IJSEM Papers in Press. Published September 3, 2010 as doi:10.1099/ijs.0.023325-0

# *Pelagibacterium halotolerans* gen. nov., sp. nov. and *Pelagibacterium luteolum* sp. nov., novel members of the family *Hyphomicrobiaceae*

Xue-Wei Xu,[1,2] Ying-Yi Huo,[3] Chun-Sheng Wang,[1,2] Aharon Oren,[4] Heng-Lin Cui[5], Eve Vedler[6] and Min Wu[3]

[1,2] Laboratory of Marine Ecosystem and Biogeochemistry[1], and Second Institute of Oceanography[2], State Oceanic Administration, Hangzhou 310012, People's Republic of China

[3] College of Life Sciences, Zhejiang University, Hangzhou 310058, People's Republic of China

[4] Institute of Life Sciences, and the Moshe Shilo Minerva Center for Marine Biogeochemistry, The Hebrew University of Jerusalem, Jerusalem 91904, Israel

[5] School of Food & Biological Engineering, Jiangsu University, Zhenjiang 212013, People's Republic of China

[6] Institute of Molecular and Cell Biology, Tartu University, Tartu 51010, Estonia

**Correspondence:** Xue-Wei Xu; Email: xuxw@sio.org.cn

**Running title:** *Pelagibacterium halotolerans* gen. nov.

**Subject category:** New taxa of *Proteobacteria*

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequence of strain B2$^T$ and 1_C16_27$^T$ are EU709017 and EF540455.

Detailed fatty acid profiles of strain B2$^T$ and 1_C16_27$^T$, transmission electron micrographs of strain B2$^T$ and 1_C16_27$^T$, maximum-parsimony and maximum-likelihood 16S rRNA gene sequence-based phylogenetic trees and five chromatograms of the polar lipids of strain B2$^T$ and 1_C16_27$^T$ as well as other related members of the family *Hyphomicrobiaceae* are available as supplementary material.

**Summary**

Two Gram-negative, motile, aerobic bacterial strains, designated B2$^T$ and 1_C16_27$^T$, were isolated from a seawater sample collected from the East China Sea and a semi-coke sample from the north-eastern region of Estonia, respectively. Their genetic, phenotypic and chemotaxonomic properties were studied. The isolates were short rods with polar flagella and were positive for catalase and oxidase activities. Q-10 was the predominant respiratory ubiquinone. The major polar lipids were phosphatidylglycerol, diphosphatidylglycerol and two unidentified glycolipids. The major fatty acids were nonadecanoic acid ($C_{19:0}$ cyclo), octadecaenoic acids ($C_{18:1}$, $C_{18:0}$ and $C_{18:0}$ 3-OH) and hexadecaenoic acid ($C_{16:0}$). The G+C content of the genomic DNA was 58.1 - 59.3 mol%. 16S rRNA gene sequence analysis revealed that these two isolates represent a distinct lineage within the family *Hyphomicrobiaceae*. The phylogenetically closest relatives were *Cucumibacter* (92.7 - 93.7 % 16S rRNA gene sequence similarity), *Devosia* (92.9 - 94.4 % similarity) and *Zhangella* (91.7 - 92.1 % similarity). Differential phenotypic properties, together with the phylogenetic and genetic distinctiveness, revealed that strain B2$^T$ and 1_C16_27$^T$ could be differentiated from each other and from the members of the genera *Cucumibacter*, *Devosia* and *Zhangella*. Therefore it is proposed that strain B2$^T$ and 1_C16_27$^T$ represent two novel species in a new genus, for which the names *Pelagibacterium halotolerans* gen. nov., sp. nov. (type strain B2$^T$ = CGMCC 1.7692$^T$ = JCM 15775$^T$) and *Pelagibacterium luteolum* sp. nov. (type strain 1_C16_27$^T$ = CGMCC 1.10267$^T$ = JCM 16552$^T$ = CELMS EEUT 1C1627$^T$) are proposed.

The family *Hyphomicrobiaceae*, belonging to the class *Alphaproteobacteria*, comprises a morphologically and metabolically heterogeneous group of microorganisms (Garrity *et al.*, 2005). The establishment of the family *Hyphomicrobiaceae* was mainly based on the phylogenetic relationship of 16S rRNA gene sequences (Garrity *et al.*, 2005; Lee *et al.*, 2005). At the time of writing, the family comprised 18 genera: including *Ancalomicrobium*, *Angulomicrobium*, *Aquabacter*, *Blastochloris*, *Cucumibacter*, *Devosia*, *Dichotomicrobium*, *Filomicrobium*, *Gemmiger*, *Hyphomicrobium*, *Maritalea*, *Methylorhabdus*, *Pedomicrobium*, *Prosthecomicrobium*, *Rhodomicrobium*, *Rhodoplanes*, *Seliberia* and *Zhangella* (Euzéby, 1997).

Most species within the family *Hyphomicrobiaceae* have been isolated from various non-marine habitats (freshwater, soil, plant roots, sewage, swamps, activated sludge, chicken, saline pond and lake sediment, etc.). Only a few have been isolated from offshore seawater: *Cucumibacter marinus*, *Filomicrobium fusiforme*, *Hyphomicrobium aestuarii* and *Zhangella mobilis* (Gliesche *et al.*, 2005; Schlesner, 2005; Hwang & Cho, 2008; Xu *et al.*, 2009). In this paper, we present a polyphasic study describing two novel chemoheterotrophic bacteria, isolated from a seawater sample off the Chinese coast and a semi-coke sample from Estonia, which belong to this family.

A sample was collected from the East China Sea (125°59′24″E, 30°58′16″N) from a depth of 70 m (temperature 16.7 °C; salinity 33.95‰). Approximately 100 µl seawater was plated on marine agar 2216 (MA). After 3 days of aerobic incubation at 30 ºC, one light yellowish colony, designated B2$^T$, was picked. Strain 1_C16_27$^T$ was isolated from a sample collected from oil shale chemical industry solid waste (semi-coke) depository area in the north-eastern region of Estonia (59°23′44″E, 27°13′5″N) in October 2003. Ten sub-samples of semi-coke from a depth of 5-15 cm were taken with a soil corer and then mixed to form a composite sample. Microbial cells were suspended from the soil sample into sterile 0.9 % NaCl solution by vortexing. After setting of the soil particles, 100 µl of the clear supernatant was plated onto minimal medium agar plate (M9-salts supplemented with trace elements) with hexadecane as the sole carbon and energy source (a piece of filter paper, soaked with hexadecane, was placed inside the cover lid of the agar plate) (Truu *et al.*, 2003). After one

week of aerobic incubation at 22 °C, one yellowish colony, designated 1_C16_27$^T$, was picked. The two strains were purified by repeated restreaking; purity was confirmed by the uniformity of cell morphology. Unless otherwise stated, strain B2$^T$ and 1_C16_27$^T$ were maintained on yeast extract broth (YEB, basal medium supplemented with 5 g $l^{-1}$ yeast extract and 8.3 g $l^{-1}$ NaCl) medium (Mikhailov *et al.*, 2006). The basal medium (BM) contained (per l distilled water): $NH_4Cl$ 1.0 g, $K_2HPO_4$ 0.044 g, $FeSO_4 \cdot 7H_2O$ 0.028 g, artificial seawater 500 ml, Tris-HCl (1 M, pH 7.5) 50 ml. Artificial seawater contained (per l distilled water): NaCl 23.4 g, $MgSO_4 \cdot 7H_2O$ 24.6 g, KCl 1.5 g, $CaCl_2$ 2.9 g.

The optimal conditions for growth were determined in PYM and YEB medium with different NaCl concentrations (0, 1.5, 3.0, 4.0, 5.0, 6.0, 8.0, 10.0, 12.0, 13.0, 14.0 and 15.0%, w/v) (Nakagawa *et al.*, 1996). The PYM medium contained (per l distilled water): peptone (BD) 10.0 g, yeast extract (BD) 2.0 g, $MgSO_4 \cdot 7H_2O$ 1.0 g, pH 7.0. The pH range for growth was determined by adding MES (pH 5.0-6.0), PIPES (pH 6.5-7.0), Tricine (pH 7.5-8.5) and CAPSO (pH 9.0-10.0) to YEB medium at a concentration of 40 mM. The temperature range for growth was determined by incubating at 4, 10, 15, 20, 25, 30, 35, 40, 42, 45 and 48 ºC. Cell motility and morphology were examined by optical microscopy (BX40, Olympus). The presence of flagella was confirmed by transmission electron microscopy (JEM-1230, JEOL).

