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Oblitimonas alkaliphila gen. nov., sp. nov., in the family *Pseudomonadaceae*, recovered from a historical collection of previously unidentified clinical strains

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Abstract

Eight Gram-stain-negative bacteria (B4199^T, C6819, C6918, D2441, D3318, E1086, E1148 and E5571) were identified during a retrospective study of unidentified strains from a historical collection held in the Special Bacteriology Reference Laboratory at the Centers for Disease Control and Prevention. The strains were isolated from eight patients: five female, two male and one not specified. No ages were indicated for the patients. The sources were urine (3), leg tissue (2), foot wound, lung tissue and deep liver. The strains originated from seven different states across the USA [Colorado, Connecticut (2), Indiana, North Carolina, Oregon and Pennsylvania]. The strains grew at 10–42 °C, were non-motile, alkalitolerant, slightly halophilic, microaerophilic, and catalase- and oxidase-positive. The DNA G+C content was 47.3–47.6 mol%. The major cellular fatty acids were tetradecanoic acid ($C_{14:0}$), hexadecanoic acid ($C_{16:0}$) and 11-octadecenoic acid ($C_{18:1}\omega7c$). Polar lipids detected were phosphatidylglycerol, phosphatidylethanolamine, diphosphatidylglycerol and unknown phospholipids; the only respiratory quinone detected was the ubiquinone Q-9 (100 %). 16S rRNA gene sequence analysis produced results with 95.6% similarity to *Pseudomonas caeni* DSM 24390^T and 95.2% similarity to *Thiopseudomonas denitrificans* X2^T. The results of the biochemical, chemotaxonomic and phylogenetic analyses between the study strains and some related type strains indicated that these strains represent a novel species of a new genus within the family *Pseudomonadaceae*, for which the name *Oblitimonas alkaliphila* gen. nov., sp. nov. is proposed. The type strain is B4199^T (=DSM 100830^T=CCUG 67636^T).

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The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains B4199^T, C6819, C6918, D2441, D3318, E1086, E1148 and E5571 are KP986583, KP986584, KP986585, KP986580, KP986581, KP986579, KP986582 and KP986578, respectively. The accession numbers for the whole genome sequences are CP012358 to CP012365, respectively.

One supplementary table is available with the online Supplementary Material.

The family *Pseudomonadaceae* consists of 13 genera (*Azomonas, Azomonotrichon, Azorhizophilus*, Azotobacter, *Cellvibrio, Chryseomonas, Flavimonas, Mesophilobacter, Pseudomonas, Rhizobacter, Rugamonas, Serpens* and *Thiopseudomonas*) at the time of writing (Tan *et al.*, 2015). Members of the family are commonly found in both fresh- and seawater, soil, plants and the environment; some genera are known to be clinically relevant (Palleroni, 1981). The common characteristics within the family *Pseudomonadaceae* are that they have rod-shaped cells that can be either straight or slightly curved, are Gram-stain-negative, have polar flagella and some are capable of fixing nitrogen (Palleroni, 1981). Eight Gram-stain-negative bacteria (B4199^T, C6819, C6918, D2441, D3318, E1086, E1148 and E5571) were identified during a retrospective study of unidentified strains from a historical collection held in the Special Bacteriology Reference Laboratory (SBRL) at the Centers for Disease Control and Prevention (CDC; http://www.cdc.gov/about/organization/mission.htm). The strains were isolated from eight patients: five female, two male and one not specified. No ages were indicated for any of the patients. The sources were urine (3), leg tissue (2), foot wound, lung tissue and deep liver. The strains originated from seven different states across different geographical locations in the USA [Colorado, Connecticut (2), Indiana, North Carolina, Oregon and Pennsylvania].

The SBRL at the CDC, has been characterizing unusual pathogenic bacteria submitted by the state laboratories for over 65 years. The SBRL has on file phenotypic characteristics of more than 60 000 of these isolates (Weyant *et al.*, 1996). The isolates have been frozen and stored in a collection that is maintained within the CDC. During a retrospective study of this historical collection, a group of eight isolates were found to have common phylogenetic and phenotypic characteristics. These previously unidentified strains were received in the SBRL from 1969 to 1979. 16S rRNA gene sequence analysis placed them in the family *Pseudomonadaceae* with 91.3–95.6% similarity to *Pseudomonas caeni* DSM 24390^T and the closely related '*Pseudomonas aeruginosa* group' (Anzai *et al.*, 2000). The other related type strains to B4199^T include: *Pseudomonas alcaliphila* DSM 17744^T (94.4% sequence similarity), *Pseudomonas pseudoalcaligenes* ATCC 17440^T(94.4% sequence similarity), *Serpens flexibilis* ATCC 29606^T (93.8% sequence similarity) and *Thiopseudomonas denitrificans* (X2^T, 95.2% sequence similarity). The eight strains shared between 99.6 and 100% similarity to each other based on the 16S rRNA gene sequence analysis. Attempts to identify the strains were also performed using the Vitek II system (bioMérieux) and the commercial test kit API 20NE (bioMérieux). The Vitek II and the API 20NE provided identifications based on their respective databases of *Pseudomonas stutzeri* and *Moraxella* sp., respectively. However, none of these results corresponded with the 16S rRNA gene sequence analysis results. The strains also shared common phenotypic traits such as the oxidation of the same carbohydrates, and the ability to grow at high pH levels and high salinity levels. In this study, the phenotypic, chemotaxonomic and genotypic analyses for the study strains are reported.

