Description of *Undibacterium oligocarboniphilum* sp. nov., isolated from purified water, and *Undibacterium pigrum* strain CCUG 49012 as the type strain of *Undibacterium parvum* sp. nov., and emended descriptions of the genus *Undibacterium* and the species *Undibacterium pigrum*

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A Gram-negative, oxidase- and catalase-positive, flagellated, rod-shaped bacterium, designated strain EM 1^T, was isolated from purified water. 16S rRNA gene sequence analysis indicated that the novel strain belonged to the family *Oxalobacteraceae* within the class *Betaproteobacteria*; the closest phylogenetic relative was *Undibacterium pigrum* DSM 19792^T (96.7 % gene sequence similarity). The new isolate could be distinguished from the type strain of *U. pigrum* DSM 19792^T (=CCUG 49009^T=CIP 109318^T) and from strain CCUG 49012^T, which has been described as a second genomovar of this species, on the basis of genotypic data and several phenotypic properties. An S-layer was present in the cell envelope in *U. pigrum* DSM 19792^T, but was absent in strains EM 1^T and CCUG 49012^T. Test conditions were established that enabled strain CCUG 49012^T to be distinguished from *U. pigrum* DSM 19792^T. As found for *U. pigrum*, the main fatty acids of strains EM 1^T and CCUG 49012^T were summed feature 3 (including unsaturated C_{16:1}ω7c), straight-chain C_{16:0} and unsaturated C_{18:1}ω7c (low percentage in strain CCUG 49012^T), and C_{10:0} 3-OH was the sole hydroxylated fatty acid. The polar lipid profile consisted of the predominant lipids phosphatidylethanolamine, diphosphatidylglycerol and phosphatidylglycerol. The polyamine profile was mainly composed of the major compound putrescine and moderate amounts of 2-hydroxyputrescine. In contrast to *U. pigrum* and strain CCUG 49012^T, where ubiquinone Q8 was reported as the sole quinone component, the quinone system of strain EM 1^T consisted of ubiquinone Q-8 (64 %) and Q-7 (36 %). The 16S rRNA gene sequence similarity, the polar lipid profile and the absence of C₁₂-hydroxylated fatty acids all indicated that strain EM 1^T was affiliated with the genus *Undibacterium*. 16S rRNA gene sequence similarity values lower than 97.0 % and several differentiating phenotypic traits demonstrated that strain EM 1^T represents a novel species for which the name *Undibacterium oligocarboniphilum* sp. nov. is proposed (type strain EM 1^T=DSM 21777^T=CCUG 57265^T). In addition, based on previously published results and this study, a separate species, *Undibacterium parvum* sp. nov., is proposed with strain CCUG 49012^T (=DSM 23061^T=CIP 109317^T) as the type strain.

Abbreviation: SEM, scanning electron microscope.

The GenBank/EMBL/DBJ accession number for the 16S rRNA gene sequence of strain EM 1^T is GQ379228.

Supplementary figures and supplementary tables are available with the online version of this paper.

The family *Oxalobacteraceae* represents a phylogenetically diverse family of typically aquatic organisms within the class *Betaproteobacteria* (Garrity *et al.*, 2005). In recent years, several new taxa have been added to this family. In addition to the genera *Oxalobacter*, *Collimonas*, *Massilia*, *Duganella*, *Janthinobacterium*, *Herbaspirillum*, *Oxalici-bacterium* and *Telluria*, the genera *Naxibacter* (Xu *et al.*, 2005), *Herminiimonas* (Fernandes *et al.*, 2005) and *Undibacterium* (Kämpfer *et al.*, 2007) have been described. Their allocation to separate genera is mainly based on 16S rRNA gene sequence analyses; in addition, phenotypic markers such as chemotaxonomic compounds are known to differentiate between genera.

Strain EM 1^T was isolated from a purified water system at Roche, Penzberg, Germany, after plating on R2A agar (Oxoid) plates and incubation at 30–35 °C for 7 days. Subcultivation was carried out on R2A agar at 28–35 °C for 32–48 h (standard conditions). On this medium, strain EM 1^T was able to grow at 10–35 °C, but not at 5 or 36 °C. Colonies of strain EM 1^T on R2A agar were tiny, cream-coloured and translucent. Strain EM 1^T was not able to grow on tryptone soy agar (Merck). The same characteristic was observed for strain CCUG 49012^T, which was described as a strain representing a genomovar of *Undibacterium pigrum* because of its low DNA–DNA relatedness with the type strain of this species, CCUG 49009^T (Kämpfer *et al.*, 2007). The temperature limit for growth for strain CCUG 49012^T is 28 °C or 30 °C (data from this study and from Kämpfer *et al.*, 2007, respectively), and growth on R2A agar plates is retarded even at 25 °C. For that reason, cultures and tests for this strain were conducted at an incubation temperature of 21 °C. Colonies of strain CCUG 49012^T on R2A agar were shiny, yellowish and translucent.

