Brevundimonas halotolerans sp. nov., *Brevundimonas poindexterae* sp. nov. and *Brevundimonas staleyi* sp. nov., prosthecate bacteria from aquatic habitats

Wolf-Rainer Abraham,[1] Andréia B. Estrela,[1] Dennis I. Nikitin,[2] John Smit[3] and Marc Vancanneyt[4]

Correspondence
Wolf-Rainer Abraham
wab@gbf.de

[1]Helmholtz Center for Infection Research, Chemical Microbiology, Inhoffenstrasse 7, 38124 Braunschweig, Germany

[2]Institute of Microbiology, Russian Academy of Sciences, Prospect 60-Letiya Octyabrya 7, korp. 2, Moscow 117811, Russia

[3]University of British Columbia, Dept of Microbiology and Immunology, Vancouver, BC, Canada

[4]BCCM/LMG Bacteria Collection and Laboratory of Microbiology, Ghent University, Ghent, Belgium

Eight strains of Gram-negative, bacteroid-shaped, prosthecate bacteria, isolated from brackish water (MCS24^T, MCS17 and MCS35), the marine environment (CM260, CM272 and CM282) and activated sludge (FWC40^T and FWC43^T), were characterized using a polyphasic approach. Analysis of 16S rRNA gene sequences determined that all strains were affiliated to the alphaproteobacterial genus *Brevundimonas*, forming three distinct phyletic lineages within the genus. The strains grew best with 5–30 g NaCl l^{-1} at 20–30 °C. DNA G+C contents for strains MCS24^T, FWC40^T and FWC43^T were between 65 and 67 mol%, in accordance with values reported previously for other species of the genus. Moreover, chemotaxonomic data and physiological and biochemical tests allowed the phenotypic differentiation of three novel species within the genus *Brevundimonas*, for which the names *Brevundimonas halotolerans* sp. nov. (type strain MCS24^T =LMG 25346^T =CCUG 58273^T), *Brevundimonas poindexterae* sp. nov. (type strain FWC40^T =LMG 25261^T =CCUG 57883^T) and *Brevundimonas staleyi* sp. nov. (type strain FWC43^T =LMG 25262^T =CCUG 57884^T) are proposed.

In a previous study, caulobacteria from a broad range of freshwater, brackish water, marine and soil habitats (Anast & Smit, 1988; MacRae & Smit, 1991; Segers *et al.*, 1994) were studied using a polyphasic approach. As a result, the descriptions of the genera *Caulobacter* and *Brevundimonas* were emended and a number of *Caulobacter* species were transferred to the genus *Brevundimonas* (Abraham *et al.*, 1999). We report here on three novel species within the genus *Brevundimonas* which emerged from this study.

Strains used in this study were obtained from the American Type Culture Collection (ATCC), the Deutsche Sammlung für Mikroorganismen und Zellkulturen (DSMZ) and the Laboratorium voor Microbiologie, Universiteit Gent, Belgium (LMG), and from one of the authors (J. S.) (CM, FWC and MCS strains) (Table 1). The strains were grown in freshwater Caulobacter medium PYEM (2 g peptone, 2 g yeast extract and 0.5 g NH_4Cl per litre MQ water). After autoclaving and cooling, 5 ml sterile-filtered riboflavin (0.2 mg ml^{-1}), 2 ml 50 % glucose (sterile), 1 ml 20 % $MgSO_4$ (sterile) and 1 ml 10 % $CaCl_2$ (sterile) were added. The strains were grown in 2 l flasks at 30 °C with shaking at 100 r.p.m. and biomass was harvested in the late exponential phase, after 72 h.

For the determination of DNA base compositions, genomic DNA was isolated from 2 ml culture using the DNeasy kit (Qiagen). DNA was digested enzymically and mean G+C contents were determined by HPLC (Tamaoka & Komagata, 1984). Calculations were carried out according to Mesbah *et al.* (1989), with non-methylated lambda phage DNA (Sigma) as a standard. For all strains, G+C contents between 64.6 and 67.0 mol% were found, within the range of those already reported for species of the genus *Brevundimonas* (Vancanneyt *et al.*, 2005).