The eight unidentified clinical strains (B4199^T, C6819, C6918, D2441, D3318, E1086, E1148 and E5571) were recovered from frozen stocks and grown on heart infusion agar (HIA) (Remel) with 5% rabbit blood in a candle jar at 35 °C for 48 h. Demographics for all strains, including type strains of closely related phylogenetic neighbours (*P. alcaliphila* DSM 17744^T, *P. caeni* DSM 24390^T, *P. pseudoalcaligenes* ATCC 17440^T, *Serpens flexibilis*

ATCC 29606^T and *T. denitrificans* X2^T), used in this study are found in Table S1 (available in the online Supplementary Material).

Gram staining was performed following procedures described previously (Weyant *et al.*, 1996). For transmission electron microscopy (TEM) imaging, cells were harvested at 1 day post-inoculation, fixed in 2.5% glutaraldehyde and embedded in an Epon-substitute/Araldite epoxy resin (Mollenhauer, 1964). Sections were stained with uranyl acetate and lead citrate prior to viewing at the microscope (Tecnai Spirit, FEI).

All tests were performed at optimal temperature (35 °C) and were read at 7 days unless otherwise stated. The strains were grown in tubes containing 5 ml heart infusion broth (HIB) (Remel) at 35 °C for 24 h. The optimal growth temperature was determined by inoculating 50 µl of the 24 h broth culture to HIA with 5% rabbit blood, streaking for isolation, and placing in candle jars at the following temperatures: 4, 10, 20, 25, 30, 35, 42 and 45 °C. The oxygen requirements were determined by inoculating 50 µl of the broth culture onto two sets of HIA plates with 5% rabbit blood, streaking for isolation, and placing into a jar with a BD Gaspak EZ Anaerobe pouch and a jar with a BD Gaspak EZ Campy pouch (Becton Dickinson), respectively, and incubating at 35 °C. The ability to grow on different media types was determined by inoculating 50 µl of the broth culture onto trypticase soy agar (TSA) with 5% sheep blood (Becton Dickinson), HIB, tryptic soy broth (TSB), Mac-Conkey agar (Remel) and *Salmonella Shigella* agar at 35 °C and observing growth. The ability to grow under saline conditions was determined by inoculating 50 µl of the broth culture in nutrient broth with sodium chloride at 0, 2, 4, 6, 8 and 10% (w/v) and incubating at 35 °C. Growth at different pH levels was determined using the buffer system described by Xu *et al.* (2005) with a range of pH between 4 and 12. All biochemical tests were performed following procedures described previously (Weyant *et al.*, 1996). API ZYM, API 50CH and API 20NE (bioMérieux,) tests strips as well as the Vitek II (bioMérieux) test system using the commercial GN ID card (bioMérieux) were used following the instructions of the manufacturer.

Cellular fatty acid analysis was performed on the MIDI system by Microbial ID, according to the method of Sasser, (1990). Analyses of respiratory quinones and polar lipids were carried out according to the methods of Bligh *et al.* (1959), Tindall (1990a, b) and Tindall *et al.* (2007).

Genomic DNA for 16S rRNA gene sequence analysis was extracted from cell mass grown overnight on HIA with 5% rabbit blood by adding 1 µl cell mass into 200 µl of 99.7% DMSO (Fisher Bioreagents). Samples were incubated overnight before PCR was run. Amplification and sequencing of a 1468–1469 bp fragment of the 16S rRNA gene was performed as described previously (Morey *et al.*, 2006) with the following amended PCR cycle: 94 °C for 5 min, followed by 35 cycles of 94 °C for 15 s, 50 °C for 15 s and 72 °C for 90 s, with a final single extension of 72 °C for 5 min, and then held at 4 °C. The 16S rRNA gene was amplified using previously described PCR primers (Gee, *et al.*, 2003; Weisburg, *et al.*, 1991; Wuyts, *et al.*, 2002). Amplicons were sequenced using a 3730 DNA Analyzer (Applied Biosystems). The 16S rRNA gene sequence was assembled with the program Geneious 7.0.4 (Biomatters) using a *de novo* assembly approach. A Clustal-W distance

matrix was created with 16S rRNA gene sequences of type strains from the order *Pseudomonadales* using *Stenotrophomonas maltophilia* (ATCC 13637^T, AB008509) as the outgroup. A phylogenetic tree was inferred using the neighbour-joining method (Saitou *et al.*, 1987), the maximum-likelihood method (Felsenstein, 1981) and the maximum–parsimony method (Fitch, 1971); topology was assessed by a bootstrap analysis of 1000 replicates (Felsenstein, 1985). The evolutionary distances were computed using the Tamura–Nei method (Tamura *et al.*, 1993) and are in the units of the number of base substitutions per site. Evolutionary analyses were conducted in MEGA6 (Tamura *et al.*, 2013).