To ascertain the morphology of cells of strain EM 1^T, cells in the exponential and stationary growth phases were analysed with scanning and transmission electron microscopic techniques. Immediately after collection from liquid medium [R2A and PYE medium; PYE medium contained (l⁻¹): 3 g peptone from casein and 3 g yeast extract] cells were fixed with 2.5 % glutaraldehyde in fixative buffer (50 mM sodium cacodylate, 2 mM MgCl₂, pH 7.0) for 1 h at room temperature. Samples were subsequently rinsed several times in fixative buffer and post-fixed at room temperature for 1 h with 1 % osmium tetroxide in fixative buffer. After two washing steps in distilled water, the cells were stained *en bloc* for 30 min with 1 % uranyl acetate in 20 % acetone. Dehydration was performed with a graded acetone series. Samples were then infiltrated and embedded in Spurr's low-viscosity resin. Ultrathin sections were cut with a diamond knife and mounted onto uncoated copper grids. The sections were post-stained with aqueous lead citrate (100 mM, pH 13.0).

For negative staining, a drop of the fixed cells at a dilution 1 : 10 with sterile Ampuwa water (Fresenius) was placed on a 400-mesh carbon-coated copper grid which had been

freshly rendered hydrophilic by glow discharge. After incubation for 2 min, the drop was quickly removed with a Pasteur pipette, the grid was air-dried and then stained with 2 % uranyl acetate and 0.01 % glucose.

Transmission electron micrographs were taken with an electron microscope (EM 912; Zeiss) equipped with an integrated OMEGA energy filter operated at 80 kV in the zero-loss mode. For scanning electron microscopy (SEM), drops of the sample were placed onto a glass slide, covered with a coverslip and rapidly frozen with liquid nitrogen. The coverslip was removed with a razor blade and the glass slide was immediately fixed with 2.5 % glutaraldehyde in 75 mM cacodylate buffer (pH 7.0), post-fixed with 1 % osmium tetroxide in fixative buffer, dehydrated in a graded series of acetone solutions and critical-point-dried after transfer to liquid CO₂. Specimens were mounted on stubs, coated with 3 nm platinum using a magnetron sputter coater (S500; BAL-TEC), and examined with either a Hitachi S-4100 field emission SEM operated at 5 kV or a Zeiss Auriga SEM operated at 1 kV.

Cells of strain EM 1^T formed small or larger aggregates depending on the age of the cultures. Individual cells were about 1.5–2.0 µm long, with a diameter of approx. 0.5 µm (see Supplementary Fig. S1a–c in IJSEM Online). Division stages were observed regularly. Ultrathin sections revealed a typical Gram-negative architecture for the bacterial envelope, which consisted of a 6–7 nm thick outer membrane, an electron-dense peptidoglycan layer measuring 2–3 nm and a cytoplasmic membrane with a thickness of 6–7 nm (see Supplementary Fig. S1d). SEM showed a slightly rough surface to the cells of strain EM 1^T, which according to TEM micrographs, could be attributed to the undulation of the outer membrane (Supplementary Fig. S1d). Electron-dense globules of varying diameters (up to 200 nm), presumably polyphosphate, were frequently observed in the cytoplasm of the cells, often with lipid body-like globules (approx. 50 nm) attached (Supplementary Fig. S1c, d). The presence of lipophilic inclusions was confirmed by Sudan Black staining. Cells observed in aggregates were interconnected by tangled filamentous structures (Supplementary Fig. S1b, g and h) which were difficult to characterize. When investigating single cells, it became obvious that there were several classes of filaments. We were able to identify two distinct classes of a) thicker and b) thinner filaments with a constant diameter and also filaments that varied in diameter and shape over their length. TEM of negatively stained bacterial preparations demonstrated the presence of flagella with diameters of 21 ± 1.3 nm and a characteristic pattern of subunits (Supplementary Fig. S1e) and pili with diameters of 9.7 ± 0.7 nm without visible substructures (Supplementary Fig. S1f).

When reaching the stationary growth phase, the ultra-structure of the cells changed. SEM investigations revealed that cell surface became strikingly 'rough' due to the formation of numerous small papillae, approx. 20–50 nm

in length and regularly occurring at a distance of about 50 nm from each other, that were formed by protuberances of the outer membrane and local changes in the periplasmic space (see Supplementary Fig. S1g, h). Most cells exhibited vacuoles formed by invaginations of the cytoplasmic membrane that engulfed smaller fractions of cytoplasm (Supplementary Fig. S1j, k). Division stages were still observed, indicating the vitality of the cells (Supplementary Fig. S1h).

