**Defluviimonas alba** sp. nov., isolated from an oilfield

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Two Gram-stain-negative, rod-shaped bacterial strains, cai42† and b45, were isolated from oil-production water taken from Xinjiang Oilfield, China. Optimum growth was observed at 30 °C, at pH 8 and with 1–3 % (w/v) NaCl. According to phylogenetic analyses, the two strains were members of the genus *Defluviimonas*, with 16S rRNA gene sequence similarities of 95.5–96.3 % with the type strains of species of the genus. The major cellular fatty acids of strains cai42† and b45 were C10:0 3-OH, C16:0 and summed feature 8 (C18:1ω7c/C18:1ω6c), and the predominant ubiquinone was Q-10, all of these data being typical for the genus *Defluviimonas*. The polar lipids were phosphatidylethanolamine, phosphatidylglycerol, glycolipid, phosphatidylcholine, two unidentified aminolipids, an unidentified phospholipid and two unidentified lipids. The mean genomic DNA G+C contents of strains cai42† and b45 were 60.8 ± 1.1 and 60.4 ± 1.0 mol%, respectively. On the basis of phylogenetic, physiological and chemotaxonomic analyses, strains cai42† and b45 represent a novel species of the genus *Defluviimonas*, for which the name *Defluviimonas alba* sp. nov. is proposed. The type strain is cai42† (=CGMCC 1.12518T=LMG 27406T).

The genus *Defluviimonas*, belonging to the family Rhodobacteraceae, was first described by Foesel et al. (2011) and, at the time of writing, comprises four recognized species, *Defluviimonas denitrificans* (Foesel et al., 2011) isolated from the biofilter of a marine aquaculture system, *Defluviimonas aestuarii* (Math et al., 2013) isolated from a tidal flat of the South Sea in Korea, *Defluviimonas indica* (Jiang et al., 2014) isolated from a deep-sea hydrothermal vent chimney collected from the South-west Indian Ridge and *Defluviimonas aquaeamixtae* (Jung et al., 2014) isolated from the junction between a freshwater spring and the ocean. Typically, cells of members of this genus are Gram-negative, moderately halophilic, catalase- and oxidase-positive rods, with the major fatty acids being summed feature 8 (C18:1ω7c/C18:1ω6c), and the respiratory quinone being Q-10. During study of the bacterial community in an oilfield in Xinjiang Province, China (Sun et al., 2014), two novel strains, cai42† and b45, were isolated and herein are shown to represent a novel species of the genus *Defluviimonas*.

Strains cai42† and b45 were isolated by the standard dilution plating method from oil-production water recovered from Xinjiang Oilfield. The isolation medium was Columbia agar base (per litre: special peptone, 23 g; starch, 1 g; NaCl, 5 g; agar, 10 g; pH 7.3 ± 0.2). The morphological, chemical and molecular analyses of the two strains were conducted after the cells were grown on Luria–Bertani agar (LB; per litre: peptone, 20 g; yeast extract, 5 g; NaCl, 10 g; agar, 15 g) for 24 h at 30 °C, pH 8, except where otherwise indicated, *D. denitrificans* DSM 18921†, *D. aestuarii* JCM 18630† and *Rhodobacter veldkampii* CGMCC 15006†, used as reference strains , were obtained from Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ), Japan Collection of Microorganisms and China General Microbiological Culture Collection Center, respectively. Reference strains *Haematobacter massiliensis* CCUG 47968† and *Haematobacter missouriensis* CCUG 52307† were kindly provided by Gejiao Wang, State
Key Laboratory of Agricultural Microbiology, Huazhong Agricultural University, China. D. denitrificans DSM 18921\textsuperscript{T}, D. aequarii JCM 18630\textsuperscript{T} and R. veldkampii CGMCC 15006\textsuperscript{T} were cultured in marine broth 2216 (MB; per litre: peptone, 5 mg; ferric phosphate, 0.1 mg; yeast extract, 1 mg; NaCl, 19.45 mg; MgCl\textsubscript{2}, 5.98 mg; Na\textsubscript{2}SO\textsubscript{4}, 3.42 mg; CaCl\textsubscript{2}, 1.8 mg; KCl, 0.55 mg; Na\textsubscript{2}CO\textsubscript{3}, 0.16 mg; KBr, 0.08 mg; Cl\textsubscript{2}Sr, 0.0034 mg; boric acid, 0.022 mg; sodium silicate, 0.004 mg; NaF, 0.0024 mg; NaNO\textsubscript{3}, 0.0016 mg; Na\textsubscript{2}HPO\textsubscript{4}, 0.008 mg; pH 7.0) at 30 °C (Math et al., 2013); H. massiliensis CCUG 47968\textsuperscript{T} and H. missouriensis CCUG 52307\textsuperscript{T} were grown under clinical culture conditions in brain heart infusion (BHI) medium (Helsel et al., 2007) at 35 °C.