Oxidase activity was determined by oxidation of 1% *p*-aminodimethylaniline oxalate. Catalase activity was determined by bubble production in 3% (v/v) $H_2O_2$ solution (Dong & Cai, 2001). Single carbon source assimilation tests were performed by using BM medium supplemented with 1.5 % (w/v) NaCl. The corresponding filter-sterilized sugar (0.2%), alcohol (0.2%), organic acid (0.1%) or amino acid (0.1%) was added into liquid medium. Acid production was tested by using the modified MOF medium supplemented with 1 % sugars or alcohols (Leifson, 1963; Xu *et al.*, 2008). Nitrate reduction, gluconate oxidation, lecithinase and urease activities and the ability to hydrolyze aesculin, casein, DNA, gelatin, Tween 40, Tween 60 and Tween 80 were determined according to Dong & Cai (2001). Susceptibility to antibiotics was detected on marine 2216 agar (MA; BD) or PYM agar plates using antibiotic discs with the following concentrations (μg unless otherwise stated):

amoxicillin (10), ampicillin (10), bacitracin (0.04 IU), cefotaxime (30), cefoxitin (30), chloramphenicol (30), erythromycin (15), kanamycin (30), neomycin (30), nitrofurantoin (300), novobiocin (30), nystatin (100), penicillin (10), polymyxin (300 IU), rifampicin (5), streptomycin (10), tetracycline (30) and tobramycin (10). Additional enzyme activities and biochemical characteristics were determined by using API 20 NE and API ZYM kits at 30 ºC as recommended by the manufacturer (bioMérieux).

Isoprenoid quinones were analyzed as described previously (Komagata & Suzuki, 1987) by reversed-phase HPLC. The fatty acids methyl esters obtained from cells grown on MA at 30 ºC and were analyzed according to the instructions of the Microbial Identification System (MIDI; Microbial ID). Polar lipids were extracted using a chloroform / methanol system and separated by two-dimensional thin-layer chromatography (TLC) using silica gel 60 $F_{254}$ aluminium-backed thin-layer plates (Merck) (Kates, 1986). The solvent system chloroform/methanol/water (65 : 24 : 4, by vol.) was used in the first dimension and chloroform/glacial acetic acid/methanol/water (80 : 12 : 15 : 4, by vol.) was used in the second dimension. The separated components were visualized by treating the plates with 10 % (w/v) molybdophosphoric acid followed by heating at 150 °C for 5 min. Genomic DNA was obtained using the method described by Marmur (1961). The purified DNA was hydrolyzed with P1 nuclease and the nucleotides dephosphorylated with calf intestine alkaline phosphatase (Mesbah & Whitman, 1989). The G+C content of the resulting deoxyribonucleosides was determined by reversed-phase HPLC and calculated from the ratio of deoxyguanosine (dG) and thymidine (dT) (Mesbah & Whitman, 1989).

The 16S rRNA gene was amplified and analyzed as described previously (Xu *et al.*, 2007). Sequence data were aligned with CLUSTAL W 1.8 (Thompson *et al.*, 1994). The sequence was compared with closely related sequences of reference organisms from the EzTaxon service (Chun *et al.*, 2007). Phylogenetic trees were constructed by the neighbor-joining (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1971) methods with the MEGA 4 program package (Tamura *et al.*, 2007) and maximum-likelihood method (Felsenstein, 1981) with the TreePuzzle 5.2 program. Evolutionary distances were calculated according to the algorithm of

the Kimura two-parameter model (Kimura, 1980) for the neighbor-joining method.

The two isolates were Gram-negative, rod-shaped, motile, oxidase-positive and possessed Q-10 as predominant quinone. Cell division occurred by binary fission. Electron micrographs of negative stained cells did not reveal prosthecae (Supplementary Fig. S1). Other physiological and chemotaxonomic characteristics of strains B2$^T$ and 1_C16_27$^T$ are summarized in the species descriptions. Phenotypic characteristics that serve to differentiate the two strains from their closest phylogenetic relatives are listed in Table 1.

The 16S rRNA gene sequence comparisons to representative bacteria with validly published names indicated that the strain B2$^T$ and 1_C16_27$^T$ were affiliated with the family *Hyphomicrobiaceae*. Based on the analysis by the EzTaxon service, these strains were most closely related to the genus *Cucumibacter* (92.7 - 93.7 % similarity), *Devosia* (92.9-94.4 % similarity) and *Zhangella* (91.7 - 92.1% similarity) as well as *Prosthecomicrobium pneumaticum* (93.1 - 93.2 % similarity), but showed < 90% sequence similarity to other described *Hyphomicrobiaceae* species. The phylogenetic trees constructed with all three treeing methods indicated that these two strains clustered with the genera *Cucumibacter* and *Zhangella* (Fig. 1 & Supplementary Fig. S2 & S3). Within this cluster, strain B2$^T$ was found to be closely related to strain 1_C16_27$^T$, as supported by a high bootstrap resampling value (99 % by the neighbour-joining method) (Fig. 1). Therefore, the low sequence similarities between the strain B2$^T$ and 1_C16_27$^T$ and their phylogenetic neighbors strongly indicate that these two strains are members of a new genus in the family *Hyphomicrobiaceae*.

The dominant fatty acid for strains B2$^T$ and 1_C16_27$^T$ was $C_{18:1}\omega7c$, charcteristic of the vast majority of species within the *Alphaproteobacteria*. The relative amounts of $C_{19:0}\omega8c$ cyclo, 11-methyl $C_{18:1}\omega7c$ and $C_{18:1}$ varied according to the age of the culture (Supplementary Table S1). The contents of $C_{18:1}\omega7c$ of strains B2$^T$ and 1_C16_27$^T$ decreased from 61.3 % and 75.3 % for one day to 58.0 % and 74.6 % for two days and 12.1 % and 59.0 % for three days. The percentages of $C_{19:0}\omega8c$ cyclo and 11-methyl $C_{18:1}\omega7c$ increased accordingly. Together, $C_{19:0}\omega8c$ cyclo, 11-methyl $C_{18:1}\omega7c$ and $C_{18:1}$ made up 67-83 % of the total fatty acids of

strains B2$^T$ and 1_C16_27$^T$. In general, the percentage of unsaturated fatty acids of strains B2$^T$ (68.2 - 75.3 %) and 1_C16_27$^T$ (68.2 - 80.4 %) were close to that of *Z. mobilis* CGMCC 1.7002$^T$ (71.8 - 83.5 %), but higher than that of *C. marinus* DSM 18995$^T$, *D. riboflavina* DSM 7230$^T$ and *D. geojensis* DSM 19414$^T$ (42.0 - 62.5 %). Presence of 10-Methyl $C_{19:0}$ was detected in extracts of strains B2$^T$ and 1_C16_27$^T$ but not in the four reference strains. iso-$C_{19:0}$ was detected in two or three day culture extracts of *C. marinus* DSM 18995$^T$ and *Z. mobilis* CGMCC 1.7002$^T$ but absent in strains B2$^T$ and 1_C16_27$^T$.

The results of two-dimensional TLC analysis of polar lipids extracted from strain B2$^T$ and 1_C16_27$^T$ as well as three reference strains, *C. marinus* DSM 18995$^T$, *D. geojensis* DSM 19414$^T$ and *Z. mobilis* CGMCC 1.7002$^T$, are shown in supplementary Fig. S4. Polar lipid profiles of all the five strains were dominated by phosphatidylglycerol (PG), diphosphatidylglycerol (DPG) and two unidentified glycolipids (GL1 and GL3). Strain B2$^T$ and 1_C16_27$^T$ did not contain a relatively large amount of an unknown lipid (L16), in marked contrast to the phylogenetically related genera *Cucumibacter* and *Zhangella*. Seven polar lipids (L1, L2, L3, L5, L6, L8 and L11) were detected in both strains B2$^T$ and 1_C16_27$^T$ in minor amounts. Four of them (L2, L3, L6 and L8) were found in three reference strains, but L5 and L11 are characteristic lipids of strains B2$^T$ and 1_C16_27$^T$. In addition, L15 found in three reference strains was not detected in strains B2$^T$ and 1_C16_27$^T$, which revealed another difference of polar lipids between these two strains and their phylogenetic neighbors. 10-methyl $C_{19:0}$ was found both in strain B2$^T$ (0.6 - 0.9 %) and 1_C16_27$^T$ (0.3 - 0.4 %), but not in related organisms (Supplementary Table S1). Furthermore, some phenotypic characteristics of strain B2$^T$ and 1_C16_27$^T$, e.g. $\beta$-galactosidase, hydrolysis of casein and utilization of gluconate, distinguish these two novel isolates from previously described species *C. marinus*, *Z. mobilis*, *D. riboflavina* and *D. geojensis* (Table 1). Therefore, chemotaxonomic and physiological features suggest that strains B2$^T$ and 1_C16_27$^T$ represents a novel genus of the family *Hyphomicrobiaceae*. The main respiratory quinone of strains B2$^T$ and 1_C16_27$^T$ is Q-10 (94.2 % and 96.2 %, respectively), while Q-9 is a minor component (5.8 % and 3.8%, respectively).