For comparison of the novel strain with its closest recognized relative, ultrastructural analyses were also performed with *U. pigrum* DSM 19792^T and strain CCUG 49012^T. Cells of *U. pigrum* DSM 19792^T and strain CCUG 49012^T were about 1.5–2.0 µm long with a diameter of approx. 0.5 µm (see Supplementary Figs S2a–c and S3a–c). Division stages were regularly observed (Supplementary Figs S2b, S3a). Ultrathin sections revealed a typical Gram-negative architecture of the bacterial envelope, which consisted of a 6–7 nm thick outer membrane, an electron-dense peptidoglycan layer measuring 2–3 nm and a cytoplasmic membrane with a thickness of 6–7 nm (see Supplementary Figs S2d and S3d). For *U. pigrum* DSM 19792^T, an S-layer with a thickness of approx. 20 nm and subunits with approx. 15 nm diameter was visible (Supplementary Fig. S2d, e). SEM investigation revealed a slightly rough surface for cells of *U. pigrum* DSM 19792^T and strain CCUG 49012^T, which, according to ultrathin sections investigated by TEM, could be attributed to the undulation of the outer membrane (Supplementary Figs S2d, S3d). Electron-dense regions of varying shapes and sizes (up to 200 nm) were frequently observed in the cytoplasm of the cells (Supplementary Figs S2c, S3c, e–f); a diffuse matrix contained both electron-dense granules (50–100 nm in diameter) and electron-transparent lipid body-like globules with diameters of about 100–150 nm, possibly poly-β-hydroxybutyrate, could be observed (Supplementary Figs S2f, S3f). SEM images of *U. pigrum* DSM 19792^T showed that cell flagellation was typically polar, monotrichous or ditrichous. TEM of negatively stained bacterial preparations of *U. pigrum* DSM 19792^T and strain CCUG 49012^T confirmed the presence of flagella with diameters of 23.1 ± 0.98 nm and 20.6 ± 1.3 nm, respectively, and a characteristic subunit pattern (see Supplementary Figs S2g, S3g), and pili with diameters of 9.2 ± 0.9 nm and 9.6 ± 0.8 nm, respectively (Supplementary Figs S2h, S3h) without visible substructures.

Strain EM 1^T exhibited Gram-negative behaviour and showed catalase- and oxidase activity. The Gram-reaction was tested by the non-staining KOH-method as described by Fluharty & Packard (1967). Oxidase activity was tested with Bactident Oxidase (Merck) and catalase activity was tested with 10 % H₂O₂.

Tolerance to NaCl was tested in R2A liquid medium supplemented with 0, 1, 2, 4, 5, 6, 8 and 10 g l⁻¹ NaCl. Strain EM 1^T grew well with 0–4 g l⁻¹ NaCl, growth with 5 and 6 g l⁻¹ NaCl was weak, and no growth was observed in the presence of 8 g l⁻¹ NaCl.

Strain EM 1^T did not react in any of the tests available in API 20NE or API 20E strips (bioMérieux) when standard conditions were applied. The same was true for oxidation of substrates in Biolog GN2 plates (AES Laboratoire). Similar inactivity has been described for the closest phylogenetic neighbours of strain EM 1^T, *U. pigrum* DSM 19792^T and strain CCUG 49012^T (Kämpfer *et al.*, 2007). Since strain EM 1^T was isolated from pure water, it seemed likely that the strain would be adapted to low NaCl and substrate concentrations. Following this idea, the solute concentrations used during testing were reduced. The NaCl content of the solution applied to prepare the inoculum suspension was used at half strength (4.25 g l⁻¹), the AUX medium used in the API 20NE and API 50CH was diluted 1:3 before use, and the concentrations of the standard mineral medium for utilization tests in tubes was reduced to (l⁻¹): 1.0 g substrate, 0.4 g K₂HPO₄, 0.1 g KH₂PO₄, 0.1 g MgSO₄ · 7H₂O, 2.5 mg CaCl₂ · 2H₂O, 0.5 g (NH₄)₂SO₄ and 25 mg NH₄-Fe(III)-citrate. By using this approach, positive reactions could be obtained for strains EM 1^T, CCUG 49012^T and *U. pigrum* DSM 19792^T in API strips (Tables 1 and 2) and growth in mineral medium with several substrates (Table 2). For the reference strain *Herbaspirillum huttiense* DSM 10281^T, the API 20NE result after the use of the diluted solutions was compared with that obtained under standard conditions. The same results were achieved for both conditions, with the exception that positive reactions were delayed for some substrates for one day when the diluted solutions were used. Strain EM 1^T was strictly aerobic and no growth was observed on R2A agar under anaerobic conditions. No acid production was detected in the oxidation/fermentation of glucose in Hugh–Leifson's medium (Cowan & Steel, 1974). The new strain could be differentiated from its phylogenetic neighbours by several reactions. In general, strain EM 1^T was less reactive than *U. pigrum* DSM 19792^T. It could be differentiated from *U. pigrum* DSM 19792^T by its ability to utilize gluconate and its inability to use mannose, N-acetylglucosamine, D-fructose, cellobiose or trehalose (Tables 1 and 2). While *U. pigrum* DSM 19792^T cleaved Tween 80, the novel isolate did not. Strain CCUG 49012^T utilized glucose weakly (turbidity visible not earlier than 4 days incubation) and utilized maltose and cellulose. When the results for the API 20E strips prepared with highly diluted NaCl solution (3 g l⁻¹) were compared with those prepared with tap water, a growth retardation was apparent even at such low NaCl concentrations. The novel strain differed from *U. pigrum* DSM 19792^T by its ability to utilize malate and by its inability to utilize several carbohydrates such as D-mannose, ribose, D-fructose, sucrose and trehalose (Tables 1 and 2). Strain CCUG 49012^T could be distinguished from strain EM 1^T by its ability to utilize malate and cellobiose and by its inability to metabolize gluconate and sucrose. Another unique feature was its sensitivity towards temperatures of 28–34 °C. The type strains of related species of the genera *Janthinobacterium* and *Herbaspirillum* displayed a broader substrate utilization spectrum than those of the three aforementioned strains.

