (depleted of all carbon and
172 energy sources) supplemented with one substrate: cellulose, D(+)cellobiose dextrin
173 D(+)galactose D(-)fructose D(+)lactose ($1 g.l^{-1}$), maltose, D(+)mannose, D(+)rhamnose, D(-
174)ribose, sucrose, L(+)arabinose, D(+)xylose, (D+)trehalose for sugars (all at 10mM), betaine
175 succinate malonate, malate, ascorbate, acetate, propionate, fumarate, lactate, citrate for organic
176 acids (all at 1 mM), ethanol, isopropanol, glycerol, D(-)sorbitol, mannitol for alcohols (all at 1%
177 w:v), L-alanine, L-arginine, L-aspartate, L-asparagine, L-cysteine, L-glutamine, L-glutamate, L-

178 glycine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-proline,
179 L-serine, L-threonine, L-tryptophane, L-tyrosine, L-valine for amino acids (all at 1mM) and
180 elastine, collagene and keratine for proteins (all at 1g.l^{-1}). Tween 80 degradation was investigated
181 on Noble agar (Sigma-Aldrich) plates prepared with the mineral basis of MB medium and
182 covered of the susbtrate (0.75mM). Metabolic properties of strain 306^T are summarized in Table
183 1 and in the species description. The ability of the strain to grow anaerobically and to ferment
184 complex organic matter or carbohydrates (yeast-extract 1 g.l^{-1}, peptone 5 g.l^{-1} and glucose 10
185 mM) was investigated under an atmosphere of N_2 (100%) on a degassed and reduced MB
186 medium. The obligately anaerobic fermentative bacterium *Aminomonas paucivorans* was used as
187 a control. Strain 306^T does not ferment proteinaceous substrates not carbohydrates. Reduction of
188 nitrate was tested in sulfate depleted mineral basis with 10 mM of nitrate as terminal electron
189 acceptor and 10 mM of succinate as carbon source. As control, *Shewanella profunda*, a nitrate
190 reducing bacterium, was used. The novel isolate does not respire nitrate.

191 Susceptibility to antibiotics was investigated, at 20°C, on MA plates using the diffusion disc
192 method. Resistance to ampicillin, vancomycin, streptomycin, chloramphenicol (diluted in
193 ethanol), kanamycin, rifampicin (diluted in DMSO), penicillin G and tetracycline was
194 investigated at 10, 30 and 100 µg. Results were read after 4 days incubation and scored as
195 positive at zone diameters above 10 mm. Strain 306^T was susceptible to all antibiotics but for
196 different quantities of antibiotic : 10 ng for chloramphenicol, ampicillin, rifampicin and penicillin
197 G, 30 ng for streptomycin, kanamycin and tetracycline, and 100 ng for vancomycin. The results
198 of the phenotypic analyses are detailed in the species description and in Table 1.
199

In conclusion, strain 306^T branches with the genus *Phaeobacter*. Its phenotypic chemotaxonomic and genotypic properties described herein generally coincide with the minimal characteristics described for this taxon (Martens *et al.,* 2006), thus confirming the belonging to this genus. Within this genus, the novel isolate is most closely related to *Phaeobacter arcticus*, but exhibits a low DNA-DNA relatedness with the type strain of this species, indicating that these strains represent two distinct genomic species. In addition to the genomic distance, strain 306^T can be distiguished from *Phaeobacter arcticus* in terms of a number of phenetic features. These distinctive criteria are detailed in Table 1. In brief, the novel taxon differs from *P. arcticus* strain 20188^T by its temperaure (4-32°C), NaCl (0.5-6%) and pH (6.5-9) ranges for growth, its DNA G+C content and its utilization profile of carbon sources. In conclusion, in view of all the above-mentioned distinctive features, we propose that the isolate 306^T should be assigned as the type strain of a novel species, for which the name *Phaeobacter leonis* sp. nov. is proposed.

Description of *Phaeobacter leonis* sp. nov

(le.o'nis., L. gen n. leonis, of a lion, named after *sinus Leonis* the Medieval Latin name of Gulf of Lions, in reference to the origin of the type strain).