Strains B2$^T$ and 1_C16_27$^T$ could be differentiated from each other by their fatty acid composition, a number of phenotypic characteristics (Table 1) and on the basis of their 16S rRNA gene sequences. The total content of $C_{19:0}\omega 8c$ cyclo, 11-methyl $C_{18:1}\omega 7c$ and $C_{18:1}$ of strain B2$^T$ (66.8 - 73.3 %) was lower than that of strain 1_C16_27$^T$ (78.1 - 79.0 %) grown under the same conditions. These two strains could also be distinguished by their different abilities to produce acid from rhamnose, their NaCl range for growth, utilization of lactose, L-ornithine, salicin and sorbitol, susceptibility to kanamycin, and enzyme activities such as alkaline phosphatase, $\alpha$-glucosidase and trypsin (Table 1). The 16S rRNA gene sequence divergence value between strain B2$^T$ and 1_C16_27$^T$ was 3.4 %, which exceeded 3 %, a commonly accepted value for the distinction of different genomic species (Stackebrandt & Goebel, 1994). On the basis of the physiological and chemotaxonomic characteristics presented and 16S rRNA gene sequence comparisons, it is proposed that strains B2$^T$ and 1_C16_27$^T$ represent two novel species in a new genus, for which the names *Pelagibacterium halotolerans* gen. nov., sp. nov. (type strain B2$^T$ = CGMCC 1.7692$^T$ = JCM 15775$^T$) and *Pelagibacterium luteolum* sp. nov. (type strain 1_C16_27$^T$ = CGMCC 1.10267$^T$ = JCM 16552$^T$ = CELMS EEUT 1C1627$^T$) are proposed.

**Description of *Pelagibacterium* gen. nov.**

*Pelagibacterium* (Pe.la.gi.bac.te'ri.um. L. n. *pelagus* the sea; Gr. n. *bakterion* a small rod; N.L. neut. n. *Pelagibacterium* a rod isolated from the sea).

Gram-negative and non-spore-forming bacteria. Divide by binary division. Motile. Catalase- and oxidase-positive. Aerobic chemoheterotrophic. The major polar lipid profiles comprise phosphatidylglycerol, diphosphatidylglycerol and two unidentified glycolipids. Small amounts of seven unidentified lipids (L1, L2, L3, L5, L6, L8 and L11) are detected. The major fatty acids include nonadecanoic acid ($C_{19:0}$ cyclo), octadecaenoic acids ($C_{18:1}$, $C_{18:0}$ and $C_{18:0}$ 3-OH) and hexadecaenoic acid ($C_{16:0}$). The main respiratory quinone is Q-10, with Q-9 as a minor component. The G+C content of the genomic DNA is 58.1 - 59.3 mol%. Belongs to the class *Alphaproteobacteria*. Analysis of 16S rRNA gene sequences showed that *Pelagibacterium* species are most closely related to the members of the genera *Cucumibacter*, *Devosia* and

*Zhangella*. The type species is *Pelagibacterium halotolerans*.

**Description of *Pelagibacterium halotolerans* sp. nov.**

*Pelagibacterium halotolerans* (ha.lo.to'le.rans. Gr. n. *hals, halos* salt; L. part. adj. *tolerans* tolerating; N.L. part. adj. *halotolerans* salt-tolerating, referring to the organism's ability to tolerate high salt concentrations).

Cells are 0.4-0.6 µm wide and 2-3 µm long. Motile by means of several polar flagella. Young cultures consist of slightly curved rods. Colonies are 1-2 mm in diameter, circular, smooth, elevated, semitransparent and light yellowish after 3 d at 30 ºC. Growth occurs at NaCl concentrations of 0-13.0 % (w/v), with optimum growth at 3.0-4.0 % (w/v). The pH and temperature ranges for growth are pH 6.0-9.5 and 10-42 ºC (optimum growth at pH 7.0 and 30 ºC). Nitrate is not reduced. Aesculin and casein are hydrolyzed. Gelatin, DNA, starch, Tween 40, Tween 60 and Tween 80 are not hydrolyzed. Gluconate oxidation, glucose fermentation, *β*-galactosidase and urease activities are positive. Negative for arginine dihydrolase, indole production and lecithinase. The following substrates are utilized for growth: acetate, L-alanine, L-arabinose, L-asparagine, L-aspartate, D-cellobiose, citrate, ethanol, D-galactose, gluconate, glucose, glycerol, L-glutamine, *myo*-inositol, lactate, malate, maltose, mannitol, D-mannose, L-ornithine, pyruvate, ribose, rhamnose, salicin, L-serine, succinate, sucrose, D-trehalose and D-xylose. The following compounds are not utilized as sole carbon sources: L-arginine, L-cysteine, glycine, formate, fumarate, L-histidine, isoleucine, lactose, L-lysine, malonate, L-methionine, propionate, raffinose, sorbitol and sorbose. Acid is produced from: L-arabinose, ethanol, D-galactose, glucose, glycerol, inositol, maltose, mannitol, D-mannose, ribose, rhamnose, salicin, sucrose, D-trehalose and D-xylose. Susceptible to (µg unless otherwise stated) amoxicillin (10), ampicillin (10), bacitracin (0.04 IU), cefotaxime (30), cefoxitin (30), chloramphenicol (30), erythromycin (15), neomycin (30), nitrofurantoin (300), novobiocin (30), penicillin (10), rifampicin (5) and tetracycline (30), but not to kanamycin (30), nystatin (100), polymyxin (300 IU), streptomycin (10) and tobramycin (10). In the API ZYM system, acid and alkaline phosphatases, *N*-acetyl-*β*-glucosaminidase, esterase (C4), esterase lipase (C8), *α*- and *β*-glucosidases, leucine arylamidase (weak reaction), naphthol-AS-*β*-1-phosphohydrolase and

trypsin activities are present, whereas *α*-chymotrypsin, cystine arylamidase, *α*-fucosidase, *α*- and *β*-galactosidases, *β*-glucuronidase, lipase (C14), *α*-mannosidase and valine arylamidase activities are absent. The major polar lipid profiles comprise phosphatidylglycerol, diphosphatidylglycerol and three unidentified glycolipids. Trace amounts of nine yet unidentified lipids were detected. The major fatty acids are $C_{19:0}\omega 8c$ cyclo, 11-methyl $C_{18:1}\omega 7c$, $C_{18:1}\omega 7c$, $C_{16:0}$ and $C_{18:0}$. The DNA G+C content is 59.3 mol% (as determined by HPLC).

The type strain, $B2^T$ (= CGMCC $1.7692^T$ = JCM $15775^T$), was isolated from a seawater sample collected from the East China Sea.

**Description of *Pelagibacterium luteolum* sp. nov.**

*Pelagibacterium luteolum* (lu.te'o.lum. L. neut. adj. *luteolum* yellowish).

Cells are 0.5-0.9 μm wide and 1.5-2.5 μm long. Short rod-shaped. Motile by means of a single polar flagellum. Colonies are 1-2 mm in diameter, circular, smooth, elevated, semitransparent and yellowish after 3 d at 30 ºC. Growth occurs at NaCl concentrations of 0-5.0 % (w/v), with optimum growth at 0.5 % (w/v). The pH and temperature ranges for growth are pH 6.0-9.5 and 4-37 ºC (optimum growth at pH 7.5 and 30 ºC). Nitrate is not reduced. Aesculin and casein are hydrolyzed. Gelatin, DNA, starch, Tween 40, Tween 60 and Tween 80 are not hydrolyzed. Gluconate oxidation, glucose fermentation, *β*-galactosidase and urease activities are positive. Negative for arginine dihydrolase and indole production. The following substrates are utilized for growth: acetate, L-alanine, D-cellobiose, citrate, ethanol, D-galactose, gluconate, glucose, glycerol, L-glutamine, *myo*-inositol, lactate, lactose, maltose, mannitol, D-mannose, pyruvate, rhamnose, L-serine, sorbitol, succinate, sucrose, D-trehalose and D-xylose. The following compounds are not utilized as sole carbon sources: L-cysteine, glycine, formate, L-histidine, isoleucine, L-lysine, malonate, L-ornithine, propionate and raffinose. Assimilation of fumarate and salicin is weakly positive. Acid is produced from: L-arabinose, ethanol, D-galactose, glucose, glycerol, inositol, maltose, mannitol, D-mannose, rhamnose, sorbitol, sucrose, D-trehalose and D-xylose. Susceptible to (μg unless otherwise stated) amoxicillin (10), ampicillin (10), bacitracin (0.04 IU), cefotaxime (30), cefoxitin (30), chloramphenicol (30),