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains MCS24^T, FWC40^T, FWC43^T, MCS17, CM260, CM272, CM282 and MCS35 are M83810, AJ227797–AJ227799 and FN397630–FN39763, respectively.

A 16S rRNA gene sequence-based UPGMA tree and details of phospho- and sulfolipids are available as supplementary material with the online version of this paper.

Table 1. Strains used in this study

Accession numbers of sequences determined in this study are in bold.

Strain	Origin	16S rRNA gene sequence accession no.
Brevundimonas halotolerans sp. nov.		
CM260	J. Poindexter, Woods Hole, MA, USA	**FN397630**
CM272	J. Poindexter, Woods Hole, MA, USA	**FN397631**
CM282	J. Poindexter, Woods Hole, MA, USA	**FN397632**
MCS17	Brackish water slough adjacent to Arness Park, Kingston, WA, USA	AJ227799
MCS24^T	Brackish water creek flowing into salt water at Carkeek Park, Seattle, WA, USA	M83810
MCS35	Water of the Baltic Sea north of Rostock, Germany	**FN397633**
Brevundimonas poindexterae sp. nov.		
FWC40^T	Secondary treatment facility, activated sludge system, Kelowna, Canada	AJ227797
Brevundimonas staleyi sp. nov.		
FWC43^T	Secondary treatment facility, activated sludge system, Calgary, Canada	AJ227798

The isolates were identified phylogenetically by sequencing of the 16S rRNA genes and by comparison of the sequences with those of type strains. For the PCR, DNA from single colonies was used which had been lysed by exposure to 100 µl TE buffer for about 10 min at 95 °C. Nearly complete 16S rRNA gene sequences were obtained as described previously (Yakimov *et al.*, 2003). The reactions were evaluated on an Applied Biosystems 377 Genetic Analyzer. The program SEQUENCHER version 4.0.5 (Gene Codes Corporation) was used to analyse the sequences. Phylogenetic analysis was made using the CLUSTAL W software (Thompson *et al.*, 1997) for the alignments and the neighbour-joining algorithm and bootstrap percentages based on 1000 replications (Fig. 1) and the UPMGA algorithm with Kimura's two-parameter model was calculated in the software MEGA 3.1. (Kumar *et al.*, 2004) (Supplementary Fig. S1, available in IJSEM Online) using sequences contained in the EMBL database (Kanz *et al.*, 2005). The 16S rRNA gene sequences determined have been deposited in the EMBL nucleotide database under the accession numbers listed in Table 1. The similarity of 16S rRNA gene sequences was 97.8, 97.5 and 97.3 % between FWC40^T and *Brevundimonas lenta* DS-18^T, *Brevundimonas subvibrioides* CB81^T and *Brevundimonas bullata* DSM 7126^T, respectively, and 96.8 and 96.5 % between MCS24^T and *Brevundimonas variabilis* ATCC 15255^T and *Brevundimonas bacteroides* CB7^T, respectively. The identity between the 16S rRNA gene sequences of FWC43^T and *B. bullata* DSM 7126^T (Kang *et al.*, 2009) was 98.6 %, but the two strains differ sharply in their cell morphology.