The morphology of the cells was observed under a transmission electron microscope (JEM-1230; JEOL). The temperature and pH range for growth were determined using LB medium at 4, 15, 20, 25, 30, 37, 40, 45, 50 and 55 °C, and at pH values from 4 to 10 (at 1 pH unit intervals), respectively (Takai et al., 2002). Salt tolerance was tested in LB medium (pH 8) ranging from 0 to 9% (w/v) NaCl (at 1% intervals).

Enzyme activities were tested using an API ZYM kit (bioMérieux) according the method described by Cai et al. (2011). Oxidase activity was examined using oxidase reagent (bioMérieux). Catalase activity and hydrolysis of Tween 80 and starch were analysed according to Dong & Cai (2001). Nitrate reduction, urease activity, indole production, hydrolysis of gelatin, glucose fermentation and utilization of carbon sources were determined using an API 20NE kit (bioMérieux) after incubation at 30 °C for 24 h. Antibiotic susceptibility tests were carried out with disc diffusion method (Fraser & Jorgensen, 1997; Andrews et al., 2008), and the compounds included amikacin (30 μg), ceftazidime (30 μg), neomycin (30 μg), cefotaxime (30 μg), kanamycin (30 μg), vancomycin (30 μg), chloramphenicol (30 μg), tetracycline (30 μg), ciprofloxacin (5 μg), ofloxacin (5 μg), gentamicin (10 μg), penicillin (10 μg), amoxicillin (10 μg), rifampicin (5 μg), erythromycin (15 μg), clindamycin (2 μg), novobiocin (5 μg) and minocycline (15 μg). After cells of strains cai42\textsuperscript{T} and b45 were cultured in 50 ml LB liquid medium at 30 °C in the dark for 24 h, pigments were extracted with acetone/methanol (7:2) and absorption peaks were detected in a UV spectrophotometer (UV-1700; Shimadzu) between 300 and 900 nm (Biebl et al., 2007). Anaerobic growth of the two isolates was evaluated in LB liquid medium covered with a 3–4 cm thickness of Vaseline and different electron acceptors (10 mM NaNO\textsubscript{3}, 10 mM Na\textsubscript{2}SO\textsubscript{4}, 2 mM NaNO\textsubscript{2}, 10 mM sodium thiosulfate or 10 mM iron (III) sulfate) (Foessel et al., 2011). D. denitrificans DSM 18921\textsuperscript{T} was tested at the same time as a reference strain. The photosynthetic reaction centre gene pufM was also tested by using the primer set pufM.557 F (5′-CGCACCTGGAACGAC) and pufM.750 R (5′-CCCATGTTCCAGGCC-AGAA) (Achenbach et al., 2001).

Isoprenoid quinones were examined by HPLC with an Agilent column (internal diameter, 4.6 mm; length, 15 cm) filled with Kromasil 100–5 C\textsubscript{18} according to the methods described by Komagata & Suzuki (1987). As D. denitrificans DSM 18921\textsuperscript{T} and D. aequarii JCM 18630\textsuperscript{T} could not growth well on trypticase soy agar (TSA; Difco) and D. aequarii JCM 18630\textsuperscript{T} could not growth on LB, cells of strains cai42\textsuperscript{T} and b45, and all the five reference strains were cultured in MB (Math et al., 2013) at 30 °C and harvested after grown for 24 h (the exponential growth phase). The fatty acids were then analysed with the standard protocol of the Microbial Identification System (MIDI, Microbial ID). For analysis of polar lipids, cells of strains cai42\textsuperscript{T} and b45 (cultured on LB agar) were harvested and polar lipids were extracted by using a chloroform/methanol system, then examined by two-dimensional TLC on Merck silica gel 60 F254 aluminium-backed thin-layer plates (Kates, 1986). TLC plates were sprayed with 1-naphthol for detection of sugars, ninhydrin for aminolipids, molybdenum blue for phospholipids, D reagent [3.5 ml bismuthyl nitrate solution (1.7%, w/v, dissolved in 20% acetic acid), 5 ml potassium iodide (40%, w/v), 20 ml acetic acid, 50 ml double distilled water] for phosphatidylcholine, phosphatidylethanolamine and phosphatidylmethylethanolamine components, and molybdatophosphoric acid for total lipids.