Cells are Gram-negative, rod- or oval-shaped, 0.7-2.5 µm x 0.4-1 µm in size and motile. Colonies on MA are beige, regularly circular, convex, translucent, smooth with an entire edge and creamy. The strain grows aerobically and produces catalase and cytochrome oxidase. Growth occurs at 4 – 32°C with an optimum at 17-22°C, pH 6.5 – 9 with an optimum at 8-9 and with NaCl concentration ranging fom 0.5 – 6 % (w:v) with an optimum at 2%. Negative for nitrate reduction, indole formation, arginine dihydrolase, urease, lysine decarboxylase, ornithine

decarboxylase, tryptophan deaminase, gelatinase, β-glucosidase, β-galactosidase, glucose assimilation, N-acetyl-glucosamine assimilation, potassium gluconate assimilation, capric acid assimilation, adipic acid assimilation, malic acid assimilation. Positive for phenylacetic assimilation. Weakly positive for trisodium citrate assimilation, phenylacetic oxidation and capric acid oxidation. The following substrates are used as sole carbon source : L(+)arabinose, succinate, propionate, fumarate, ethanol, L-alanine, L-asparagine, L-threonine, L-leucine, L-phenylalanine, keratine, collagene, D(-)trehalose, D(-)fructose, D(+)mannose, D(+)rhamnose, D(-)ribose, sucrose, citrate, D-mannitol, L-arginine, L-glutamine, L-methionine, L-serine, L-proline, L-glycine, L-tryptophan, L-tyrosine, L-isoleucine, keratine and collagene. The following substrates are not used as sole carbon source : dextrine, cellulose, D(+)cellobiose, D(+)glucose, maltose, D(+)xylose, betaine, malonate, acetate, malate, ascorbate, lactate, isoropanol, glycerol, sorbitol, L-glutamate, L-histidine, L-valine, L-cysteine, L-leucine, L-aspartate and elastine.

The main fatty acids comprise $C_{18:1}$ ω7-c and $C_{16:00}$.The main polar lipids are diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylcholine, an unidentified aminolipid and 2 unidentified polar lipids. Ubiquinone-10 (Q-10) is the only isoprenoid quinone. The DNA G + C content is around 59mol%.

The type strain, 306^T (DSM 25627^T, CIP 110369^T, UBOCC 3187^T) was isolated from sediments collected at 109 cm below the sea floor, in the Gulf of Lions, in the Western Mediterranean Sea.