Table 1. Characteristics of strain EM 1^T and its closest phylogenetic neighbours in the API 20NE test system

Taxa: 1, strain EM 1^T; 2, strain CCUG 49012^T; 3, *Undibacterium pigrum* DSM 19792^T; 4, *J. agaricidamnorum* DSM 9628^T; 5, *H. rubrisubalbicans* DSM 9440^T; 6, *H. huttiense* DSM 10281^T. Cells were suspended in 4.25 g l⁻¹ NaCl and the AUX-medium used to test for substrate utilization was diluted 1:3 in deionized water. Results after 2 days and after 6 days (8 days for strain CCUG 49012^T) after slash, if different from the 2 day result. All strains were negative for indole production, glucose acidification, arginine dihydrolase activity and aesculin hydrolysis and positive for oxidase. +, Positive; -, negative; w, weak reaction.

Characteristic	1	2	3	4	5	6
N-Acetylglucosamine	-	-	+	-	-	+
Adipate	-	-	-	-	-	-/w
Arabinose	-	-	-	-	+	+
Caprate	-	-	-	-	+	+
Citrate	-	-	-	-	+	-/w
β-Galactosidase	-	-	-	-	+	+
Gelatin hydrolysis	-/+	-/w	+	+	-	-
Gluconate	+	-	-	-	+	+
Glucose	+	-/+	w/+	+	+	+
Malate	-	+	-	+	+	+
Maltose	+	+	+	-	-	-
Mannitol	-	-	-	+	+	+
Mannose	-	-	+	+	+	-
Nitrate reduction	+	+	+	-	+	-
Phenylacetate	-	-	-	-	+	+
Urease	-	-	-	-	w/+	-/+

The 16S rRNA gene sequence of strain EM 1^T, comprising 1526 nt, was inserted into an ARB database based upon the SILVA SSU Ref release 98 (Pruesse *et al.*, 2007) using the respective alignment and analysis tools of the ARB software package (Ludwig *et al.*, 2004). The database contained about 250 000 sequence entries. Phylogenetic tree reconstruction and topology evaluation were performed by applying the maximum-parsimony (ARB_PARS) and maximum-likelihood (RAXML; Stamatakis, 2006) facilities as implemented in the ARB package in combination with various positional variability filters and models of evolution. Non-redundant subsets of 30 000 and 5000 complete and high quality sequence data balanced with respect to the representation of the major lines of descent were selected for tree topology evaluation by applying the maximum-parsimony and maximum-likelihood approaches, respectively. These analyses showed that strain EM 1^T was a member of the family *Oxalobacteraceae* of the class *Betaproteobacteria* and was most closely related to *U. pigrum* DSM 19792^T (96.7% 16S rRNA gene sequence similarity; see Supplementary Table S1). A consensus tree is shown in Fig. 1. Multifurcations indicate that a common relative branching order was not significantly supported by the various phylogenetic analyses performed.

