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

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Two Gram-negative, motile, aerobic bacterial strains, designated B2^T and 1_C16_27^T, were respectively isolated from a seawater sample collected from the East China Sea and a semi-coke sample from north-eastern Estonia. 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 ($C_{19:0}$ cyclo), octadecanoic ($C_{18:0}$ and $C_{18:0}$ 3-OH), octadecenoic ($C_{18:1}$) and hexadecanoic ($C_{16:0}$) acids. The G+C content of the genomic DNA was 58.1–59.3 mol%. 16S rRNA gene sequence analysis revealed that the 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 %) and *Zhangella* (91.7–92.1 %). Differential phenotypic properties, together with phylogenetic and genetic distinctiveness, revealed that strains B2^T and 1_C16_27^T could be differentiated from each other and from members of the genera *Cucumibacter*, *Devosia* and *Zhangella*. Therefore, 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. (the type species; 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*, class *Alphaproteobacteria*, comprises a morphologically and metabolically heterogeneous group of micro-organisms (Garrity *et al.*, 2005). The establishment of the family *Hyphomicrobiaceae* was based mainly on phylogenetic relationships of 16S rRNA gene sequences (Garrity *et al.*, 2005; Lee *et al.*, 2005). At the time of writing, the family encompassed 18 genera: *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

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains B2^T and 1_C16_27^T are EU709017 and EF540455, respectively.

Four supplementary figures and a supplementary table are available with the online version of this paper.

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 (30° 58′ 16″ N 125° 59′ 24″ E) 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 °C, one light-yellowish colony, designated B2^T, was picked. Strain 1_C16_27^T was isolated from a sample collected from an oil shale chemical industry solid waste (semi-coke) depository area in north-eastern Estonia (59° 23′ 44″ N 27° 13′ 05″ E) in October 2003. Ten subsamples 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 plates (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 a 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, strains B2^T and 1_C16_27^T were maintained in yeast extract broth (YEB; basal medium supplemented with 5 g yeast extract and 8.3 g NaCl l^{-1}) (Mikhailov *et al.*, 2006). The basal medium (BM) contained (per l distilled water) 1.0 g NH_4Cl, 0.044 g K_2HPO_4, 0.028 g $FeSO_4.7H_2O$, 500 ml artificial seawater and 50 ml Tris/HCl (1 M, pH 7.5). The artificial seawater contained (per l distilled water) 23.4 g NaCl, 24.6 g $MgSO_4.7H_2O$, 1.5 g KCl and 2.9 g $CaCl_2$.

Optimal conditions for growth were determined in PYM and YEB medium with 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) NaCl (Nakagawa *et al.*, 1996). The PYM medium contained (per l distilled water) 10.0 g peptone (BD), 2.0 g yeast extract (BD) and 1.0 g $MgSO_4.7H_2O$, 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) or 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 °C. 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 to liquid medium. Acid production was tested by using 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 hydrolyse aesculin, casein, DNA, gelatin and Tweens 40, 60 and 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 containing the following amounts (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 °C as recommended by the manufacturer (bioMérieux).

Isoprenoid quinones were analysed as described previously (Komagata & Suzuki, 1987) by reversed-phase HPLC. Fatty acid methyl esters obtained from cells grown on MA at 30 °C were analysed 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 TLC using silica gel 60 F_{254} aluminium-backed thin-layer plates (Merck) (Kates, 1986). The solvent systems chloroform/methanol/water (65 : 24 : 4, by vol.) and chloroform/glacial acetic acid/methanol/water (80 : 12 : 15 : 4, by vol.) were used in the first and second dimensions. Separated components were visualized by treating the plates with 10 % (w/v) molybdatophosphoric 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 hydrolysed with P1 nuclease and the nucleotides were 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 analysed 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 neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1971) methods with the MEGA 4 program package (Tamura *et al.*, 2007) and the maximum-likelihood method (Felsenstein, 1981) with the TreePuzzle 5.2 program. Evolutionary distances were calculated according to the algorithm of Kimura's two-parameter model (Kimura, 1980) for the neighbour-joining method.

The two isolates were Gram-negative, rod-shaped, motile, oxidase-positive and possessed Q-10 as the predominant quinone. Cell division occurred by binary fission. Electron micrographs of negative stained cells did not reveal prosthecae (Supplementary Fig. S1, available in IJSEM Online). 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.