DNA G+C content (mol%) of the whole genome was determined using MEGA6. *In silico* DNA–DNA hybridization (dDDH) was performed according to published methods (Auch *et al.*, 2010; Meier-Kolthoff *et al.*, 2013). The average nucleotide identity (ANI) was calculated using EZgenome.

Strains originated from seven different states across different geographical locations in the USA (Table S1). The strains were received during the years 1969–1979. There was no common site of infection, cultures were obtained from: urine (3), leg tissue (2), deep liver, foot wound and lung tissue. Microscopic morphology, Gram and TEM staining (Fig. 1a), showed that the bacteria were wide, Gram-stain-negative, non-motile, pleomorphic bacilli (sometimes more coccoid in shape) and grouped in chains or pairs. Colonies of B4199^T on HIA with 5% rabbit blood plates were convex, circular and 1–2 mm in size. Growth was observed from temperatures of 10–42 °C. The strains grew aerobically and microaerophilically, but not anaerobically. Growth was observed at 24 h on HIA with 5% rabbit blood, TSA with 5% sheep blood, HIB, TSB, MacConkey agar and *Salmonella Shigella* agar. Optimal growth occurred at 48 h on HIA with 5% rabbit blood at 35 °C. All strains grew with 0–6% NaCl, except E1086 and E5571 (0–8 %); all strains, except D3318 (pH 6–10), grew in alkaline conditions (pH 6–11). On heart infusion tyrosine agar (HIT), a light brown pigment was observed. The strains oxidized glucose and xylose, but not mannitol, lactose, sucrose or maltose. The strains were catalase- and oxidase-positive. They were negative for the production of urea, indole, hydrogen sulfide and gelatinase, the utilization of citrate as a sole carbon source, the reduction of nitrate and nitrite, and the hydrolysis of aesculin. The organisms did not grow on cetrimide agar and were not motile (Table 1). Using the API ZYM test, all strains were positive for leucine arylamidase; strain E1086 was positive for esterase lipase and D3318, E1086 and E5571 were positive for acid phosphatase. All strains were negative for alkaline phosphatase, lipase, valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, naphtol-AS-BI-phosphohydrolase, α-galactosidase, α-glucosidase, α-mannosidase, α-fucosidase, β-galactosidase, β-glucuronidase, β-glucosidase and *N*-acetyl-β-glucosaminidase. Using the API 50CH test, all strains oxidized the following carbohydrates: L-arabinose, D-fucose, D-galactose, D-glucose, D-mannose, D-ribose and D-xylose. E1086, E1148 and E5571 oxidized D-arabinose; B4199^T, E1086 and E1148 oxidized L-rhamnose and E1148 oxidized melibiose. All other carbohydrates in the panel were negative. C6918, D2441 and E1148 were positive for the hydrolysis of ferric citrate aesculin (Table 2).

Fatty acid methyl ester analysis showed that the strains consisted predominantly of tetradecanoic acid ($C_{14:0}$), hexadecanoic acid ($C_{16:0}$) and 11-octadecenoic acid

($C_{18:1}\omega 7c$). The only respiratory quinone detected was the ubiquinone Q-9 (100%). Polar lipids detected were phosphatidylglycerol, phosphatidylethanolamine, diphosphatidylglycerol and an unknown phospholipid (Fig. 2).

Complete 16S rRNA gene sequences of 1468–1469 bp were obtained. Strains showed 99.6–100% similarity to each other. Genetic analysis showed the closest phylogenetic relative to each strain to be *P. caeni* DSM 24390^T at 95.6–95.8% similarity. The neighbour-joining tree showed that the study strains formed a monophyletic clade with the closest type strain being *P. caeni* DSM 24390^T within the family *Pseudomonadaceae* (Fig. 3). Similar results were obtained using the maximum-likelihood and maximum-parsimony methods (data not shown).

The DNA G+C content of the whole genomes ranged from 47.3–47.6mol% (Lauer *et al.*, 2015). dDDH showed greater than 70% hybridization between all strains (86.8–92.6 %) indicating they belong to the same species. All strains were above the 95–96% ANI threshold for species delineation (Richter & Rosselló-Móra, 2009).

Based on the analysis of the 16S rRNA gene sequences, it was determined that the organisms are in the family *Pseudomonadaceae*, the most similar sequence being from *P. caeni* DSM 24390^T (NR116388, 95.6 %). Typical of the family *Pseudomonadaceae*, the strains were all oxidase positive. Many members of the family *Pseudomonadaceae*, including *Pseudomonas aeruginosa* ATCC 10145^T, are motile. However, none of the eight strains within this study were motile. Further distinguishing the strains from the species of the genus *Pseudomonas* was the inability to reduce nitrogen. Many members of the family *Pseudomonadaceae* do not grow at a high pH or in the presence of high NaCl concentrations; these novel strains, however, grew at a pH of 11 and in the presence of 6% NaCl. Uniform morphology of the strains showed the cells to be pleomorphic, alternating between both cocci and bacillary forms, depending on the age of the culture; this characteristic may serve as a tool to help with identification of the strains.