The 16S rRNA gene-sequence based analyses clearly showed a closer relationship between strain EM 1^T and

U. pigrum DSM 19792^T (=CCUG 49009^T, GenBank accession no. AM397630) than to strain CCUG 49012^T (AM397629; 94.6% 16S rRNA gene sequence similarity, Supplementary Table S1). The data presented here enabled clear differentiation of strain EM 1^T by phenotypic characteristics and justified the creation of a novel species within the genus *Undibacterium*.

For determination of the G + C content, DNA of strain EM 1^T was degraded to nucleosides by using P1 nuclease and bovine intestinal mucosa alkaline phosphatase, as described by Mesbah *et al.* (1989). The nucleosides were separated by reversed-phase HPLC (Shimadzu apparatus) according to the method described by Tamaoka & Komagata (1984), and the G + C content was calculated from the ratio of deoxyguanosine to thymidine. The G + C content of strain EM 1^T was found to be 52.4 mol%.

For the analysis of fatty acids, cells were grown on R2A agar for 2 days at 28 °C and 21 °C in the case of strain CCUG 49012^T. Whole-cell fatty acid methyl esters were obtained using methods described previously (Kämpfer & Kroppenstedt, 1996) and were separated using GC (model 5898A; Hewlett Packard). Peaks were automatically integrated and fatty acid names and percentages were determined using the Microbial Identification standard software package (MIDI; Sasser, 1990). The major fatty acids of strain EM 1^T were summed feature 3 (C_{16:1}ω7c and/or iso-C_{15:0} 2-OH), straight chain C_{16:0} and unsaturated C_{18:1}ω7c (not CCUG 49012^T) (see Supplementary Table S2). Since no significant amounts of branched fatty acids were detected, it appears likely that this organism does not possess the capacity for biosynthesis of iso-branched fatty acids and hence summed feature 3 can be considered to represent only C_{16:1}ω7c. The sole hydroxylated fatty acid detected was C_{10:0} 3-OH. This overall composition was highly similar to those of the closest phylogenetic neighbours *U. pigrum* DSM 19792^T and strain CCUG 49012^T, a fact which confirmed the taxonomic relationship between these three taxa. The related species *Janthinobacterium agaricidamnorum* DSM 9628^T, *Herbaspirillum rubrisubalbicans* DSM 9440^T and *Herbaspirillum huttiense* DSM 10281^T also contained the hydroxylated compound C_{12:0} 2-OH. Moreover, the two species of the genus *Herbaspirillum* differed from the other species by the presence of C_{12:0} 3-OH, C_{14:0} 2-OH and C_{18:0} and a high proportion of C_{18:1}ω7c (Supplementary Table S2). The discrepancy between our data and those given by Kämpfer *et al.* (2007) for the compounds C_{16:1}ω7c and C_{17:0} cyclo in *J. agaricidamnorum* DSM 9628^T may be due to differing physiological states of the cultures prior to analysis. For example, unsaturated fatty acids such as C_{16:1}ω7c may be transformed to the respective odd-numbered cyclopropane derivatives by cyclopropyl fatty acid synthases at the onset of starvation (Huisman *et al.*, 1996).

For extraction of quinones, polar lipids and polyamines, biomass was grown in R2A medium. Quinones and polar lipids were extracted and analysed as described by Tindall

Table 2. Utilization patterns in API 50CH strips or in mineral medium (MM) for strain EM 1^T and its closest phylogenetic neighbours

Taxa: 1, strain EM 1^T; 2, strain CCUG 49012^T; 3, *U. pigrum* DSM 19792^T; 4, *J. agaricidamnorum* DSM 9628^T; 5, *H. rubrisubalbicans* DSM 9440^T; 6, *H. huttiense* DSM 10281^T. Cells were suspended in 4.25 g l⁻¹ NaCl. The AUX-medium supplied for substrate utilization in the API-strips was diluted 1:3. Results are for the API 50CH test after 2 days and after 6 days (or 8 days for CCUG 49012^T) after the slash if different from the 2 day result. MM and substrate concentrations were diluted to half strength of the standard, i.e. substrate concentrations were 1 g l⁻¹. Incubation was for up to 10 days. Substrates without an entry in the columns for mineral medium were not tested. +, Positive; -, negative; w, weak reaction. In the API 50CH test system, all taxa were negative for L-xylose, L-sorbose, L-rhamnose, dulcitol, amygdalin, arbutin, lactose, melibiose, inulin, melezitose, raffinose, glycogen, turanose and D-tagatose.