erythromycin (15), kanamycin (30), neomycin (30), nitrofurantoin (300), novobiocin (30), penicillin (10), rifampicin (5) and tetracycline (30), but not to nystatin (100), polymyxin (300 IU), streptomycin (10) and tobramycin (10). In the API ZYM system, acid phosphatase, *N*-acetyl-*β*-glucosaminidase, esterase (C4), esterase lipase (C8), *β*-glucosidase, leucine arylamidase (weak reaction), naphthol-AS-*β*-1-phosphohydrolase and activities are present, whereas alkaline phosphatase, *α*-chymotrypsin, cystine arylamidase, *α*-fucosidase, *α*- and *β*-galactosidases, *α*-glucosidase, *β*-glucuronidase, lipase (C14), *α*-mannosidase, trypsin and valine arylamidase activities are absent. The major polar lipid profiles comprise phosphatidylglycerol, diphosphatidylglycerol and two unidentified glycolipids. Trace amounts of ten yet unidentified lipids were detected. The major fatty acids are $C_{18:1}\omega7c$, $C_{19:0}\omega8c$ cyclo, $C_{18:0}$ and 11-methyl $C_{18:1}\omega7c$. The DNA G+C content is 58.1 mol% (as determined by HPLC).

The type strain, 1_C16_27$^T$ (= CGMCC 1.10267$^T$ = JCM 16552$^T$ = CELMS EEUT 1C1627$^T$), was isolated from a semi-coke sample collected from the north eastern region of Estonia.

**Acknowledgements**

This work was supported by grants from the Ministry of Science and Technology of China (863 Program, 2007AA021305), the National Natural Science Foundation of China (40806066), the Zhejiang Provincial Natural Science Foundation of China (Y5080060) and the Chinese Offshore Investigation and Assessment (908-ZC-I-02).

**References**

**Chun, J., Lee, J.-H., Jung, Y., Kim, M., Kim, S., Kim, B. K. & Lim, Y.-W. (2007).** EzTaxon: a web-based tool for the identification of prokaryotes based on 16S ribosomal RNA gene sequences. *Int J Syst Evol Microbiol* **57**, 2259-2261.

**Dong, X.-Z. & Cai, M.-Y. (2001).** *Determinative Manual for Routine Bacteriology*. Beijing: Scientific Press (English translation).

**Euzéby, J. P. (1997).** List of Bacterial Names with Standing in Nomenclature: a folder available on the Internet. *Int J Syst Bacteriol* **47**, 590-592. (List of Prokaryotic names

with Standing in Nomenclature. Last full update January 13, 2010. URL: http://www.bacterio.cict.fr).

**Felsenstein, J. (1981).** Evolutionary trees from DNA sequences: a maximum likelihood approach. *J Mol Evol* **17**:368-376.

**Fitch, W. M. (1971).** Toward defining the course of evolution: minimum change for a specific tree topology. *Syst Zool* **20**, 406-416.

**Garrity, G. M., Bell, J. A. & Lilburn, T. (2005).** Family VIII. *Hyphomicrobiaceae* Babudieri 1950, 589. In *Bergey's Manual of Systematic Bacteriology*, 2nd edn, vol. 2, *The Proteobacteria, Part C, The Alpha-, Beta-, Delta-, and Epsilonproteobacteria,* p. 476. Edited by D. J. Brenner, N. R. Krieg, J. T. Staley & G. M. Garrity. New York: Springer.

**Gliesche, C., Fesefeldt, A. & Hirsch, P. (2005).** Genus I. *Hyphomicrobium* Stutzer and Hartleb 1898, 76$^{AL}$. In *Bergey's Manual of Systematic Bacteriology*, 2nd edn, vol. 2, *The Proteobacteria, Part C, The Alpha-, Beta-, Delta-, and Epsilonproteobacteria,* pp. 476-494. Edited by D. J. Brenner, N. R. Krieg, J. T. Staley & G. M. Garrity. New York: Springer.

**Hwang, C. Y. & Cho, B. C. (2008).** *Cucumibacter marinus* gen. nov., sp. nov., a marine bacterium in the family *Hyphomicrobiaceae*. *Int J Syst Evol Microbiol* **58**, 1591-1597.

**Kates, M. (1986).** *Techniques of lipidology*, 2nd edn. Amsterdam: Elsevier.

**Kimura, M. (1980).** A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* **16**, 111-120.

**Komagata, K. & Suzuki, K. (1987).** Lipids and cell-wall analysis in bacterial systematics. *Methods Microbiol* **19**, 161-207.

**Lee, K.-B., Liu, C.-T., Anzai, Y., Kim, H., Aono, T. & Oyaizu, H. (2005).** The hierarchical system of the '*Alphaproteobacteria*': description of *Hyphomonadaceae* fam. nov., *Xanthobacteraceae* fam. nov. and *Erythrobacteraceae* fam. nov. *Int J Syst Evol Microbiol* **55**, 1907-1919.

**Leifson, E. (1963).** Determination of carbohydrate metabolism of marine bacteria. *J Bacteriol* **85**, 1183-1184.

**Marmur, J. (1961).** A procedure for the isolation of deoxyribonucleic acid from microorganisms. *J Mol Biol* **3**, 208-218.

**Mesbah, M. & Whitman, W. B. (1989).** Measurement of deoxyguanosine/thymidine ratios in complex mixtures by high-performance liquid chromatography for determination of the mole percentage guanine + cytosine of DNA. *J Chromatogr* **479**, 297-306.

**Mikhailov, V. V., Romanenko, L. A. & Ivanova E. P. (2006).** The genus *Alteromonas* and related proteobacteria. In *The Prokaryotes*: a Handbook on the Biology of Bacteria, 3rd edn, vol. 6, pp. 597-645. Edited by M. Dworkin, S. Falkow, E. Rosenberg, K. H. Schleifer & E. Stackebrandt. New York: Springer.

**Nakagawa, Y., Sakane, T. & Yokota, A. (1996).** Transfer of "*Pseudomonas riboflavina*" (Foster 1944), a Gram-negative, motile rod with long-chain 3-hydroxy fatty acids, to *Devosia riboflavina* gen. nov., sp. nov., nom. rev. *Int J Syst Bacteriol* **46**, 16-22.

**Ryu, S. H., Chung, B. S., Le, N. T., Jang, H. H., Yun, P. Y., Park, W. & Jeon, C. O. (2008).** *Devosia geojensis* sp. nov., isolated from diesel-contaminated soil in Korea. *Int J Syst Evol Microbiol* **58**, 633-636.

**Schlesner, H. (2005).** Genus X. *Filomicrobium* Schlesner 1988, 220$^{VP}$ (Effective publication: Schlesner 1987, 65). In *Bergey's Manual of Systematic Bacteriology*, 2nd edn, vol. 2, *The Proteobacteria, Part C, The Alpha-, Beta-, Delta-, and Epsilonproteobacteria*, pp. 518-520. Edited by D. J. Brenner, N. R. Krieg, J. T. Staley & G. M. Garrity. New York: Springer.

**Saitou, N. & Nei, M. (1987).** The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406-425.

**Stackebrandt, E. & Goebel, B. M. (1994).** Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int J Syst Bacteriol* **44**, 846–849.

**Tamura, K., Dudley, J., Nei, M. & Kumar, S. (2007).** MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Mol Biol Evol* **24**, 1596-1599.

**Thompson, J. D., Higgins, D. G. & Gibson, T. J. (1994).** CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acid Res* **22**, 4673-4680.

**Truu, J., Kärme, L., Talpsep, E., Heinaru, E., Vedler, E. & Heinaru, A. (2003).**

Phytoremediation of solid oil shale waste from the chemical industry. *Acta Biotechnol* **23**, 301-307.

**Xu X.-W., Wu Y.-H. Zhou Z. Wang C.-S. Zhou Y.-G. Zhang H.-B. Wang Y. & Wu M. (2007).** *Halomonas saccharevitans* sp. nov., *Halomonas arcis* sp. nov., and *Halomonas subterranea* sp. nov., halophilic bacteria isolated from hypersaline environments of China. *Int J Syst Evol Microbiol* **57**, 1619-1624.