16S rRNA gene sequence comparisons to representative bacteria with validly published names indicated that strains B2^T and 1_C16_27^T were affiliated with the family *Hyphomicrobiaceae*. Based on analysis by the EzTaxon service, the two strains were related most closely to type strains of the genera *Cucumibacter* (92.7–93.7 % similarity), *Devosia* (92.9–94.4 %) and *Zhangella* (91.7–92.1 %) as well as *Prosthecomicrobium pneumaticum* (93.1–93.2 %), and they showed <90 % sequence similarity to other described species of the *Hyphomicrobiaceae*. Phylogenetic trees constructed with all three treeing methods indicated that the two strains clustered with the genera *Cucumibacter* and *Zhangella* (Fig. 1 and Supplementary Figs S2 and 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 relatively low sequence similarities between strains B2^T and 1_C16_27^T and their phylogenetic neighbours indicate strongly 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 $C_{18:1}\omega7c$, characteristic of the vast majority of species within the *Alphaproteobacteria*. The relative amounts of $C_{19:0}$ cyclo $\omega8c$, 11-methyl $C_{18:1}\omega7c$ and $C_{18:1}$ varied according to the age of the culture (Supplementary Table S1). The respective contents of $C_{18:1}\omega7c$ of strains B2^T and 1_C16_27^T decreased from 61.3 and 75.3 % after 1 day to 58.0 and 74.6 % after 2 days and 12.1 and 59.0 % after 3 days. The proportions of $C_{19:0}$ cyclo $\omega8c$ and 11-methyl $C_{18:1}\omega7c$ increased accordingly. Together, $C_{19:0}$ cyclo $\omega8c$, 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 percentages 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 *Zhangella mobilis* CGMCC 1.7002^T (71.8–83.5 %) and were greater than those of *Cucumibacter marinus* DSM 18995^T, *Devosia riboflavina* DSM 7230^T and *Devosia geojensis* DSM 19414^T (42.0–62.5 %). 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 extracts from 2- and 3-day cultures of *C. marinus* DSM 18995^T and *Z. mobilis* CGMCC 1.7002^T but was absent in strains B2^T and 1_C16_27^T.

The results of two-dimensional TLC analysis of polar lipids extracted from strains B2^T and 1_C16_27^T as well as three

Table 1. Taxonomic characteristics that differentiate strains B2^T and 1_C16_27^T from 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. To determine substrate utilization for growth, all strains except *C. marinus* DSM 18995^T were grown in BM broth; *C. marinus* DSM 18995^T was grown on BM agar plates because its growth in BM broth was very slow. To determine susceptibility to antibiotics, all strains except *D. riboflavina* DSM 7230^T were grown on MA (BD) plates; *D. riboflavina* DSM 7230^T was grown on PYM agar plates because its growth on MA was very slow. +, Positive; −, negative; W, weakly positive; NA, no data available.

Characteristic	1	2	3	4	5	6
Colony colour*	LY	Y	C	PY	C	WH
Growth at 10 % NaCl (w/v)	+	−	+[a]†	−[b]	−	NA
Nitrate reduction	−	−	−	+	−	−
Urease	+	+	−	−	+	+
Hydrolysis of:						
Casein	+	+	−	−	−	−
DNA	−	−	−	−	−	+
Acid production from:						
Ethanol	+	+	NA	−	−	−
Glycerol	+	+	NA	−	+	+
Raffinose	−	−	NA	−	+	+
Rhamnose	+	−	NA	−	+	+
Utilization of:						
Gluconate	+	+	−	−	−	−
Lactose	−	+	−	−	+	+
Mannitol	+	+	−	−	+	+
L-Ornithine	+	−	+	+	−	−
Salicin	+	−	+	+	+	+
Sorbitol	−	+	−	−	+	+
Succinate	+	+	−	+	+	−
Sensitivity to:						
Bacitracin (0.04 IU)	+	+	−	+	−	−
Kanamycin (30 μg)	−	+	+	+[b]	−	−
Neomycin (30 μg)	+	+	+	+	+	−
Novobiocin (30 μg)	+	+	+	+	+	−
API ZYM results						
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[a]	53.1[b]	61.4[c]	60.8[d]

*C, Cream; LY, light yellow; PY, pale yellow; WH, white; Y, yellow.
†Data from: *a*, Hwang & Cho (2008); *b*, Xu *et al.* (2009); *c*, Nakagawa *et al.* (1996); *d*, Ryu *et al.* (2008).

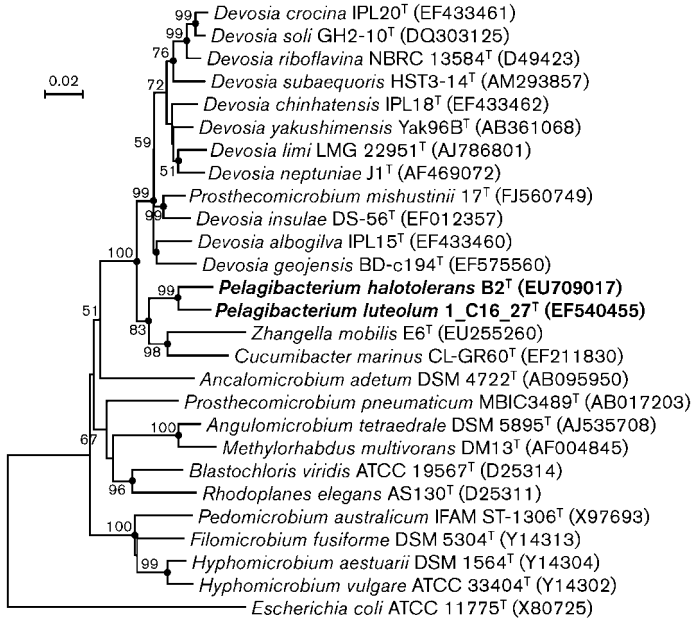

Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequences, showing the phylogenetic relationships of the novel isolates and related members of the *Hyphomicrobiaceae*. Bootstrap values are based on 1000 replicates; values >50 % are shown. Filled circles indicate nodes recovered in both maximum-likelihood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