Characteristic	1		2		3		4		5		6	
	API	MM	API	MM	API	MM	API	MM	API	MM	API	MM
N-Acetylglucosamine	-	-	-	-	-/w	+	-	-	-	-	+	+
Adonitol	-		-		-		-		+		+	
Aesculin	w		-		w/+		-		-		-	
D-Arabinose	-	-	-	-	-	-	-		-/+		-/+	
L-Arabinose	-	-	-	-	-	-	-		+	+	+	+
D-Arabitol	-		-		-		+	+	+		+	
L-Arabitol	-		-		-		-		+	+	+	+
Cellobiose	-	-	+	+	+	+	-	-/+	-	-	-	-
Erythritol	-		-		-		-		-/+		+	
D-Fructose	-	-	-	-	+	+	+	+	-/+		-/+	
D-Fucose	-		-		-		-		-/+		-	
L-Fucose	-		-		-		-		+		-/+	
Galactose	-	-	-		-/+	-	-	-	+	+	+	+
β -Gentiobiose	-	-	-	-	+	+	w		-		-	
Gluconate	+	+	-	-	-	-	-	-	+	+	+	+
D-Glucose	+		-/+	+	+		+		+		+	
Glycerol	-		-		-		+	+	+		+	
Inositol	-		-		-		+	+	-		-	
2-Ketogluconate	-		-		-		-		-/+		-	
5-Ketogluconate	-		-		-		-		-/+		+	
D-Lyxose	-		-		-		-		-/+		-/w	
Maltose	+	+	+	+	+	+	-	-	-		-	
Mannitol	-	-	-	-	-	-	+	+	+	+	+	+
D-Mannose	-	-	-	-	+	+	-/+	w	+	+	-/+	+
Methyl α -D-glucoside	-	w	-	-	+	+	-		-		-	
Methyl α -D-mannoside	-		-		-/+		-		-		-	
β -Methyl xyloside	-		-		-		-		-/+		-	
Ribose	-/+	w	-		+		+		-/+		+	
Sorbitol	-		-		-		-		-/+		+	
Starch	+		-		w		-		-		-	
Sucrose	+	+	-	-	+		+		-	-	-	-
Trehalose	-	-	-	-	+	+	+	+	-		-	
Xylitol	-		-		-		-		+	+	+	+
D-Xylose	-		-		-		-		+	+	+	+

(1990a, b) and Altenburger *et al.* (1996) and polyamines were analysed as described by Busse & Auling (1988). HPLC analysis was carried out using the equipment described by Stolz *et al.* (2007). Re-evaluation of the quinone system of *U. pigrum* DSM 19792^T and strain CCUG 49012^T showed that these two strains contained only small amounts of Q-7 (1 % and 2 %, respectively). The quinone system of strain EM 1^T consisted of ubiquinone Q-8 (64 %) and Q-7 (36 %). The relatively high content of Q-7 clearly distinguished strain EM 1^T from *U. pigrum*

DSM 19792^T and strain CCUG 49012^T (Kämpfer *et al.*, 2007). The polar lipid profile (see Supplementary Figure S4) consisted of the major lipid phosphatidylethanolamine, relatively high amounts of diphosphatidylglycerol and phosphatidylglycerol, small amounts of phosphatidylserine and an unidentified aminolipid, AL1, and an unknown lipid, L. This lipid profile was highly similar to those of *U. pigrum* DSM 19792^T and strain CCUG 49012^T, but the unidentified aminolipids AL2 and AL3 (Kämpfer *et al.*, 2007) were not detectable. In addition, the polyamine

Fig. 1. 16S rRNA gene sequence-based consensus phylogenetic tree reflecting the relationships of strain EM 1^T and the type species of the family Oxalobacteraceae. Bar, 10% estimated sequence divergence.

pattern of strain EM 1^T was most similar to those reported for *U. pigrum* DSM 19792^T and strain CCUG 49012^T (Kämpfer *et al.*, 2007). It consisted of putrescine [87.5 µmol (g dry weight)⁻¹], 2-hydroxyputrescine [29.7 µmol (g dry weight)⁻¹], spermidine [1.4 µmol (g dry weight)⁻¹] and spermine [0.9 µmol (g dry weight)⁻¹].

The polyamine pattern, major characteristics of the polar lipid and fatty acid profiles and 16S rRNA gene sequence analysis suggested the placement of strain EM 1^T in the genus *Undibacterium*. Strain EM 1^T could be distinguished from *U. pigrum* DSM 19792^T by a 16S rRNA gene sequence similarity of 96.7%, a relatively high content of ubiquinone Q-7, its ability to utilize gluconate and its inability to decompose Tween 80 or to use mannose, *N*-acetylglucosamine, D-fructose, cellobiose or trehalose and by the absence of an S-layer. In conclusion, strain EM 1^T represents a novel species of the genus *Undibacterium* for which the name *Undibacterium oligocarboniphilum* sp. nov. is proposed.