For whole-cell fatty acid analysis, cells were saponified [15 % (w/v) NaOH, 30 min, 100 °C], methylated to fatty acid methyl esters (methanolic HCl, 10 min, 80 °C) and extracted (hexane/methyl-tert-butyl ether; 1 : 1, v/v) as described in detail by Osterhout *et al.* (1991). Fatty acid methyl esters were analysed on an HP 5890A gas chromatograph. Separation of fatty acid methyl esters was achieved with a fused-silica capillary column (25 m by 0.2 mm) with cross-linked 5 % phenyl methyl silicone (film thickness 0.33 µm; HP Ultra 2). The computer-controlled parameters were the same as those described by Osterhout *et al.* (1991). The instrument was equipped with a flame-ionization detector and an autosampler (HP 7673). The main fatty acids were summed feature 7 (one or more of $C_{18:1}\omega7c$, $C_{18:1}\omega9t$ and $C_{18:1}\omega12t$) and $C_{16:0}$ for all strains; the main hydroxy fatty acid was always $C_{12:0}$ 3-OH. Strain FWC40^T had a rather large amount of 11-methyl-12-*trans*-octadecanoic acid (11-methyl $C_{18:1}\omega5t$; ECL 18.080) (Abraham *et al.*, 2008), the highest yet reported for a *Brevundimonas* strain (Table 2).

Polar lipid fatty acid analysis with fast-atom-bombardment mass spectrometry (FAB-MS) was performed in the negative mode on the first of two mass spectrometers of a tandem high-resolution instrument in an $E_1B_1E_2B_2$ configuration (JMS-HX/HX110A; JEOL) using the conditions reported by Abraham *et al.* (1997). In all strains, phosphatidylglycerol, 1,2-di-*O*-acyl-3-*O*-[β-D-glucopyranosyl-(1→4)-α-D-glucopyranuronosyl]glycerol, *lyso*-phosphoglucolipid and 1,2-di-*O*-acyl-3-*O*-[6′-(*sn*-1″,2″-di-*O*-acyl-glycero-3″-phosphoryl)-α-D-glucopyranosyl]-*sn*-glycerol were present. Furthermore, sulfoquinovosyl diacylglycerols could be detected in strains CM260, CM272, CM280, MCS17 and MCS24^T (Supplementary Table S1).

For phenotypic characterization, strains were grown in 20 ml PYEM medium amended with 0, 5, 10, 20, 30, 40, 60, 80 or 100 g NaCl l^{-1} at 30 °C. The OD_{600} of the cell

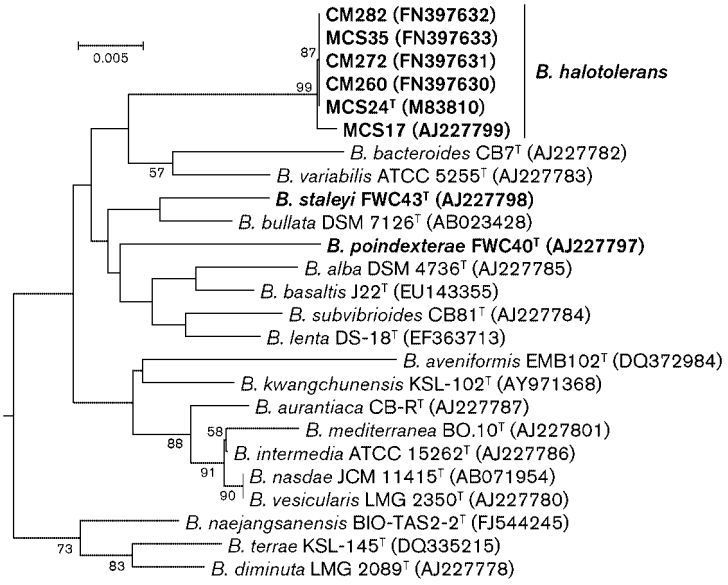

Fig. 1. Unrooted neighbour-joining dendrogram of the phylogenetic relationships between *Brevundimonas poindexterae* sp. nov. FWC40^T, *Brevundimonas staleyi* sp. nov. FWC43^T, *Brevundimonas halotolerans* sp. nov. MCS24^T (and five other strains) and all recognized type strains of the genus *Brevundimonas* based on a distance-matrix analysis of 16S rRNA gene sequences. GenBank accession numbers are given in parentheses. The sequence of *Hirschia baltica* ATCC 49814^T was used as an outgroup (not shown). Bootstrap percentages >50 % are indicated at tree branching points. Bar, 0.005 substitutions per nucleotide position.

suspension was determined at the beginning of the experiment and after 2 days. The difference between these two measurements was used to determine salt tolerance. All strains could grow with salt concentrations of 5–30 g l^{-1} but not with 100 g l^{-1}. Strain MCS24^T also showed growth, albeit slow, with salt concentrations up to 80 g l^{-1}. Strain FWC40^T showed reduced growth without NaCl.