**Xu, X.-W., Wu, Y.-H., Wang, C.-S., Yang, J.-Y., Oren, A. & Wu, M. (2008).** *Marinobacter pelagius* sp. nov., a moderately halophilic bacterium. *Int J Syst Evol Microbiol* **58**, 637-640.

**Xu, H.-Y., Chen, L.-P., Fu, S.-Z., Fan, H.-X., Zhou, Y.-G., Liu, S.-J. & Liu, Z.-P. (2009).** *Zhangella mobilis* gen. nov., sp. nov., a new member of the family *Hyphomicrobiaceae* isolated from coastal seawater. *Int J Syst Evol Microbiol* **59**, 2297-2301.

Table 1. Taxonomic characteristics differentiating strains B2$^T$ and 1_C16_27$^T$ from other related members of the family *Hyphomicrobiaceae*. Strains: 1, B2$^T$; 2, 1_C16_27$^T$; 3, *Cucumibacter marinus* DSM 18995$^T$; 4, *Zhangella mobilis* CGMCC 1.7002$^T$; 5, *Devosia riboflavina* DSM 7230$^T$; 6, *Devosia geojensis* DSM 19414$^T$. Unless stated otherwise, data were obtained from this study under identical growth conditions. +, Positive; -, negative; W, weakly positive; NA, no data available.

| Characteristic | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| Colony colour* | LY | Y | C | PY | C | W |
| Grow at 10 % NaCl (w/v) | + | - | +† | -‡ | - | NA |
| Nitrate reduction | - | - | - | + | - | - |
| Urease | + | + | - | - | + | + |
| Hydrolysis of: | | | | | | |
| Casein | + | + | - | - | - | - |
| DNA | - | - | - | - | - | + |
| Acid produce: | | | | | | |
| Ethanol | + | + | NA | - | - | - |
| Glycerol | + | + | NA | - | + | + |
| D-Raffinose | - | - | NA | - | + | + |
| Rhamnose | + | - | NA | - | + | + |
| Utilization of: | | | | | | |
| Gluconate ¶ | + | + | - | - | - | - |
| Lactose ¶ | - | + | - | - | + | + |
| Mannitol ¶ | + | + | - | - | + | + |
| L-Ornithine ¶ | + | - | + | + | - | - |
| Salicin ¶ | + | - | + | + | + | + |
| Sorbitol ¶ | - | + | - | - | + | + |
| Succinate ¶ | + | + | - | + | + | - |
| Sensitive to: | | | | | | |
| Bacitracin (0.04 IU) # | + | + | - | + | - | - |
| Kanamycin (30 μg) # | - | + | + | +‡ | - | - |
| Neomycin (30 μg) # | + | + | + | + | + | - |
| Novobiocin (30 μg) # | + | + | + | + | + | - |
| API ZYM | | | | | | |
| Alkaline phosphatase | + | - | + | + | + | + |
| *α*-Chymotrypsin | - | - | W | W | W | - |
| Cystine arylamidase | - | - | + | + | W | - |
| *β*-Galactosidase | - | - | + | + | + | + |
| *α*-Glucosidase | + | - | + | + | - | W |
| Trypsin | + | - | + | + | + | - |
| DNA G+C content (mol%) | 59.3 | 58.1 | 62.9† | 53.1‡ | 61.4§ | 60.8¦ |

*LY, light-yellow; Y, yellow; C, cream; PY, pale-yellow; W, white.

†Data from Hwang & Cho, 2008.

‡Data from Xu *et al.*, 2009.

§Data from Nakagawa *et al.*, 1996.

¦Data from Ryu *et al.*, 2008.

¶ All strains except for *C. marinus* DSM 18995$^T$ grew in BM broth for determining the substrate utility for growth. *C. marinus* DSM 18995$^T$ grew on BM agar plate because its growth in BM broth is very slow.

#All strains except for *D. riboflavina* DSM 7230$^T$ grew on MA (BD) plate for determining the susceptibility to antibiotics. *D. riboflavina* DSM 7230$^T$ grew on PYM agar plate because its growth on MA is very slow.

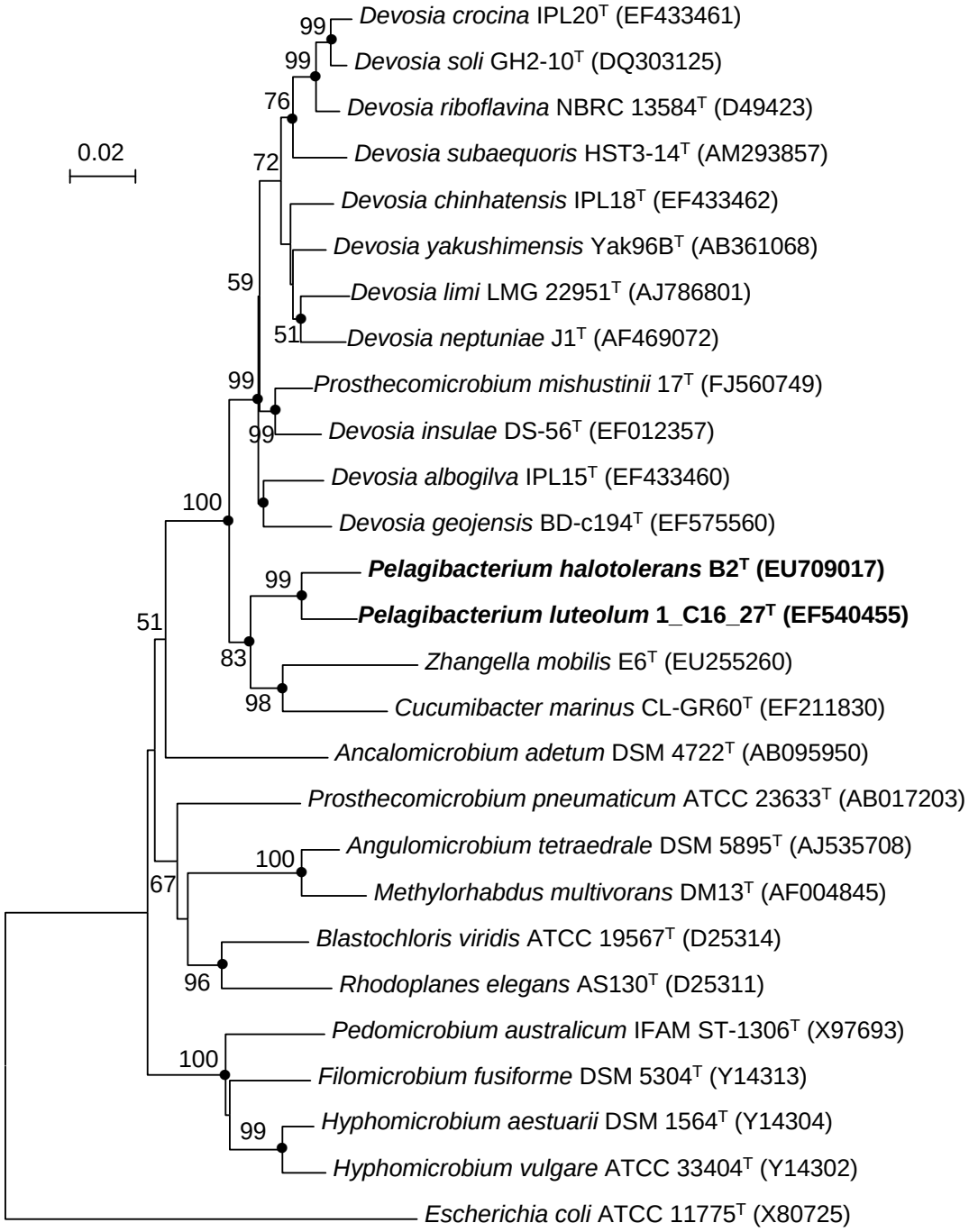
406 **Legends to the Figure:**

407

408 Fig. 1. Neighbor-joining tree based on 16S rRNA gene sequences, showing the
409 phylogenetic relationships of the novel isolates and related *Hyphomicrobiaceae* taxa.
410 Bootstrap values are based on 1000 replicates; values > 50% are shown. Filled circles
411 indicate nodes recovered in both maximum-likelihood and maximum-parsimony trees.
412 Bar, 0.02 substitutions per nucleotide position.