Further distinguishing characteristics of the strains that differ from those of currently classified genera in the family *Pseudomonadaceae* are DNA G+C content and the size of the genome. The DNA G+C content of *P. alcaliphila* DSM 17744^T, *P. caeni* DSM 24390^T, *P. pseudoalcaligenes* ATCC 1744^T and *Serpens flexibilis* ATCC 29606^T ranges from 50.1 to 65.8 mol% (Hespell, 1977; Romanenko *et al.*, 2005; Saha *et al.*, 2010; Xiao *et al.*, 2009; Yumoto *et al.*, 2001). The DNA G+C content of the study strains ranged from 47.3 to 47.6 mol%. We also found that the length of the genome of the study strains, around 2.3 Mbp (Lauer *et al.*, 2015) was decidedly shorter than that of some of the closely related type strains (*P. alcaliphila* DSM 17744^T, *P. caeni* DSM 24390^T, *P. pseudoalcaligenes* ATCC 17440^T and *Serpens flexibilis* ATCC 29606^T), 3.02–5.43 Mbp (Lenneman & Barney, 2014; Santopolo *et al.*, 2013).

Since the last strain in this group was received in 1979 in our lab, it is hypothesized that automated systems could be incorrectly identifying these organisms. The analytical profile index (API) system was introduced in the 1970s, and has been subsequently updated as more organisms are identified. Under normal circumstances, bacterial isolates have to first pass

through a hospital and then a state laboratory, before an isolate reaches the CDC. If the hospital or state health department labs utilize automated or rapid identification systems, such as the Vitek II or the API 20NE, it is possible that the identifications provided are sufficient to treat the patient and require no further testing, but ultimately are incorrect. For example, the Vitek II system gives three different identification scores with 'Excellent' being the highest match percentage. When the study strains were run on the Vitek II system, the score of 'Excellent identification' of *P. stutzeri*, 96% probability, was given instead of an unknown identification. An identification of *Moraxella* sp. occurred when the API 20NE rapid identification system was used. Further studies are needed to describe this unusual aspect. The results of the physiological properties of the study strains, low level of 16S rRNA gene sequence similarities with other genera within the family *Pseudomonadaceae*, the lack of motility, the ability to grow at a high pH or in the presence of high concentrations of sodium chloride, dDDH and ANI results, low DNA G+C content and small genome size compared with the type strains within our study suggest that these strains represent a novel species of a new genus within the family *Pseudomonadaceae*, for which the name *Oblitimonas alkaliphila* gen. nov., sp. nov. is proposed.

Description of *Oblitimonas* gen. nov

Oblitimonas gen. nov. (Ob.li.ti.mo′nas. L. part. adj. *oblitus* forgotten; L. fem. n. *monas* unit, monad; N.L. fem. n. *Oblitimonas* a forgotten monad).

Cells are Gram-stain-negative, aerobic, oxidase- and catalase-positive and non-motile. The major fatty acids (>1 %) are tetradecanoic acid ($C_{14:0}$, hexadecanoic acid ($C_{16:0}$) and 11-octadecenoic acid ($C_{18:1}\omega 7c$); polar lipids detected are phosphatidylglycerol, phosphatidylethanolamine, diphosphatidylglycerol and unknown phospholipids. The DNA G+C content is 47.3–47.6 mol%. The only respiratory quinone detected is ubiquinone Q-9. Phylogenetically, the genus is affiliated to the family *Pseudomonadaceae*. The type species is *Oblitimonas alkaliphila*.

Description of *Oblitimonas alkaliphila* sp. nov

Oblitimonas alkaliphila [al.ka.li′phi.la. N.L. n. *alkali* soda ash; N.L. adj. *philus -a -um* (from Gr. adj. *philos -ê -on*) friend, loving; N.L. fem. adj. *alkalphila* alkaline-loving).

Exhibits the following properties in addition to those given in the genus description. On HIA with 5% rabbit blood, cells are convex, circular in shape and around 1–2 mm in size. Oxidizes both glucose and xylose but is negative for utilization of mannitol, lactose, sucrose and maltose. Cannot use citrate as a sole source of carbon. Does not hydrolyse aesculin, gelatin or urea. Does not reduce nitrate or nitrite. Negative for the production of indole. Growth occurs aerobically and in a Campy environment but not anaerobically. Strains are able to grow under alkalitolerant conditions; up to pH 11 (optimal is pH 7.5). Growth occurs in the presence of 0–8% (w/v) NaCl (optimum, 4–6 %). Optimal growth occurs after incubation for 48 h at 35 °C in a candle jar. Growth occurs at 10–42 °C (optimum, 20–35 °C). Strains grow on both MacConkey agar and *Salmonella Shigella* agar, and produce a light brown pigment on HIT agar. Positive for leucine arylamidase, variable for esterase