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. The polar lipid profiles of all five strains were dominated by phosphatidylglycerol, diphosphatidylglycerol and two unidentified glycolipids (GL1 and GL3). Strains B2^T and 1_C16_27^T did not contain relatively large amounts of 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 also found in the three reference strains, but L5 and L11 are characteristic lipids of strains B2^T and 1_C16_27^T. In addition, L15, found in the three reference strains, was not detected in strains B2^T and 1_C16_27^T. 10-Methyl $C_{19:0}$ was found in both strains B2^T (0.6–0.9 %) and 1_C16_27^T (0.3–0.4 %), but not in related organisms (Supplementary Table S1). In addition to these differences, some phenotypic characteristics of strain B2^T and 1_C16_27^T, e.g. β-galactosidase, hydrolysis of casein and utilization of gluconate, distinguish the two novel isolates from the 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 represent a novel genus of the family *Hyphomicrobiaceae*. The main respiratory quinone of strains B2^T and 1_C16_27^T was Q-10 (94.2 and 96.2 %, respectively), while Q-9 was 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 compositions and a number of phenotypic characteristics (Table 1) and on the basis of their 16S rRNA gene sequences. The total content of $C_{19:0}$ cyclo $\omega 8c$, 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. The 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, α-glucosidase and trypsin (Table 1). The 16S rRNA gene sequence divergence between strain B2^T and 1_C16_27^T was 3.4 %, which exceeded the commonly accepted threshold of 3 % 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. and *Pelagibacterium luteolum* sp. nov. are proposed.

Description of *Pelagibacterium* gen. nov.

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

Gram-negative, non-spore-forming bacteria. Divide by binary division. Motile. Catalase- and oxidase-positive. Aerobic chemoheterotrophs. Major polar lipids are phosphatidylglycerol, diphosphatidylglycerol and two unidentified glycolipids. Small amounts of seven unidentified lipids (L1, L2, L3, L5, L6, L8 and L11) are detected. Major fatty acids include nonadecanoic ($C_{19:0}$ cyclo), octadecanoic ($C_{18:0}$ and $C_{18:0}$ 3-OH), octadecenoic ($C_{18:1}$) and hexadecanoic ($C_{16:0}$) acids. 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, semi-transparent and light yellowish after 3 days at 30 °C. Growth occurs at 0–13.0 % (w/v) NaCl, with optimum growth at 3.0–4.0 % (w/v). Grows at pH 6.0–9.5 and 10–42 °C (optimum growth at pH 7.0 and 30 °C). Nitrate is not reduced. Aesculin and casein are hydrolysed. Gelatin, DNA, starch and Tweens 40, 60 and 80 are not hydrolysed. 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, 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, 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, trehalose and D-xylose. Susceptible to (μg per disc 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) or 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-BI-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. In addition to the major polar lipids described for the genus, contains a third unidentified glycolipid. Trace amounts of nine unidentified lipids are detected. The major fatty acids are $C_{19:0}$ cyclo $\omega 8c$, 11-methyl $C_{18:1}\omega 7c$, $C_{18:1}\omega 7c$, $C_{16:0}$ and $C_{18:0}$. The DNA G+C content of the type strain is 59.3 mol% (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, semi-transparent and yellowish after 3 days at 30 °C. Growth occurs at 0–5.0 % (w/v) NaCl, with optimum growth at 0.5 % (w/v). Grows at pH 6.0–9.5 and 4–37 °C (optimum growth at pH 7.5 and 30 °C). Nitrate is not reduced. Aesculin and casein are hydrolysed. Gelatin, DNA, starch and Tweens 40, 60 and 80 are not hydrolysed. 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, 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, 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, trehalose and D-xylose. Susceptible to (μg per disc 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) or tobramycin (10). In the API ZYM system, acid phosphatase, *N*-acetyl-β-glucosaminidase, esterase (C4), esterase lipase (C8), β-glucosidase, leucine arylamidase (weak reaction) and naphthol-AS-BI-phosphohydrolase activities are present, whereas alkaline phosphatase, α-chymotrypsin, cystine arylamidase, α-fucosidase, α- and β-galactosidase, α-glucosidase, β-glucuronidase, lipase (C14), α-mannosidase, trypsin and valine arylamidase activities are absent. Trace amounts of ten unidentified lipids are detected. The major fatty acids are $C_{18:1}\omega 7c$, $C_{19:0}$ cyclo $\omega 8c$, $C_{18:0}$ and 11-methyl $C_{18:1}\omega 7c$. The DNA G+C content of the type strain is 58.1 mol% (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 north-eastern Estonia.

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