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303

304 ## Tables and figures

305

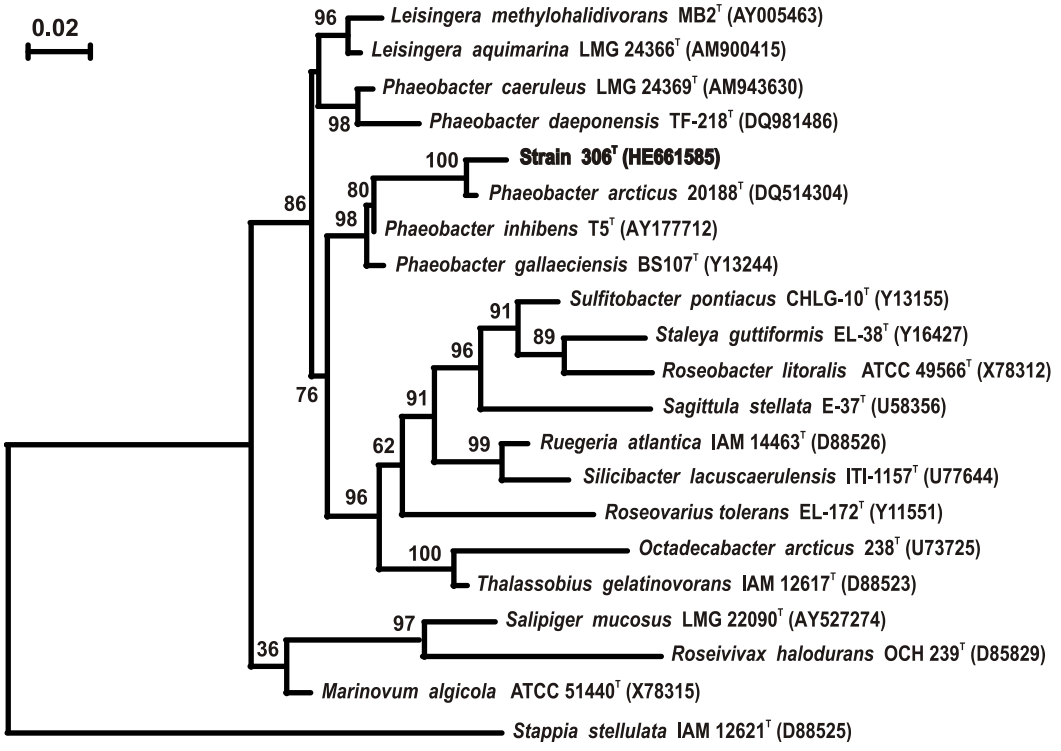

FIG. 1. Phylogenetic tree based on 16S rRNA gene sequences showing the relationships between strain 306^T and its nearest phylogenetic neighbours. The topology shown was calculated with the maximum likelihood algorithm. Accession numbers are indicated in brackets. Bootstrap values (%) are indicated at the branch nodes and were calculated from1000 resample datasets. *Stappia Stellulata* was used as an outgroup.

Table 1: Phenotypic characteristics that differentiate strain 306^T from related species of the genus *Phaeobacter*.

Only the data related to strain 306^T were experimentally obtained. Data concerning strains 2 to 6 were obtained from literature.

Taxa: 1, strain 306^T ; 2, *P. arcticus* (Zhang *et al*., 2008); 3, *P. gallaeciensis* (Ruiz-Ponte *et al*., 1998 ; Martens *et al*., 2006) ; 4, *P. inhibens* (Martens *et al*., 2006) ; 5, *P. daeponensis* (Yoon *et al*., 2007); 6, *P. caeruleus* (Vandecandelaere *et al., 2009)* +, Positive ; —, Negative ; ND, no data available ; Y, Yes.

Characteristic	1	2	3	4	5	6
Motility	Y	Y	Y	Y	Y	Y
Size (µm)	2.5-0.7x 1-0.4	6.1-2.6x0.5-0.3	1x1.5	2.5-1.7x1-0.7	0.4-0.9x0.7-2.0	0.9x1.8
- Colony	Beige	Yellow	Brown	Brown	No pigment	Blue
- Temperature range (opt)	4 – 32 (17-22)	0 – 25 (20)	<4 – 35 (28)	15 – 37 (23-27)	4 – 42 (ND)	4 – 45† (20–28)
- pH range (opt)	6.5 – 9 (8-9)	5 – 10 (6-9)	6 – 9.5 (7.5)	7 – 10 (7)	5.5 – 8 (7-8)	6 – 9 (6.5-8)
- NaCl range %w:v (opt)	0.5 – 6 (2)	2 – 9	0.06 – 9 (3-4)	0.6 – 11.5 (1.2)	ND – 8‡ (ND)	2 – 5† (3-4)
- Hydrolysis of :						
Tween 80	-	nd	-	+	-	+$
D(+)cellobiose	+	-	-	+	-	ND
- Growth with:						
L(+)arabinose	+	+§	nd	-	-	-$
D(+)cellobiose	-	-§	+	+*	-	ND
D(-)fructose	-	+§	+	+*	-	ND
D(+)galactose	-	-§	+	+*	-	ND
D(-)maltose	-	+§	+	+	-	-
Sucrose	+	+§	+	-	-	ND
D(+)trehalose	+	-§	+	+	-	ND
Betaine	-	ND	+	+*	ND	ND
D(-)lactate	+	-§	+	+*	ND	ND
D(-)succinate	+	-§	-	+*	+	ND
Sorbitol	-	-§	+	+	-	ND
L-glutamate	-	-§	+	+	-	ND
L-leucine	-	-§	+	+	+	ND
L-serine	+	-§	-	+	+	ND
- DNA G+C content (mol %)	58.8	59.6	57.6-58	55.7	64.9	63.6

* Utilization of these C sources was investigated in ASW medium with 10 mg.l^{-1} casein hydrolysate.

† These characteristics were tested in R2A medium.

‡ These characteristics were tested in trypticase soy broth medium.

§ These characteristics were determined with Api20NE or Api20E galleries.