lipase and acid phosphastase. Negative for alkaline phosphatase, lipase, valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, naphtol-AS-BI-phosphohydrolase, α-galactosidase, α-glucosidase, α-mannosidase, α-fucosidase, β-galactosidase, β-glucuronidase, β-glucosidase and *N*-acetyl-β-glucosaminidase (API ZYM test strip). Oxidizes the following carbohydrates: L-arabinose, D-fucose, D-galactose, D-glucose, D-mannose, D-ribose and D-xylose. Variable for the oxidation of D-arabinose, L-rhamnose and melibiose. Variable for the hydrolysis of ferric citrate aesculin. Negative for the rest of the carbohydrates (API 50CH test strip): glycerol, erythritol, L-xylose, D-adonitol, methyl β-D-xylopyranoside, D-fructose, L-sorbose, dulcitol, inositol, D-mannitol, D-sorbitol, methyl α-D-mannopyranoside, *N*-acetylglucosamine, amygdalin, arbutin, salicin, cellobiose, maltose, lactose, sucrose, trehalose, inulin, melezitose, raffinose, starch, glycogen, xylitol, gentiobiose, turanose, D-lyxose, D-tagatose, L-fucose, D-arabitol, L-arabitol, potassium gluconate, potassium 2-ketogluconate and potassium 5-ketogluconate. The only respiratory quinone is Q9. The major fatty acids (>1 %) are $C_{12:0}$ $C_{14:0}$ $C_{15:0}$ $C_{16:0}$ $C_{18:1}\omega7c$ and $C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$. The polar lipids consist of phosphatidylglycerol, phosphatidylethanolamine, diphosphatidylglycerol and unknown phospholipids.

The type strain is B4199^T (=DSM 100830^T=CCUG 67636^T). The DNA G+C content of the type strain is 47.3–47.6 mol%.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

ANI	average nucleotide identity
CDC	Centers for Disease Control and Prevention
dDDH	*in silico* DNA-DNA hybridization
SBRL	Special Bacteriology Reference Laboratory
TEM	transmission electron microscopy

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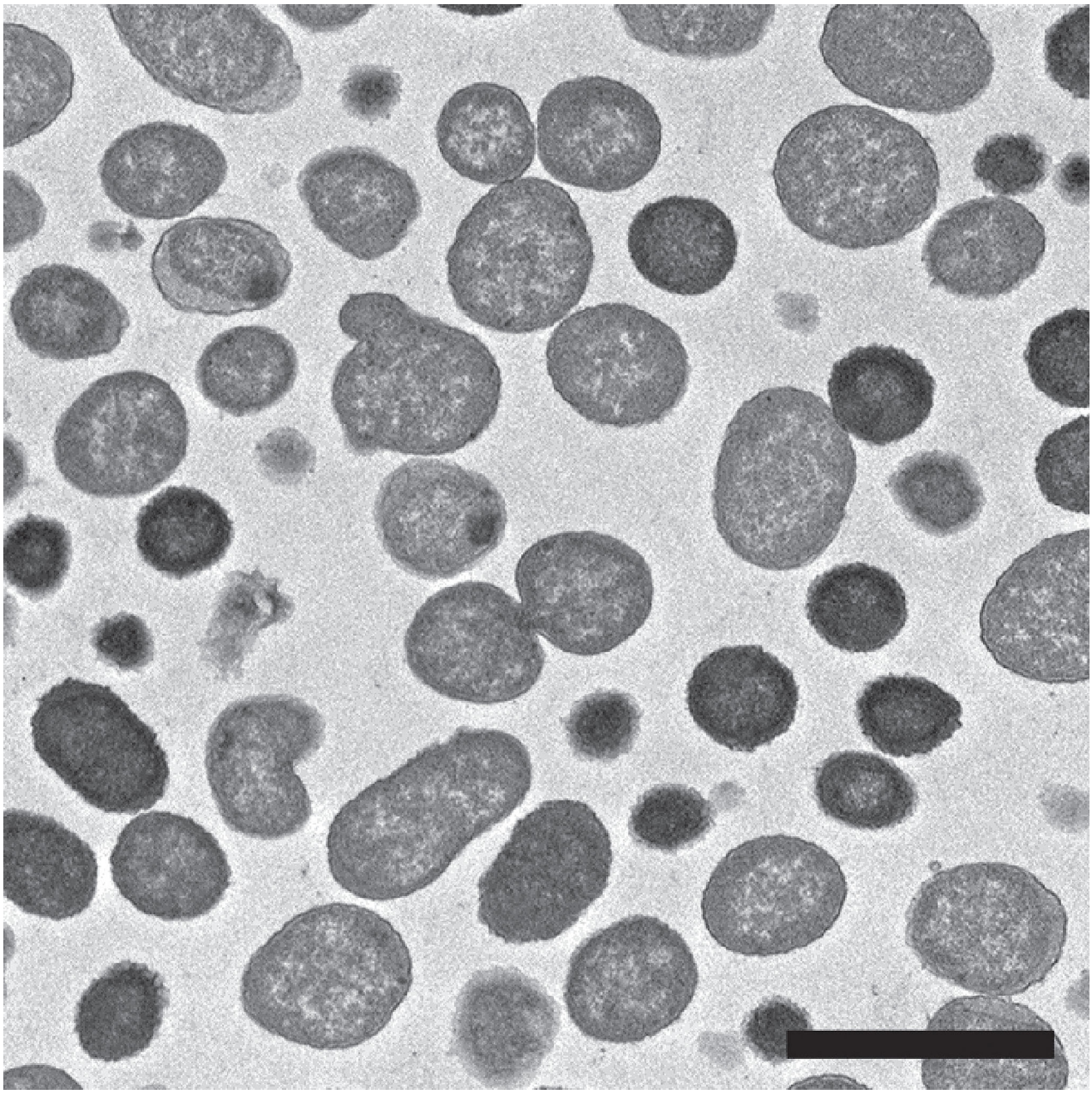