Substrate specificity tests were conducted by the use of API Biotype 100 and API 20 NE test strips (bioMérieux) using

Table 2. Fatty acid contents of whole-cell hydrolysates of *Brevundimonas* strains

Strains: 1, *B. alba* ATCC 15265^T; 2, *B. aurantiaca* ATCC 15266^T; 3, *B. bacteroides* LMG 15096^T; 4, *B. diminuta* LMG 2089^T; 5, *B. intermedia* ATCC 15262^T; 6–10, *B. halotolerans* sp. nov. strains CM260 (6), CM272 (7), CM282 (8), MCS17 (9) and MCS24^T (10); 11, *B. poindexterae* sp. nov. FWC40^T; 10, *B. staleyi* sp. nov. FWC43^T; 13, *B. subvibrioides* LMG 14903^T; 14, *B. variabilis* ATCC 15255^T; 15, *B. vesicularis* LMG 2350^T; 16, *B. bullata* DSM 7126^T. Data were obtained in this study. Values are percentages of total fatty acids; only fatty acids accounting for more than 1.0 % (mean amount) are indicated. The following strains contained significant amounts (>1.0 %) of additional fatty acids: *B. subvibrioides* LMG 14903^T also contained 20 : 2ω6,9*c* (1.6 %); *B. alba* also contained 16 : 1ω9*c* (2.7 %). tr, Trace amount (<1.0 %); −, not detected; ECL, unknown fatty acid identified by equivalent chain length.

Fatty acid	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
12 : 0 3-OH	1.1	2.3	2.3	1.5	1.7	1.3	1.2	1.2	1.2	1.0	3.1	2.2	2.8	2.3	1.9	1.3
12 : 1 3-OH	tr	tr	−	tr	−	−	−	−	−	−	−	−	−	−	−	tr
14 : 0	1.0	3.4	3.0	tr	1.5	1.5	1.4	1.4	1.0	1.3	1.7	3.2	4.6	2.5	2.4	tr
15 : 0	5.4	3.0	tr	7.6	2.8	6.5	6.7	7.7	6.7	1.2	2.8	6.4	1.5	5.4	4.0	5.1
ECL 15.275	2.0	−	−	−	−	tr	tr	tr	1.5	1.2	−	−	−	−	−	−
16 : 0	16.8	21.3	12.8	10.1	24.3	16.7	16.3	16.4	12.3	13.8	19.2	14.8	15.9	13.5	20.7	18.9
Summed feature 4*	5.9	4.0	6.7	1.0	7.5	5.6	5.9	5.2	6.4	12.8	4.1	10.6	11.2	6.2	5.1	4.5
17 : 1ω6c	2.5	1.7	tr	8.5	1.6	2.8	2.6	3.6	3	tr	tr	1.6	tr	2.2	2.4	1.6
17 : 1ω8c	1.5	1.0	tr	6.2	1.1	1.6	1.7	1.9	3	tr	tr	2.2	tr	2.5	1.4	1.5
17 : 1	7.7	1.6	1.4	10.8	2.0	4.1	4.2	4.6	7.5	1.6	2.6	5.6	1.8	5.7	2.6	3.3
ECL 17.897	1.4	1.3	tr	1.1	tr	−	−	−	1.5	tr	1.3	1.1	tr	tr	1.1	1.1
18 : 0	tr	tr	tr	tr	tr	−	tr	tr	−	tr	−	−	1.4	tr	tr	tr
Summed feature 7*	43.2	56.5	69.4	38.7	49.4	51.5	50.0	48.4	51.7	60.4	52.8	50.2	56.7	55.8	53.7	55.5
18 : 1ω9*c*	1.0	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
11-Methyl 18 : 1ω5*t*	2.7	3.5	tr	−	6.3	3.3	4.0	3.6	1.1	1.9	8.2	tr	tr	−	4.5	1.6
ECL 18.797	tr	−	−	3.1	−	tr	tr	tr	−	−	−	tr	−	1.0	−	tr
19 : 0 cyclo ω8c	2.4	−	−	6.2	−	−	−	−	−	−	−	−	−	−	−	1.1