413

**Figure 1**


0.02
99
99
76
72
59
51
99
99
100
99
51
83
98
100
67
96
100
99
Devosia crocina IPL20T (EF433461)
Devosia soli GH2-10T (DQ303125)
Devosia riboflavina NBRC 13584T (D49423)
Devosia subaequoris HST3-14T (AM293857)
Devosia chinhatensis IPL18T (EF433462)
Devosia yakushimensis Yak96BT (AB361068)
Devosia limi LMG 22951T (AJ786801)
Devosia neptuniae J1T (AF469072)
Prosthecomicrobium mishustinii 17T (FJ560749)
Devosia insulae DS-56T (EF012357)
Devosia albogilva IPL15T (EF433460)
Devosia geojensis BD-c194T (EF575560)
Pelagibacterium halotolerans B2T (EU709017)
Pelagibacterium luteolum 1_C16_27T (EF540455)
Zhangella mobilis E6T (EU255260)
Cucumibacter marinus CL-GR60T (EF211830)
Ancalomicrobium adetum DSM 4722T (AB095950)
Prosthecomicrobium pneumaticum ATCC 23633T (AB017203)
Angulomicrobium tetraedrale DSM 5895T (AJ535708)
Methylorhabdus multivorans DM13T (AF004845)
Blastochloris viridis ATCC 19567T (D25314)
Rhodoplanes elegans AS130T (D25311)
Pedomicrobium australicum IFAM ST-1306T (X97693)
Filomicrobium fusiforme DSM 5304T (Y14313)
Hyphomicrobium aestuarii DSM 1564T (Y14304)
Hyphomicrobium vulgare ATCC 33404T (Y14302)
Escherichia coli ATCC 11775T (X80725)

**Legends to the Supplementary Figures:**

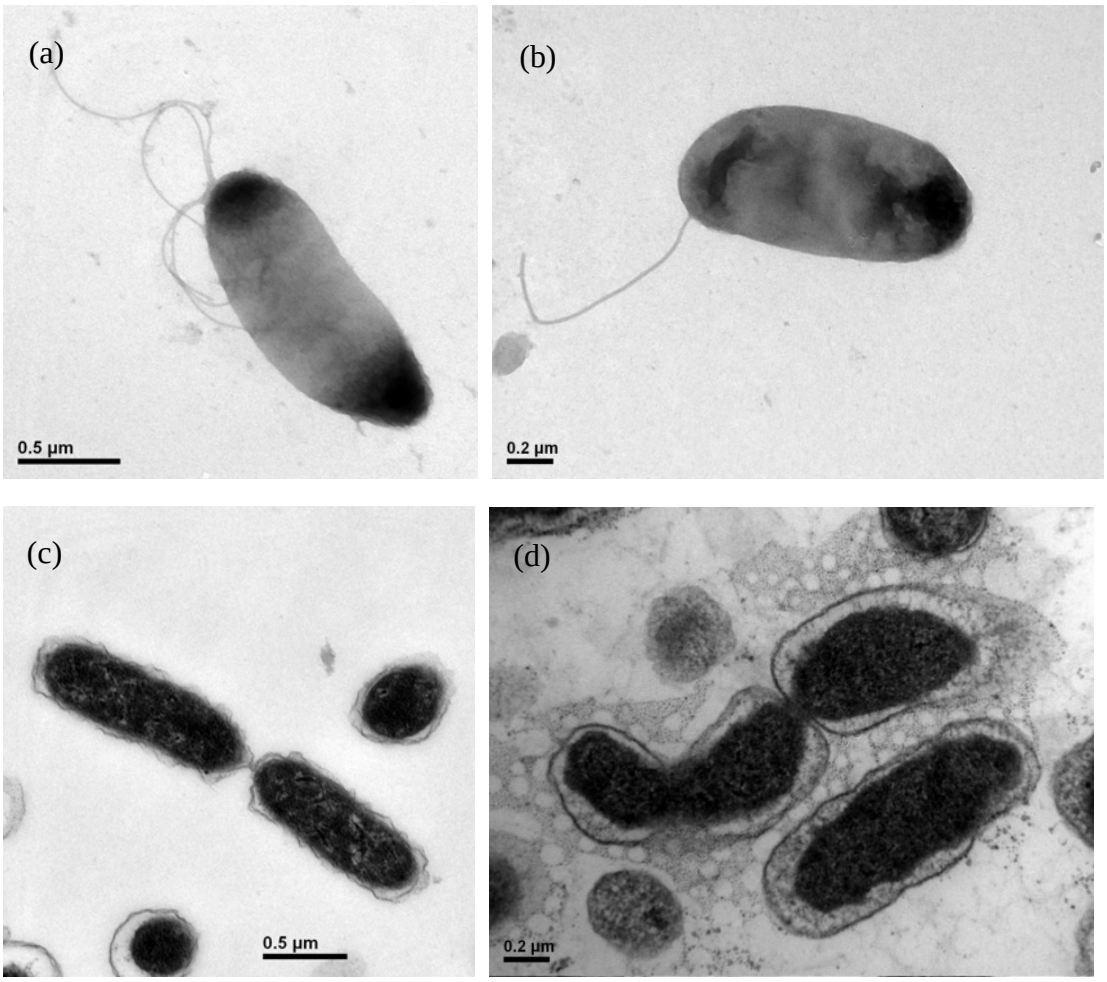
Supplementary Fig. S1. Transmission electron micrographs of strain B2$^T$ and 1_C16_27$^T$. (a) transmission electron micrograph of strain B2$^T$ showing slightly ovoid rods with several polar flagella; (b) transmission electron micrograph of strain 1_C16_27$^T$ showing short rods with a single flagellum; (c) transmission electron micrograph of an ultrathin section of strain B2$^T$ showing outer and cytoplasmic membrane of the cell; (d) transmission electron micrograph of an ultrathin section of strain 1_C16_27$^T$ showing outer and cytoplasmic membrane of the cell.

Supplementary Fig. S2. Phylogenetic tree based on 16S rRNA gene sequences using the maximum-parsimony method. Numbers at branching points refer to bootstrap values (1000 resamplings; only values above 50% are shown).

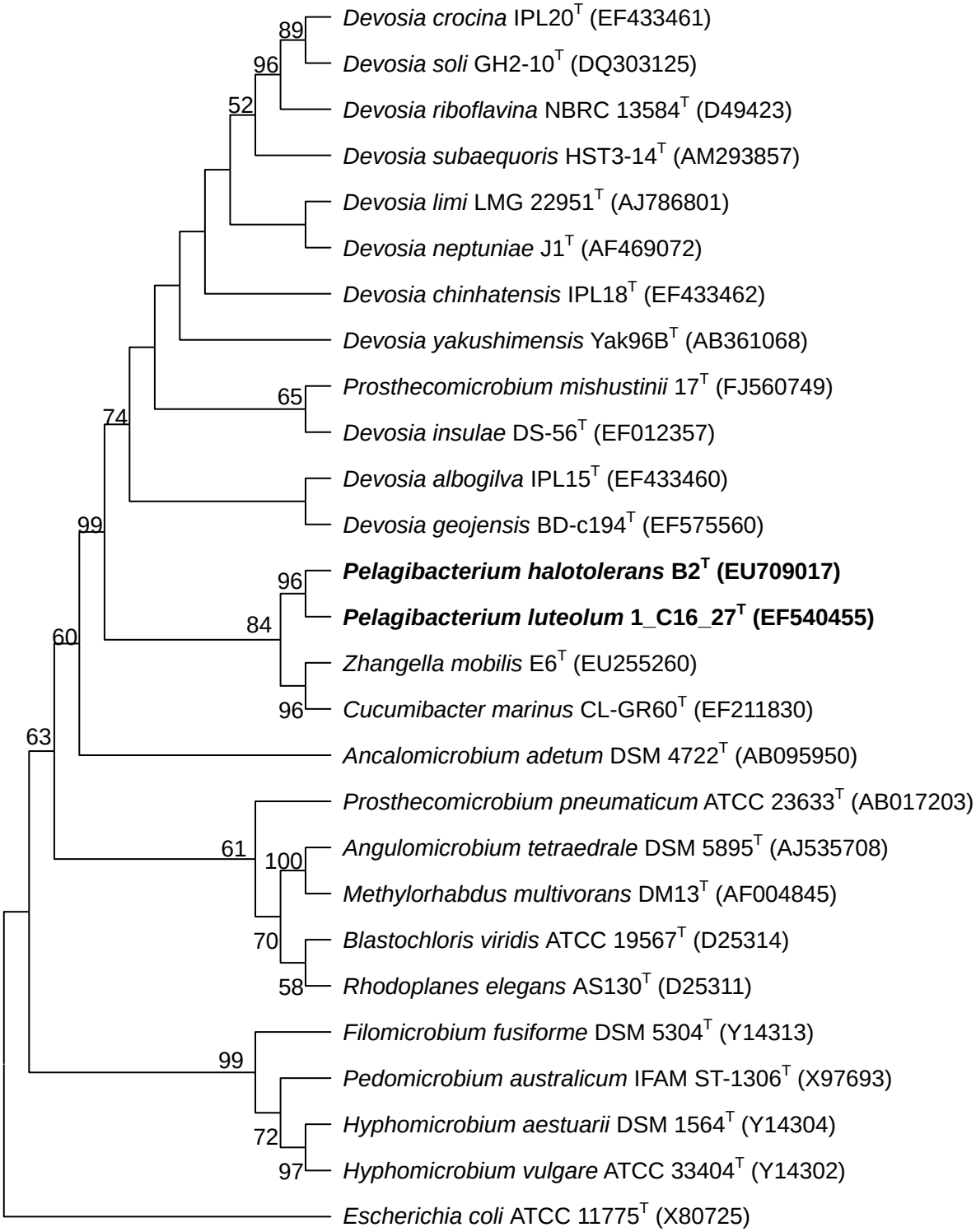
Supplementary Fig. S3. Phylogenetic tree based on 16S rRNA gene sequences using the maximum-likelihood method. Bar, 0.1 substitutions per nucleotide position.