Fig. 1.
TEM of cells of strain B4199^{T} at ×1000 magnification; bar, 2 μm.

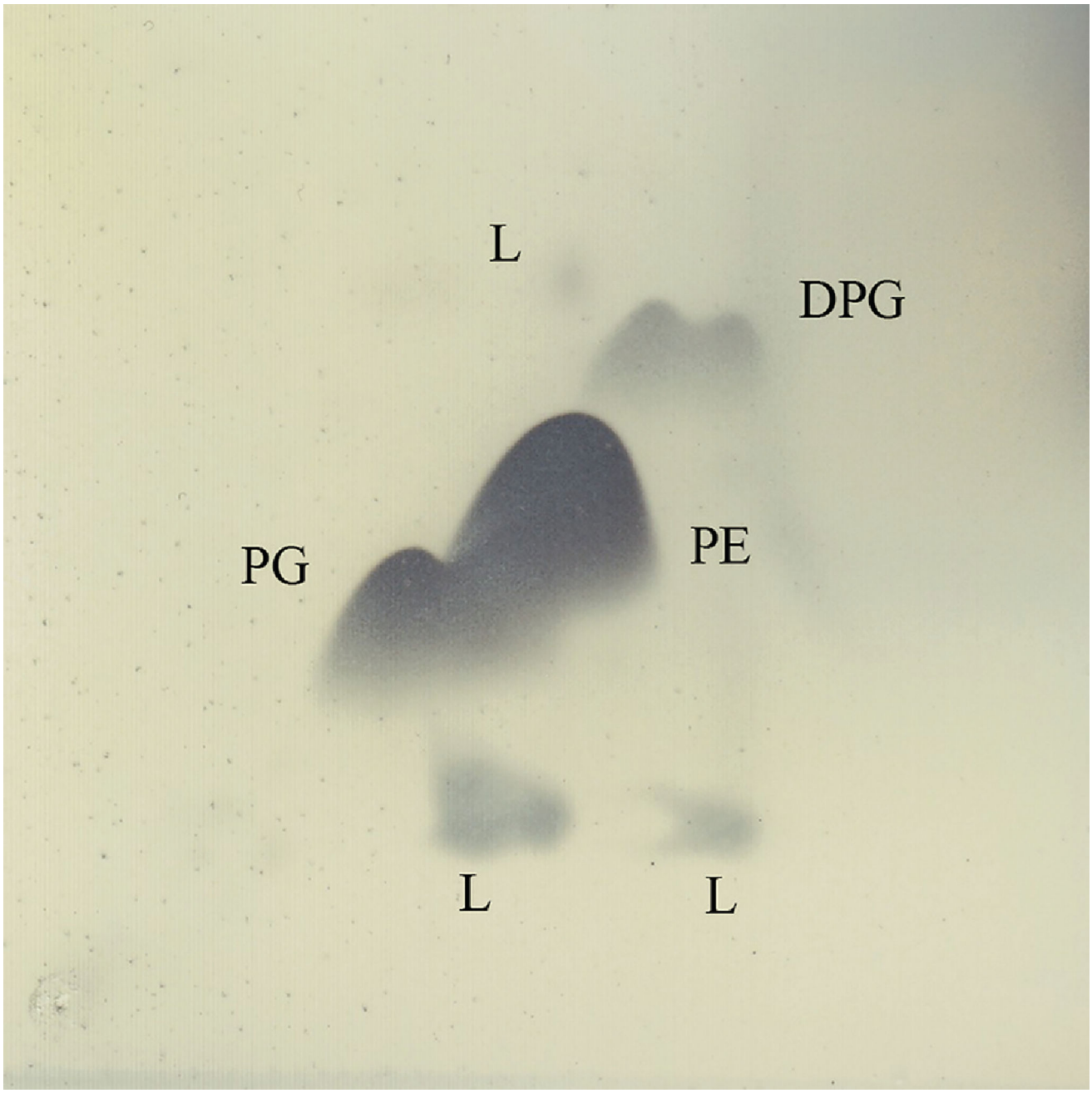

Fig. 2.

Polar lipids of strain. strain B4199^{T}. DPG, diphosphatidylglycerol; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; L, lipid.