*Summed features consist of one or more fatty acids that could not be separated by the Microbial Identification System. Summed feature 4, one or more of iso-15 : 0 2-OH, 16 : 1ω7*c* and 16 : 1ω7*t*; summed feature 7, one or more of 18 : 1ω7*c*, 18 : 1ω9*t* and 18 : 1ω12*t*.

the protocols supplied by the manufacturer. The test strips were incubated at 30 °C for 14 days and monitored three times a week. A test was considered positive if the interface between sample well and air was visibly turbid due to bacterial growth after incubation for 14 days (Rüger & Krambeck, 1994). Only strain MCS24^T could reduce nitrate to nitrite. The results for individual strains are given in the species descriptions.

For enzyme activity tests, API ZYM test strips (bioMérieux) were used according to the protocol supplied by the manufacturer. Strains MCS24^T and FWC43^T showed weak lipase (C_{14}) activity, which was absent from FWC40^T. Leucine arylamidase activity was strong in all strains tested. Cystine arylamidase activity was strong in MCS24^T, moderate in FWC43^T but weak in FWC40^T; acid phosphatase activity was weak in MCS24^T but strong in all other tested strains. The strains also differed in α-glucosidase activity, which was strong in MCS24^T and FWC43^T but absent from FWC40^T. β-Glucosidase activity was found only in FWC43^T.

Due to the heterogeneity of the isolates, three different taxa can be discerned and proposals are made of three novel species of *Brevundimonas*. Discriminating characteristics of the newly proposed species and their nearest neighbours are summarized in Table 3.

Description of *Brevundimonas halotolerans* sp. nov.

Brevundimonas halotolerans (ha.lo.to′le.rans. Gr. n. *hal*, *halos* salt; L. part. adj. *tolerans* tolerating; N.L. part. adj. *halotolerans* salt tolerating).

The description is the same as that given for the genus (Segers *et al.*, 1994; emended by Abraham *et al.*, 1999), with the following additional characteristics. Gram-negative, aerobic, non-spore-forming, prosthecate cells; colonies white coloured, cells bacteroid. *B. halotolerans* has complex substrate requirements and shows optimal growth on peptone-yeast extract medium with 0–40 g NaCl l^{-1}. With 60–80 g NaCl l^{-1}, growth is observed, although reduced; no growth is found with 100 g NaCl l^{-1}. Grows optimally

Table 3. Characteristics that are useful in discerning the novel species

Strains: 1, *B. poindexterae* sp. nov. FWC40^T; 2, *B. staleyi* sp. nov. FWC43^T; 3, *B. halotolerans* sp. nov. MCS24^T; 4, *B. terrae* KSL-145^T (data from Yoon *et al.*, 2006); 5, *B. diminuta* LMG 2089^T; 6, *B. bullata* DSM 7126^T (data in columns 5 and 6 from Fritz, 2000); 7, *B. variabilis* ATCC 15255^T; 8, *B. bacteroides* CB7^T; 9, *B. lenta* DS-18^T (data in columns 7–9 from Yoon *et al.*, 2007). ++, Strongly positive; +, positive; W, weakly positive; −, negative; ND, no data available.