The position of strain CCUG 49012^T in the genus *Undibacterium* was determined previously by chemotaxonomic and molecular methods (Kämpfer *et al.*, 2007). Based on the differences in several characteristics from the type strains of *U. pigrum* and *U. oligocarboniphilum* sp. nov. (results presented by Kämpfer *et al.*, 2007 and data from this study), we suggest that this strain should be assigned as the type strain of a novel species, *Undibacterium parvum* sp. nov. In addition to its narrow temperature range for growth (no growth above 28 °C), it showed a unique substrate utilization pattern by which it could be distinguished from the two other species (Tables 1 and 2).

Description of *Undibacterium oligocarboniphilum* sp. nov.

Undibacterium oligocarboniphilum (o.li.go.car.bo.ni'phi. lum. Gr. adj. *oligos* few, little, small; L. n. *carbo* -onis charcoal and in chemistry carbon; Gr. adj. *philos* loving; N.L. neut. adj. *oligocarboniphilum* loving low carbon substrate concentrations).

Cells form small and large aggregates and are non-spore-forming. Single cells are about 1.5–2.0 µm long with a diameter of about 0.5 µm. Cells reveal a typical Gram-negative architecture with a 6–7 nm thick outer membrane, a 2–3 nm peptidoglycan layer and a 6–7 nm thick cytoplasmic membrane. Electron-dense globules of varying diameters (up to 200 nm) are often observed in the cytoplasm of the cells, frequently with lipid body-like globules (approx. 50 nm) attached. Flagella with diameters of 21 ± 1.3 nm and pili with diameters of 9.7 ± 0.7 nm are present. Stationary phase cells show the presence of papillae with lengths of 20–50 nm and vacuoles formed by invaginations of the cytoplasmic membrane engulfing smaller fractions of the cytoplasm. On R2A agar, cream-coloured, translucent, shining, convex colonies with entire edges form that reach 1.5 mm after 2 days at 28 °C. When ageing (from 3 days on), the culture gives a characteristic brownish hue to the agar. No growth on TSA. Cells are strictly aerobic, oxidase- and catalase-positive and non-fluorescent. Reduces nitrate to nitrite, but does not denitrify. No acid production in O/F test. In API 20NE and API 20E tests under reduced salt conditions and prolonged incubation period, weak reactions are noted for the hydrolysis of gelatin, the Vosges–Proskauer reaction

and citrate alkalization (API 20E). Negative reactions for arginine dihydrolase, lysine or ornithine decarboxylase, β -galactosidase, tryptophan deaminase, hydrolysis of urea or aesculin and production of hydrogen sulfide or indole. Hydrolyses starch, but not DNA, casein or Tween 80 on standard media. Temperature range for growth is 10–35 °C, no growth occurs at 5 °C or 36 °C. Sensitive to medium NaCl concentrations, growth in R2A with 5 g l⁻¹ and 6 g l⁻¹ NaCl is weak, no growth occurs in the presence of 8 g l⁻¹ NaCl. Weak growth in mineral medium with 2 g l⁻¹ glucose, good growth with 1 g l⁻¹; sensitive to higher substrate concentrations. Correspondingly, no growth on TSA and no response to physiological tests under standard conditions, but positive reactions occur when reduced concentrations of salts and substrates are applied. Utilizes D-glucose, maltose, ribose, sucrose, starch and gluconate. Other physiological properties are given in Table 1 and Table 2. Main fatty acids are summed feature 3 (including unsaturated C_{16:1}ω7c), unsaturated C_{18:1}ω7c and straight chain C_{16:0}, the sole hydroxylated fatty acid is C_{10:0} 3-OH. Other fatty acids are listed in Supplementary Table S2. The quinone system is composed of ubiquinone Q-8 and Q-7 (approx. 2:1). The polar lipid profile contains the major lipids phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol and minor amounts of phosphatidylserine, an unidentified aminolipid and an unidentified polar lipid not containing an amino-, phospho- or sugar group. The polyamine pattern contains the predominant compounds putrescine and 2-hydroxyputrescine.

The type strain, EM 1^T (=DSM 21777^T=CCUG 57265^T) was isolated from a pure water system. The DNA G+C content of the type strain is 52.4 mol%.

Description of *Undibacterium parvum* sp. nov.

Undibacterium parvum (par'vum. L. neut. adj. *parvum* small).