Supplementary tables and figures

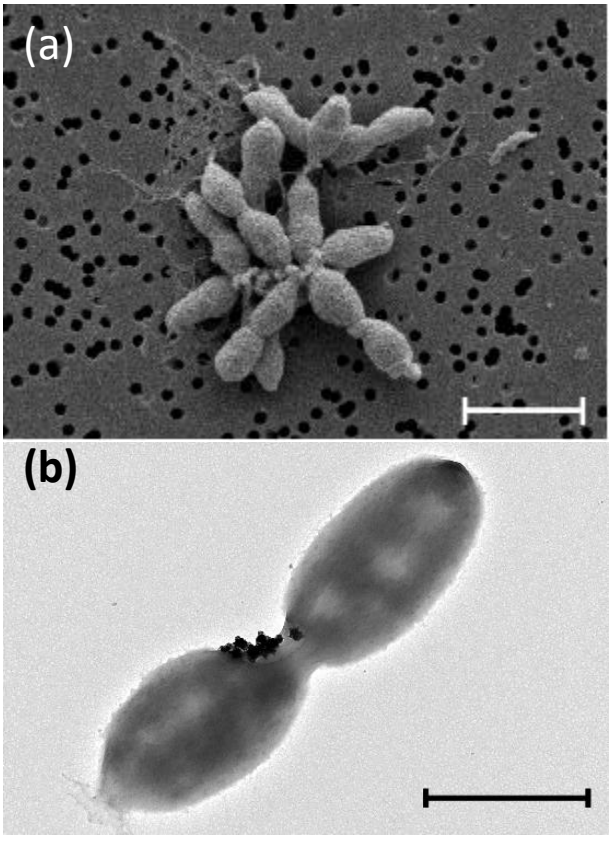

Fig. S1. Scanning (a) and transmission (b) electron micrographs of cells of strain 306^T in exponential growth phase showing cellular aggregates (a) and division by constriction (b). Bars, 2 µm (a) and 1 µm (b).

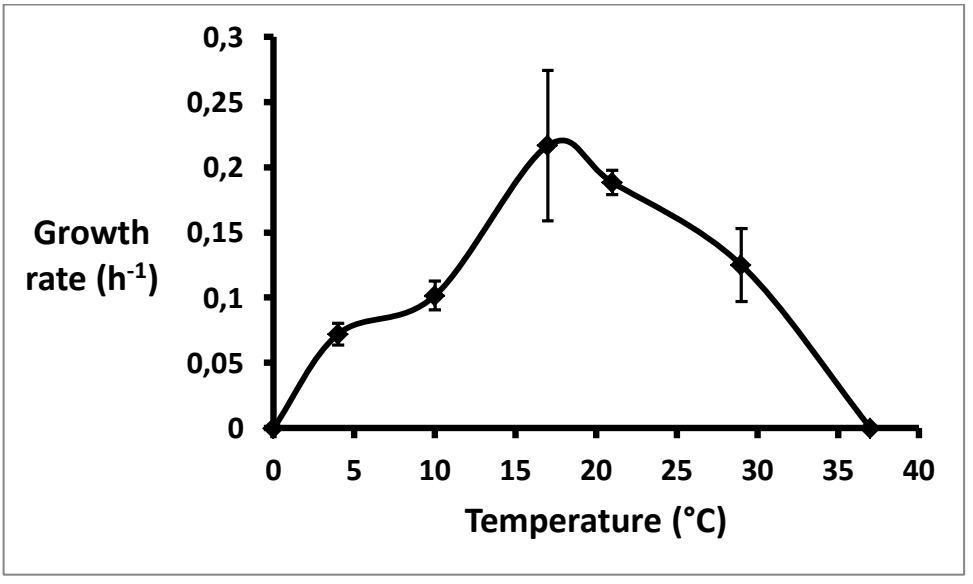

Fig. S2. Effects of temperature on the maximum growth rate of strain 306^{T}. Bars indicate confidence intervals.

Fig. S3. Polar lipids of strain 306^T following separation by two-dimensional TLC. PE, Phosphatidylethanolamine; PG, Phosphatidylglycerol; PC, Phosphatidylcholine; DPG, Diphosphatidylglycerol; AL1, Unidentified aminolipid; L1-L2, Unidentified lipids.