Characteristic	1	2	3	4	5	6	7	8	9
Utilization of:									
α-D-Glucose	+	+	−	−	−	+	−	−	−
D-Galactose	−	−	−	−	−	−	−	W	−
D-Mannose	−	+	−	−	−	−	−	−	−
Maltotriose	+	−	−	ND	−	−	+	−	ND
Maltose	+	−	−	−	−	−	−	−	−
Cellobiose	+	+	−	−	−	−	−	−	−
Aesculin	+	+	+	−	−	ND	+	+	+
DL-Lactate	−	+	−	−	−	−	+	−	−
Succinate	+	+	−	−	−	−	−	−	−
Fumarate	+	+	−	ND	−	−	−	+	ND
L-Proline	+	+	−	+	++	+	−	−	+
D-Alanine	−	−	−	ND	+	+	−	−	−
L-Alanine	−	W	−	ND	+	+	−	−	−
Activity of:									
Lipase (C_{14})	−	++	W	ND	−	−	W	−	−
Valine arylamidase	+	++	++	ND	−	++	+	+	−
Cystine arylamidase	W	+	++	ND	−	−	−	−	−
α-Chymotrypsin	W	+	−	ND	W	W	W	W	−
Acid phosphatase	++	++	W	ND	++	++	W	W	+
Naphthol-AS-BI-phosphohydrolase	++	++	++	ND	++	++	W	W	+
α-Glucosidase	−	+	++	ND	−	W	−	−	+
β-Glucosidase	−	+	−	ND	−	−	−	−	−
Protease	−	+	+	ND	ND	+	ND	ND	+
β-Galactosidase	−	+	−	−	ND	−	ND	ND	−
Reduction of nitrates to nitrites	−	−	+	−	ND	−	ND	ND	−
DNA G+C content (mol%)	67.0	66.5	64.6	61.8	67	66.7	ND	66	68.7

at 20–40 °C; slow growth at 10 °C and no growth at 5 or 50 °C. Nitrate is reduced to nitrite but not to nitrogen. Shows strong activities of alkaline phosphatase, esterase (C_4), esterase/lipase (C_8), naphthol-AS-BI-phosphohydrolase, leucine, valine and cystine arylamidases, trypsin, α-glucosidase and protease and weak activities of lipase (C_{14}) and acid phosphatase. All strains are characterized by two major fatty acids, $C_{18:1}\omega7$ and $C_{16:0}$; minor fatty acids are $C_{15:0}$, $C_{16:1}\omega7$, iso-$C_{17:1}\omega8$, $C_{17:0}$ and 11-methyl $C_{18:1}\omega5c$. Polar lipids are α-D-glucopyranosyl, α-D-glucuronopyranosyl, D-glucopyranosyl-(1→4)-α-D-glucopyranuronosyl, sulfoquinovosyl, phosphatidyl and 6-phosphatidyl-α-D-glucopyranosyl diacylglycerols. The G+C content of the type strain is 64.6 mol%. Known isolates have been obtained from brackish water or seawater.

The type strain, MCS24^T (=LMG 25346^T =CCUG 58273^T), was isolated from brackish water of a creek flowing into salt water at Carkeek Park, Seattle, WA, USA. The species is widespread in the marine environment and is known from the Canadian Pacific coast (strains MCS17 and MCS24^T), the North American Atlantic coast (strains CM260, CM272 and CM282), the Tasman Sea near New Zealand (strains CDF5, CDF18 and CDF35) (Fenton, 1994) and the Baltic Sea (strain MCS35). Its ability to grow both in fresh water and in ocean water may be one reason for this wide distribution.

Description of *Brevundimonas poindexterae* sp. nov.

Brevundimonas poindexterae (poin.dex′ter.ae. N.L. gen. n. *poindexterae* of Poindexter, named to honour Jeanne S. Poindexter, who contributed much to our current understanding of the *Caulobacterales*).