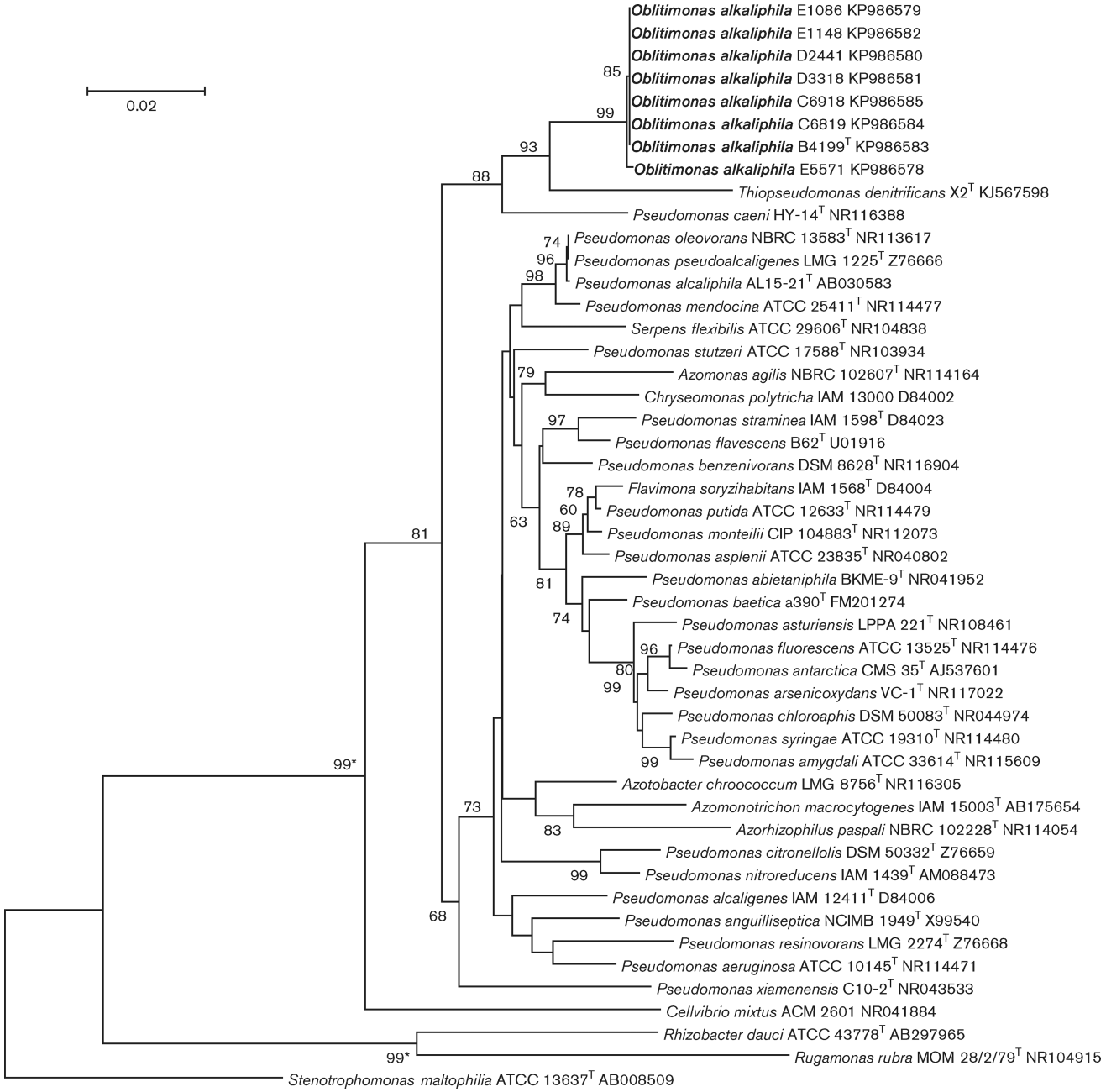

Fig. 3.

Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences (1420 bp) showing the relationship of *Oblitimonas alkaliphila* gen. nov., sp. nov. (strains B4199^T, D3318, C6918, D2441, E1148, E1086, C6819 and E5571) to members of the order *Pseudomonadales*. Bootstrap values (percentages of 1000 replications) of above 65% are shown at branch points. Astrisks denote nodes that were also recovered using maximum-likelihood methods. Bar, 0.02 base substitutions per site.

Table 1

Phenotypic and chemotaxonomic data of *Oblitimonas alkaliphila* gen. nov., sp. nov. and the nearest phylogenetic neighbours

Strains: 1, B4199^T; 2 (n=7), C6819, C6918, D2441, D3318, E1086, E1148 and E5571; 3, *P. alcaliphila* DSM 17744^T; 4, *P. caeni* DSM 24390^T; 5, *P. pseudoalcaligenes* ATCC 17440^T; 6, *Serpens flexibilis* ATCC 29606^T; 7, *T. denitrificans* X2^T. All strains positive: catalase, oxidase, growth at 10 °C, 25 °C, 35 °C, pH 7.5, pH 8.0, pH 9.0, and in nutrient broth with 2% NaCl; all strains negative: TSI slant acid, TSI butt acid, gelatin and aesculin hydrolysis, growth with 10% NaCl, utilization of D-mannitol, lactose, sucrose, maltose. +, Positive; −, negative; ND, no data.