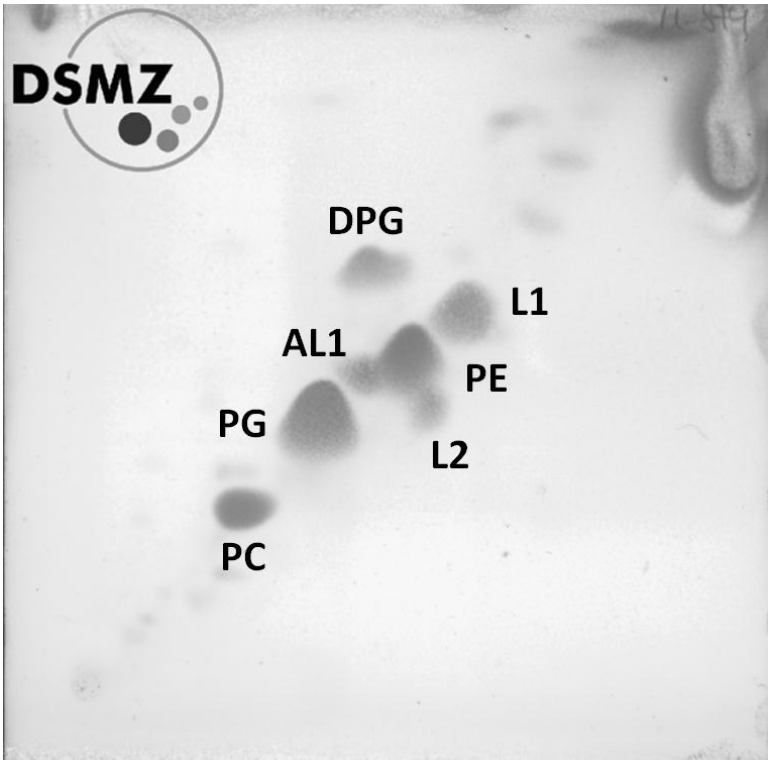

Table S1. Whole-cell fatty acid profile of mid to late exponential phase of growth cells of strain 306^T cultivated on MB2216 medium. 92.28% of the fatty acid peaks could be assigned by the Sherlock Microbial Identification System (MIDI Inc, Newark, USA). Major fatty acids are indicated in bold. All data have been obtained on exponential phase of growth cultures grown on Marine Broth 2216 (Difco) or Marine Agar 2216 (Difco).

Species: 1, Strain 306^T; 2, *Phaeobacter arcticus* 20188^T (Zhang *et al*.); 3, *Phaeobacter gallaeciensis* (Yoon *et al.*, 2007); 4, *Phaeobacter inhibens* (Yoon *et al.*, 2007); 5, *Phaeobacter daeponensis* (Yoon *et al.*, 2007); 6, *P. caeruleus* **(**Vandecandelaere *et al.,* 2009).
Values are percentages of the total fatty acids. ND, Not detected.
Legend: ECL, equivalent chain-length. * Data from Ruiz-Ponte *et al*., 1998.**Summed feature 3 contains $C_{16:1}$ $\omega 7c$ and/or 2-OH iso-$C_{15:0}$ and summed feature 5 contains $C_{18:2}$ $\omega 6,9c$ and/or ante-$C_{18:0}$.

	Proportion (%)					
Fatty acid	**1**	**2**	**3**	**4**	**5**	**6**
Saturated						
$C_{12:0}$	0.23	0.86	0.4	0.4	1.2	<1%
$C_{16:0}$	**6.05**	**9.69**	**6.3**	**5.2**	**8.6**	**4.2**
$C_{17:0}$	0.1	ND	0.2	0.2	0.6	ND
$C_{18:0}$	1.56	0.53	1.3	2.0	2.4	1.0
Monounsaturated						
$C_{18:1}\omega 9c$*	0.14	ND	0.92	1.11	ND	ND
$C_{18:1}\omega 7c$	**84.11**	**44.63**	**74.5**	**70.8**	**57.7**	**81.5**
$C_{18:1}\omega 5c$	0.13	ND	ND	ND	ND	ND
$C_{20:1}\omega 7c$	0.1	ND	ND	ND	ND	ND
Hydroxy						
3-OH $C_{10:0}$	1.75	6.75	1.9	1.8	1.7	2.8

2-OH $C_{16:0}$	0.65	3.95	2.7	2.8	5.6	2.4
2-OH $C_{18:1}$	0.12	0.59	ND	ND	ND	ND
Methyl-substituted						
11-methyl $C_{18:1}\omega 7c$	2.11	18.1	7.8	11.8	16.6	1.3
Summed featured						
Summed feature 3**	0.18	2.30	0.3	0.2	0.5	ND
Summed feature 5**	0.16	ND	ND	ND	ND	ND
Unknown component in the MIS database						
ECL 11.799	2.60	10.88	2.6	2.7	2.3	2.3