The description is the same as that given for the genus (Segers *et al.*, 1994; emended by Abraham *et al.*, 1999), with the following additional characteristics. Gram-negative, aerobic, non-spore-forming, prosthecate cells; colonies tan coloured, cells bacteroid. No S layer is detected and multiple bands of polysaccharides are observed (Walker *et al.*, 1992). The species can grow on peptone-yeast extract medium without NaCl, but optimal growth occurs with 5–30 g NaCl l^{-1}. Can not tolerate salt concentrations above 60 g NaCl l^{-1}. Grows optimally at 20–30 °C; slow growth at 40 °C, no growth at 10 or 50 °C. Can use pyruvate, maltotriose, maltose, 1-*O*-methyl α-galactopyranoside, cellobiose, aesculin, xylose, glucose, rhamnose, malate, galacturonate, succinate, fumarate, 3-hydroxybutyrate, asparagine, L-glutamate and L-proline. In contrast, β-(+)-D-fructose, (+)-D-galactose, trehalose, (+)-D-mannose, (+)-L-sorbose, (+)-melibiose, sucrose, (+)-raffinose, lactose, lactulose, 1-*O*-methyl β-galactopyranoside, (+)-gentiobiose, 1-*O*-methyl β-glucopyranoside, (−)-D-ribose, (+)-L-arabinose, palatinose, α-L-fucose, (+)-melezitose, (+)-D- and (−)-L-arabitol, xylitol, dulcitol, D-tagatose, glycerol, *myo*-inositol, D-mannitol, maltitol, (+)-turanose, D-sorbitol, adonitol, D-lyxose, i-erythritol, 1-*O*-methyl and 3-*O*-methyl α-D-glucopyranoside, saccharate, mucate, (+)-L-, (−)-D- and *meso*-tartrate, (+)-D-malate, *cis*- and *trans*-aconitate, tricarballylate, citrate, D-glucuronate, 2-keto-D-gluconate, *N*-acetyl-D-glucosamine, D-gluconate, phenylacetate, protocatechuate, 4-hydroxybenzoate, (−)-quinate, gentisate, 3-hydroxybenzoate, benzoate, *m*-coumarate, trigonelline, betaine, putrescine, 4-aminobutryate, histamine, DL-lactate, glutarate, DL-glycerate, 5-aminovalerate, ethanolamine, tryptamine, itaconate, L-aspartate, L-glutamate, D- and L-alanine, L-serine, malonate, L-tyrosine and 2-ketoglutarate are not used. Enzymic activity of alkaline and acid phosphatases, esterase (C_4), esterase lipase (C_8), leucine arylamidase, valine arylamidase, trypsin, naphthol-AS-BI-phosphohydrolase and β-glucosidase is present. Nitrate is not reduced to nitrite or to nitrogen. Polar lipids are α-D-glucopyranosyl, α-D-glucuronopyranosyl, D-glucopyranosyl-(1→4)-α-D-glucopyranuronosyl, phosphatidyl and 6-phosphatidyl-α-D-glucopyranosyl diacylglycerols. Characterized by two major fatty acids, $C_{18:1}\omega7$ and $C_{16:0}$; minor fatty acids are $C_{15:0}$, $C_{16:1}\omega7$, $C_{17:1}\omega8$ and 11-methyl $C_{18:1}\omega5$. The main hydroxy-fatty acid is $C_{12:0}$ 3-OH. The G+C content of the type strain is 67.0 mol%.

The type strain is FWC40^T (=LMG 25261^T =CCUG 57883^T), isolated from activated sludge from a secondary treatment facility at Kelowna, British Columbia, Canada.

Description of *Brevundimonas staleyi* sp. nov.

Brevundimonas staleyi (sta′ley.i. N.L. gen. n. *staleyi* of Staley, named to honour the American microbiologist James T. Staley for his contribution to the knowledge of the caulobacteria).