Test performed	**1**	**2***	**3**	**4**	**5**	**6**	**7†**
Oxidation of:							
D-Glucose	+	7(100)	+	−	−	−	−
D-Xylose	+	7(100)	+	−	−	−	ND
Urea, Christensen's	−	0(0)	+	−	−	−	−
Nitrate reduction	−	0(0)	+	−	+	+	+
H_2S (lead acetate paper)	−	0(0)	−	−	+	−	ND
Growth temperature:							
4 °C	−	0(0)	−	+	−	−	−
42 °C	+	6(85.7)	−	−	−	+	−
45 °C	−	0(0)	−	−	−	+	−
Growth conditions:							
Anaerobe Jar	−	0(0)	+	+	+	+	+
Nutrient broth, 0% NaCl	+	7(100)	+	−	+	+	+
Nutrient broth, 4% NaCl	+	7(100)	+	−	+	+	−
Nutrient broth, 6% NaCl	+	7(100)	+	−	+	−	−
Nutrient broth, 8% NaCl	−	2(28.6)	+	−	−	−	−
Growth on:							
MacConkey agar	+	7(100)	+	−	+	+	ND
Salmonella Shigella agar	+	7(100)	+	−	+	−	ND
Cetrimide agar	−	0(0)	+	−	+	−	ND
Citrate utilization	−	0(0)	+	−	+	−	−
Motility	−	0(0)	+	+	+	+	+
Growth at pH:							
6.0	+	7(100)	+	−	+	−	+

Test performed	1	2*	3	4	5	6	7†
10.0	+	7(100)	+	–	+	+	+
11.0	+	6(85.7)	+	–	+	–	–

* *n*=7, Number positive (%).

† Data from Tan *et al.* (2015).

Table 2

Phenotypic and chemotaxonomic data of *Oblitimonas alkaliphila* gen. nov., sp. nov. and the nearest phylogenetic neighbours

Strains: 1, B4199^T; 2 (n=7), C6819, C6918, D2441, D3318, E1086, E1148 and E5571; 3, *P. alcaliphila* DSM 17744^T; 4, *P. caeni* DSM 24390^T; 5, *P. pseudoalcaligenes* ATCC 17440^T; 6, *Serpens flexibilis* ATCC 29606^T; 7, *.T. denitrificans* X2^T. $C_{12:0}$=dodecanoic acid, $C_{14:0}$=myristic acid, $C_{15:0}$=pentadecanoic acid, $C_{16:0}$=palmitic acid. +, Positive; −, negative; ND, no data; W, weakly positive.

Test performed	1	2	3	4	5	6	7*
Fatty acid							
$C_{12:0}$	5.34	4.46–5.53	10.13	9.53	9.75	4.85	7.47
$C_{14:0}$	10.79	7.96–13.66	<1	1.62	<1	2.11	6.80
$C_{15:0}$	5.01	1.51–5.01	<1	<1	<1	4.88	ND
$C_{16:0}$	21.08	20.64–23.16	23.82	35.29	22.38	22.27	29.13
$C_{18:1}\omega 7c$	34.48	33.3–38.36	31.05	8.59	36.19	20.93	27.38
Summed feature 3†	9.77	8.54–12.09	17.54	33.09	12.08	4.68	25.36
Quinones	Q9	Q9	Q9	Q9	Q9	Q9	Q8 & Q9
API ZYM test:‡							
Alkaline phosphatase	−	0(0)	+	−	−	−	−
Esterase lipase (C8)	−	1(14)	+	+	+	+	W
Leucine arylamidase	+	7(100)	+	+	+	+	ND
Acid phosphatase	−	3(43)	−	−	−	−	−
API 50CH test:‡							
L-Arabinose	+	7(100)	−	−	−	−	ND
D-Fucose	+	7(100)	−	−	−	−	ND
D-Galactose	+	7(100)	−	−	−	−	ND
D-Glucose	+	7(100)	−	−	−	−	ND
D-Mannose	+	7(100)	−	−	−	−	ND
D-Ribose	+	7(100)	−	−	−	−	ND
D-Xylose	+	7(100)	−	−	−	−	ND
D-Arabinose	−	3(43)	−	−	−	−	ND
L-Rhamnose	+	2(29)	−	−	−	−	ND
Melibiose	−	1(14)	−	−	−	−	ND

Test performed	1	2	3	4	5	6	7*
Ferric citrate aesculin	–	3(43)	–	–	–	–	ND
DNA G+C content (mol%)§	47.4	47.3–47.6	63.2	50.1–51.1	62.2	65.8	59

*Data from Tan *et al.* (2015).

†Summed feature 3 comprises $C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$.

‡*n*=7, Number positive (%).

§Data for strains 3–6 from Hespell (1977), Romanenko *et al.* (2005), Saha *et al.* (2010), Xiao *et al.* (2009) and Yumoto *et al.* (2001).