Supplementary Fig. S4. Two-dimensional thin-layer chromatograms after staining with molybdophosphoric acid showing the total polar lipid profiles of strains (a), B2$^T$; (b), 1_C16_27$^T$; (c), *C. marinus* DSM 18995$^T$; (d), *Z. mobilis* CGMCC 1.7002$^T$; (e), *D. geojensis* DSM 19414$^T$. PG, phosphatidylglycerol; DPG, diphosphatidylglycerol; GL, glycolipid; PL, phospholipid; L, unidentified lipid.

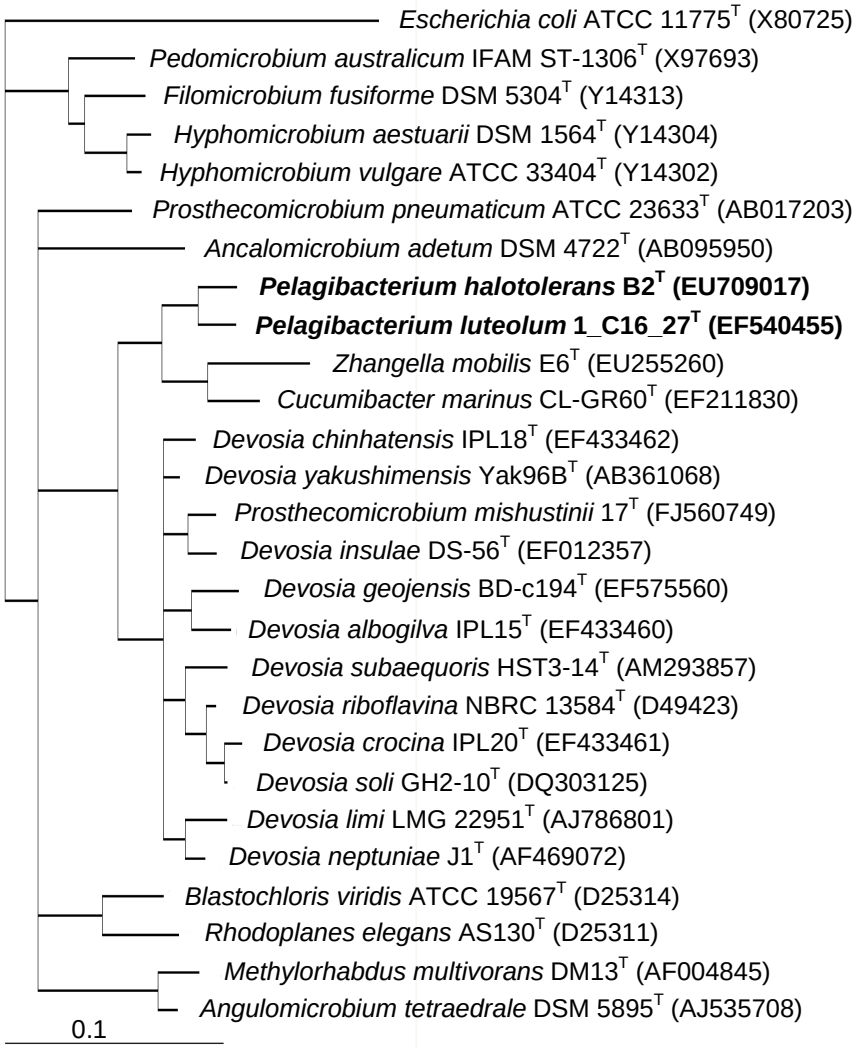
Supplementary Fig. S1.



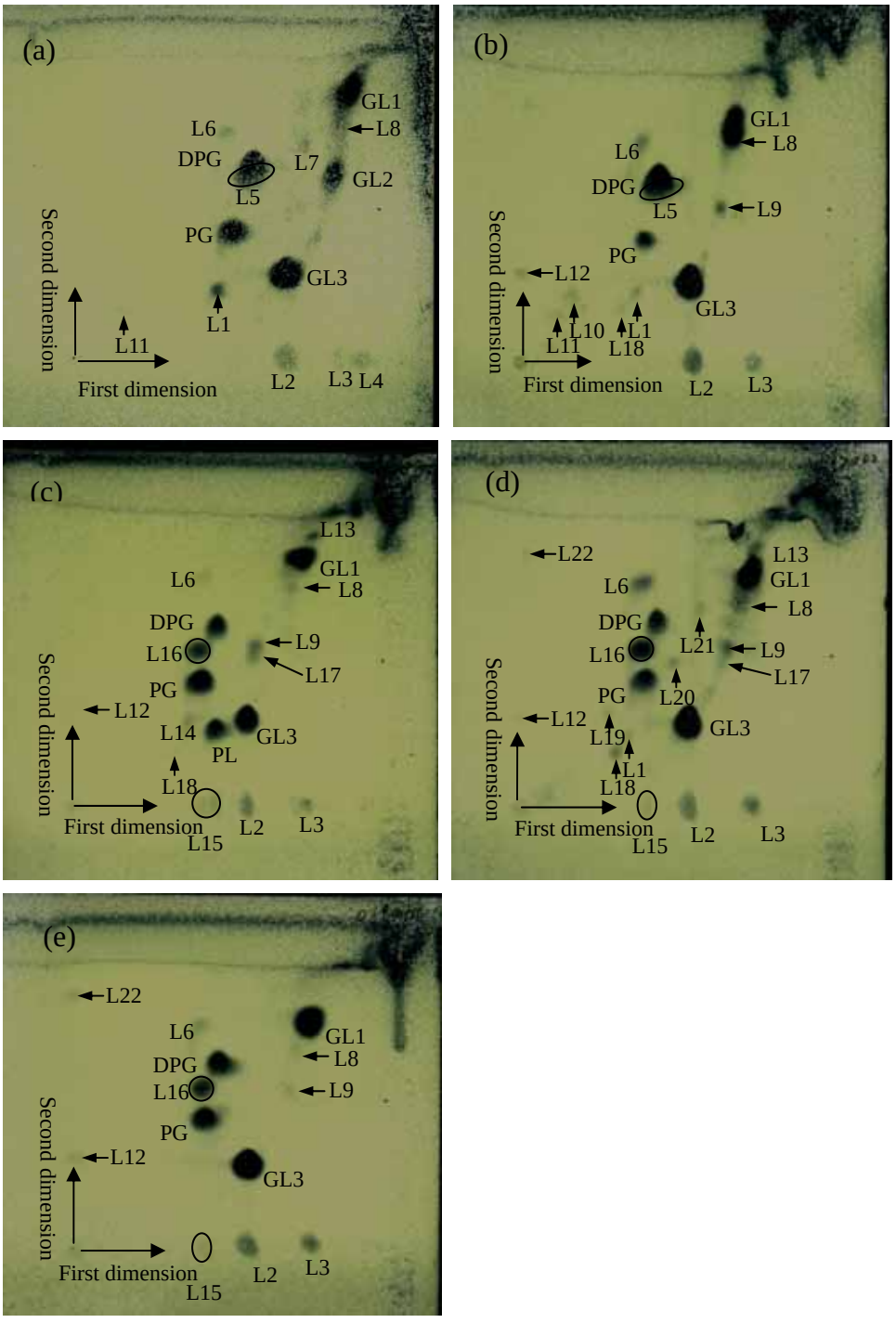
Supplementary Fig. S2.



Supplementary Fig. S3.



Supplementary Fig. S4.