The description is the same as that given for the genus (Segers *et al.*, 1994; emended by Abraham *et al.*, 1999), with the following additional characteristics. Gram-negative, aerobic, non-spore-forming, prosthecate cells; colonies bright yellow coloured, cells bacteroid. No S layer is detected and multiple bands of polysaccharides are observed (Walker *et al.*, 1992). Can grow on peptone-yeast extract medium with 0–40 g NaCl l^{-1}, with optimal growth at 5–30 g NaCl l^{-1}. Does not tolerate salt concentrations above 60 g NaCl l^{-1}. Grows best at 20–40 °C; slow growth at 10 °C and no growth at 5 or 50 °C. D-Glucose, D-mannose, cellobiose, gentiobiose, 1-*O*-methyl β-glucopyranoside, aesculin, α-L-rhamnose, gentisate, DL-lactate, succinate, fumarate, 3-hydroxybutyrate, L-aspartate, L-glutamate, L-proline, L-alanine, malonate and L-tyrosine are used as substrates. β-(+)-D-Fructose, (+)-D-galactose, (+)-trehalose, (+)-L-sorbose, (+)-melibiose, sucrose, (+)-raffinose, maltotriose, maltose, lactose, lactulose, 1-*O*-methyl α- and β-galactopyranoside, (+)-gentiobiose, (−)-D-ribose, (+)-L-arabinose, (+)-D-xylose, palatinose, α-L-fucose, (+)-melezitose, (+)-D- and (−)-L-

arabitol, xylitol, dulcitol, D-tagatose, glycerol, *myo*-inositol, D-mannitol, maltitol, (+)-turanose, D-sorbitol, adonitol, D-lyxose, i-erythritol, 1-*O*-methyl and 3-*O*-methyl α-D-glucopyranoside, saccharate, mucate, (+)-L-, (−)-D- and *meso*-tartrate, (+)-D- and (−)-L-malate, *cis*- and *trans*-aconitate, tricarballylate, citrate, D-glucuronate, D-galacturonate, 2-keto-D-gluconate, *N*-acetyl-D-glucosamine, D-gluconate, phenylacetate, protocatechuate, 4-hydroxybenzoate, (−)-quinate, gentisate, 3-hydroxybenzoate, benzoate, *m*-coumarate, trigonelline, betaine, putrescine, 4-aminobutyrate, histamine, glutarate, DL-glycerate, 5-aminovalerate, ethanolamine, tryptamine, itaconate, L-glutamate, D-alanine, L-serine, malonate, L-tyrosine and 2-ketoglutarate are not oxidized. Activities of alkaline and acid phosphatases, esterase (C_4), esterase lipase (C_8), leucine and valine arylamidases, trypsin, phosphatase, naphthol-AS-BI-phosphohydrolase, α-glucosidase, β-glucosidase, β-galactosidase and oxidase and weak activities of lipase (C_{14}), cystine arylamidase and α-chymotrypsin are present. Nitrate is not reduced to nitrite or to nitrogen. Polar lipids are α-D-glucopyranosyl, α-D-glucuronopyranosyl, D-glucopyranosyl-(1→4)-α-D-glucopyranuronosyl, phosphatidyl and 6-phosphatidyl-α-D-glucopyranosyl diacylglycerols. Characterized by two major fatty acids, $C_{18:1}\omega7$ and $C_{16:0}$; minor fatty acids are $C_{15:0}$, $C_{16:1}\omega7$, $C_{17:1}\omega8$, $C_{14:0}$ and $C_{12:0}$ 3-OH. The G+C content of the type strain is 66.5 mol%; genome size of the type strain is 2.2×10^9 Da. Closely related to *B. bullata* by 16S rRNA gene sequence similarity, but clearly distinct by morphology and cell cycle.

The type strain is FWC43^T (=LMG 25262^T =CCUG 57884^T), isolated from activated sludge of a secondary treatment facility at Calgary, Alberta, Canada.

Acknowledgements

We are indebted to Dagmar Wenderoth, Annette Krüger and Peter Wolff for their excellent technical assistance. This work was supported by grants of the German Federal Ministry for Science, Education and Research (projects no. 0319433C and 01 KI 07 96) and the European Union within the T-project 'High Resolution Automated Microbial Identification and Application to Biotechnologically Relevant Ecosystems'.

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