Growth and chemotaxonomic characteristics are as those listed in the description by Kämpfer *et al.* (2007). Cells are non-spore-forming; single cells are about 1.5–2.0 µm long with a diameter of about 0.5 µm. Cells tend to grow on glass surfaces when cultivated in liquid R2A and PYE medium. Cells reveal a typical Gram-negative architecture with a 6–7 nm thick outer membrane, a 2–3 nm peptidoglycan layer and a 6–7 nm thick cytoplasmic membrane. Electron-dense globules of varying diameters (up to 200 nm) are often observed in the cytoplasm of the cells, frequently with lipid body-like globules (approx. 50 nm) attached. Flagella with diameters of 20.6 ± 1.3 nm and pili with diameters of 9.6 ± 0.8 nm are present. Colonies of the type strain on R2A agar are shiny, yellowish and translucent, convex with a narrow and delicate lacerated rim and 0.5 mm in diameter after 3 days. No growth on TSA. Cells are oxidase- and catalase-positive. In API 20NE and API 20E tests under reduced salt conditions, positive for reduction of nitrate to nitrite,

citrate alkalization (API 20E) and malate utilization, weak hydrolysis of gelatin; negative responses for arginine dihydrolase, lysine and ornithine decarboxylase, acetoin production, β -galactosidase, tryptophan deaminase, hydrolysis of urea and aesculin and production of hydrogen sulfide and indole. Hydrolyses Tween 80, but not DNA, casein or starch on standard media. Temperature range for growth is 5–27 °C, no growth at 28 °C (data from this study) or 30 °C (Kämpfer *et al.*, 2007). Utilizes malate, maltose and cellobiose and weakly utilizes D-glucose when substrate concentrations of 1 g l⁻¹ are supplied. Other physiological properties are given in Tables 1 and 2. Main fatty acids are summed feature 3 (including unsaturated C_{16:1}ω7c), unsaturated C_{18:1}ω7c and straight chain C_{16:0}, the sole hydroxylated fatty acid is C_{10:0} 3-OH. Other fatty acids are listed in Supplementary Table S2. The quinone system is ubiquinone Q-8 with small amounts of Q-7, the polar lipid profile is composed of the major components phosphatidylethanolamine, phosphatidylglycerol and diphosphatidylglycerol. The polyamine pattern contains predominantly putrescine and 2-hydroxyputrescine.

The type strain, CCUG 49012^T (=DSM 23061^T=CIP 109317^T), was isolated from drinking water. The DNA G+C content of the type strain is 50.6 mol% as described by Kämpfer *et al.* (2007).

Emended description of the genus *Undibacterium* Kämpfer *et al.* 2007

Undibacterium (Un'di.bac.te'ri.um. L. n. *unda* water; L. neut. n. *bacterium* rod; N.L. neut. n. *Undibacterium* a rod of water).

Cells are motile by flagellation; pili are present. Sensitive to standard laboratory salt concentrations thus non-reactive in API or Biolog systems under standard conditions [e.g. inoculum preparation for biochemical tests in 8.5 g l⁻¹ NaCl, substrate content 0.2 %, AUX medium for API strips as provided by the manufacturer], but displaying biochemical reactions under reduced salt and substrate concentrations. Correspondingly, no response to physiological tests under standard conditions. Positive reactions may be achieved when reduced concentrations of salts and substrates are applied. Other characteristics are as given by Kämpfer *et al.* (2007). The type species is *Undibacterium pigrum*.

Emended description of *Undibacterium pigrum* Kämpfer *et al.* 2007

Undibacterium pigrum (pig'rum. L. neut. adj. *pigrum* inactive).

In addition to the description given by Kämpfer *et al.* (2007), also shows the following characteristics. Single cells are about 1.5–2.0 µm long with a diameter of about 0.5 µm. Cells reveal a typical Gram-negative architecture with a 6–7 nm thick outer membrane, a 2–3 nm peptidoglycan layer

and a 6–7 nm thick cytoplasmic membrane. Typically, an S-layer is visible with a thickness of approx. 20 nm and subunits with approx. 15 nm diameter. Electron-dense regions of varying shapes and sizes (up to 200 nm) are frequently observed in the cytoplasm of the cells. Cell flagellation is typically polar, monotrichous or ditrichous; flagella with diameters of 23.1 ± 0.98 nm and pili with diameters of 9.2 ± 0.9 nm are present. Polyamine pattern, quinone system, polar lipid profile, fatty acid patterns and growth characteristics are those given in the genus and species description by Kämpfer *et al.* (2007). At reduced salt and substrate concentrations, able to reduce nitrate to nitrite, to hydrolyse gelatin and to utilize D-glucose, mannose, maltose, N-acetylglucosamine, ribose, galactose, D-fructose, cellobiose, sucrose, trehalose, β -gentiobiose and starch (weakly). No growth on R2A agar under anaerobic conditions and no acid production from glucose or other carbohydrates. Some of the substrates that are not utilized under the conditions described by Kämpfer *et al.* (1991) may be used when tested with low salt and substrate concentrations. The type strain is CCUG 49009^T (=DSM 19792^T = CIP 109318^T).

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