**Supplementary material**

Supplementary Table S1. Fatty acid composition (%) of novel isolates and other related species. Strains: 1, B2$^T$; 2, 1_C16_27$^T$; 3, *C. marinus* DSM 18995$^T$; 4, *Z. mobilis* CGMCC 1.7002$^T$; 5, *D. riboflavina* DSM 7230$^T$; 6, *D. geojensis* DSM 19414$^T$. -, Not detected; ECL, equivalent chain length. All strains were grown under identical conditions on MA for 1, 2 and 3 days at 30 ºC. Fatty acids representing less than 0.1 % in all strains were omitted.

| Fatty acid | MA for 1 day‡ | | | | MA for 2 days | | | | | | MA for 3 days | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 4 | 6 | 1 | 2 | 3 | 4 | 5 | 6 | 1 | 2 | 3 | 4 | 5 | 6 |
| Straight-chain fatty acids | | | | | | | | | | | | | | | | |
| $C_{10:0}$ | - | - | - | - | - | - | 0.1 | 0.1 | - | - | - | - | 0.2 | 0.3 | - | - |
| $C_{12:0}$ | - | - | - | - | - | - | 0.2 | - | 0.3 | - | - | - | - | - | - | - |
| $C_{14:0}$ | 0.5 | 0.7 | - | 0.8 | 0.5 | 0.4 | 0.3 | 0.5 | 0.7 | 0.7 | 0.2 | 0.2 | - | - | 0.5 | 0.3 |
| $C_{15:0}$ | 0.2 | 0.2 | - | 0.7 | 0.2 | - | - | 0.1 | 0.1 | 0.4 | - | - | - | - | - | 0.3 |
| $C_{16:0}$ | 11.3 | 6.4 | 4.7 | 16.7 | 10.4 | 5.0 | 8.2 | 6.2 | 27.4 | 19.4 | 12.4 | 4.4 | 7.7 | 5.6 | 29.9 | 17.1 |
| $C_{17:0}$ | 2.3 | 0.7 | 2.5 | 9.6 | 2.2 | 0.3 | 0.6 | 2.5 | 0.4 | 6.4 | 1.0 | 0.4 | 0.4 | 2.7 | 0.3 | 4.5 |
| $C_{17:0}$ cyclo | - | - | - | - | - | - | - | - | 1.9 | - | 0.3 | - | - | - | 1.8 | - |
| $C_{18:0}$ | 5.6 | 5.2 | 3.2 | 8.5 | 5.6 | 4.1 | 19.4 | 4.4 | 2.2 | 9.5 | 5.3 | 6.9 | 23.3 | 5.4 | 2.6 | 10.2 |
| $C_{19:0}$ | - | - | - | 0.3 | - | - | - | - | - | 0.2 | - | - | - | - | - | 0.2 |
| Branched fatty acids | | | | | | | | | | | | | | | | |
| iso-$C_{16:0}$ | - | - | - | - | - | - | | - | - | - | 0.2 | 0.1 | - | 0.2 | - | - |
| iso-$C_{19:0}$ | - | - | - | - | - | - | 2.5 | 0.5 | | - | - | - | 4.0 | 0.9 | - | - |
| iso-$C_{15:1}$ | - | 0.4 | - | - | 0.3 | - | 0.2 | - | 0.2 | 0.2 | - | - | - | - | - | - |
| Unsaturated fatty acids | | | | | | | | | | | | | | | | |
| $C_{17:1}\omega 8c$ | 0.7 | 0.5 | 1.2 | 0.2 | 0.6 | - | - | 1.0 | - | - | 0.2 | 0.2 | - | 0.8 | - | - |

| | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $C_{17:1}\omega 6c$ | 0.9 | 0.5 | 0.8 | 0.9 | 0.8 | - | - | 0.6 | - | 0.2 | - | 0.2 | - | - | - | - |
| $C_{18:1}\omega 9c$ | 0.3 | 0.6 | - | - | - | - | - | - | 0.4 | - | - | - | - | - | - | - |
| $C_{18:1}\omega 7c$ | 61.3 | 75.3 | 73.7 | 36.5 | 58.0 | 74.6 | 33.8 | 60.6 | 17.8 | 12.7 | 12.1 | 59.0 | 17.6 | 66.9 | 11.6 | 12.5 |
| $C_{18:1}\omega 5c$ | 0.4 | 0.5 | - | - | 0.4 | 0.5 | 0.1 | - | 0.1 | 0.2 | 0.5 | 0.4 | - | - | - | - |
| $C_{20:1}\omega 7c$ | - | - | 0.3 | 0.9 | 0.6 | 0.5 | - | - | - | 0.5 | 0.5 | 0.8 | - | 0.5 | 0.6 | 0.8 |
| $C_{20:2}\omega 6,9c$ | - | - | - | - | - | - | - | - | - | - | 0.7 | 0.2 | - | - | - | 0.2 |
| Hydroxy fatty acids | | | | | | | | | | | | | | | | |
| $C_{8:0}$ 3-OH | - | - | - | 0.4 | - | - | - | - | - | 0.2 | - | - | - | - | - | - |
| $C_{10:0}$ 3-OH | 0.5 | 0.4 | 2.6 | - | - | 0.2 | - | 2.6 | 2.0 | - | 0.3 | 0.2 | -† | - | 1.6 | - |
| $C_{16:0}$ 3-OH | - | - | - | - | - | - | - | 0.1 | - | - | 0.1 | - | - | 0.3 | - | - |
| $C_{17:0}$ 3-OH | 0.3 | - | - | - | 0.3 | - | - | 0.1 | - | - | 0.2 | - | -† | 0.4 | - | - |
| $C_{18:0}$ 3-OH | - | 2.8 | 0.2 | - | 3.8 | 3.2 | - | 0.2 | 0.5 | - | 4.9 | 3.4 | -† | - | 0.5 | - |
| iso-$C_{13:0}$ 3-OH | - | 0.3 | - | - | - | 0.2 | - | 0.3 | 0.2 | 0.3 | - | - | - | - | - | - |
| iso-$C_{17:0}$ 3-OH | 1.6 | - | - | - | - | - | 4.8 | 4.1 | 1.5 | - | - | - | - | - | - | - |
| 11-Methyl $C_{18:1}\omega 7c$ | 5.2 | 1.4 | 7.5 | 22.8 | 6.8 | 3.8 | 25.2 | 9.6 | 38.9 | 28.4 | 13.5 | 5.5 | 23.7 | 10.5 | 43.1 | 21.1 |
| 10-Methyl $C_{19:0}$ | 0.6 | 0.3 | - | - | 0.6 | 0.4 | - | - | - | - | 0.9 | 0.4 | - | - | - | - |
| $C_{19:0}\omega 8c$ cyclo | 6.1 | 0.3 | - | 1.2 | 7.8 | 3.3 | 1.7 | - | 3.2 | 6.8 | 40.7 | 14.1 | 7.7 | - | 5.6 | 8.2 |
| Unknown ECL 14.959 | - | - | - | - | - | - | - | - | - | - | 0.3 | 0.1 | - | - | - | - |
| Unknown ECL 18.814 | 0.9 | - | - | - | - | - | 2.1 | 1.2 | - | 13.6 | - | - | 14.8 | 1.5 | - | 24.3 |
| Summed features* | | | | | | | | | | | | | | | | |
| 1 | 0.1 | 0.2 | - | - | 0.2 | - | - | 0.2 | 0.1 | 0.1 | - | - | - | - | - | - |
| 2 | - | - | - | - | - | - | 0.5 | - | - | - | - | - | 0.5 | - | - | - |
| 3 | 1.1 | 3.3 | 1.3 | 0.5 | 1.0 | 3.6 | 0.4 | 1.1 | 1.7 | 0.3 | 0.9 | 2.8 | 0.2 | 0.9 | 1.0 | 0.3 |
| 7 | - | - | 1.9 | - | - | | | 3.2 | 0.4 | - | 4.9 | 0.4 | - | 2.8 | 1.0 | - |

*Summed features represent groups could not be separated by GLC with MIDI system. Summed feature 1 contains one or more of iso-$C_{13:0}$

3-OH and/or $C_{15:1}$ iso H; summed feature 2 contains one or more of unknown ECL 10.928, iso-$C_{14:0}$ 3-OH and/or $C_{16:1}$ iso I; summed feature 3 contains iso-$C_{15:0}$ 2-OH/$C_{16:1}\omega7c$; summed feature 7 contains one or more of ECL 18.846, $C_{19:0}$ cyclo and/or $C_{19:1}\omega6c$.

†Fatty acids were detected via manual analysis but their compositions (%) were less than 0.1 %.

‡The total biomass of *C. marinus* DSM 18995$^T$ and *D. riboflavina* DSM 7230$^T$ obtained from 30 MA plates (standard 9-cm diameter petri dishes) was insufficient for fatty acid analysis.