The genomic DNA of strains cai42\textsuperscript{T} and b45 was extracted according to Marmur (1961) from cells grown in LB medium for 24 h at pH 8 and 30 °C. The 16S rRNA gene was PCR-amplified using the universal bacterial primer set 8 F and 1492 R (Embley, 1991). After sequencing, the 16S rRNA gene sequences were compared with sequences obtained from the EzTaxon-e database (Kim et al., 2012). Phylogenetic trees were reconstructed and evaluated using MEGA software (Tamura et al., 2011) with the neighbour-joining (Saitou & Nei, 1987), minimum-evolution (Rzhetsky & Nei, 1993) and maximum-likelihood (Felsenstein, 1981) algorithms, and the bootstrap analysis was based on 1000 resamplings.

The genomic DNA G+C contents of strains cai42\textsuperscript{T} and b45 were determined from the midpoint value (T\textsubscript{m}) of the thermal denaturation profile (Mandel et al., 1970) with Escherichia coli K-12 as reference. DNA–DNA hybridization between strains cai42\textsuperscript{T} and b45 was performed in triplicate according to the methods of De Ley et al. (1970) and Huss et al. (1983).

Strains cai42\textsuperscript{T} and b45 shared a similarity of 99.9% in the nearly complete 16S rRNA gene sequence (1428 nt), and the mean (± SD) level of DNA–DNA relatedness between the two strains was 73.9±8.2%. The results showed that they were different strains belonging to the same species. The two strains shared 96.3–96.4% 16S rRNA gene sequence similarity with R. veldkampii CGMCC 15006\textsuperscript{T}, 96.0–96.2% with strains in the genus Haematobacter and 95.5–96.3% with strains in the genus Defluviimonas. However, in the phylogenetic trees reconstructed using the neighbour-joining (Fig. 1), minimum-evolution (Fig. S1, available in the online Supplementary Material) and
maximum-likelihood algorithms (Fig. S2), strains cai42T and b45 formed a stable and distinct clade with species of the genus Defluviimonas and separated from other genera. For better confirmation of the taxonomic position of the two new isolates, the closest related type strains in the genera Defluviimonas, Haematobacter and Rhodobacter were used as reference strains, namely D. denitrificans DSM 18921T (95.5–95.6% 16S rRNA gene sequence similarity), D. aestuarii JCM 18630T (96.2–96.3%), H. missouriensis CCUG 52307T (96.0–96.1%), H. massiliensis CCUG 47968T (95.8–95.9%) and R. veldkampii CGMCC 15006T. The mean genomic DNA G+C contents of strains cai42T and b45 were 60.8 ± 1.1 and 60.4 ± 1.0 mol%, respectively, values similar to that of D. aestuarii JCM 18630T (61.6 mol%), but significantly lower than the four other reference strains.

After growth on LB agar (pH 8) for 48 h, colonies of strains cai42T and b45 were creamy, circular and with a diameter of 0.5–1.0 mm. Cells of the two strains were Gram-stain-negative, rod-shaped, 1.0–1.5 μm in length and 0.5–0.7 μm in width (Fig. 2). Cells of strain cai42T had a cluster of one polar pili, which was different from D. denitrificans DSM 18921T and D. aestuarii JCM 18630T.

Growth was observed at 20–37 °C, at pH 7–9 and with 0–5% (w/v) NaCl. Optimum growth occurred at 30 °C, at
In accordance with the genera and b45 and the reference strains are listed in Table 1. However, strains cai42T and b45 lacked C18:0 3-OH and C19:0 cyclo ω8c (trace amount), components that were found in D. aeurii JCM 18630T and D. denitrificans DSM 18921T (Table 2). Furthermore, the two new isolates contained specific components present in trace amounts, namely summed feature 5 (C18:2ω6, 9c/anteiso-C18:0, 0.8%), which also differed from D. aeurii JCM 18630T and D. denitrificans DSM 18921T. H. massiliensis CCUG 47968T and H. missouriensis CCUG 52307T lacked 11-methyl C18:1ω7c but contained C19:0 cyclo ω8c and summed feature 2 (C14:0 3-OH/iso-C16:1 I; 1.5–1.6%) compared with strains cai42T and b45. R. veldkampii CGMCC 15006T showed clear differences in fatty acid features with the two new isolates. The polar lipids of strains cai42T and b45 were phosphatidylethanolamine (PE), phosphatidylglycerol (PG), glycolipid (GL), phosphatidylcholine (PC), two unidentified aminolipids (AL1 and 2), an unidentified phospholipid (PL2) and two unidentified lipids (L1 and 2) (Figs 3 and S3). Although strains cai42T and b45 shared similar PE, PG, AL2 and PC components with D. denitrificans DSM 18921T, D. aeurii JCM 18630T (Math et al., 2013), H. massiliensis CCUG 47968T and H. missouriensis CCUG 52307T (Wang et al., 2014), they contained unique PL, GL, AL1 and two other unknown lipids.

From the above analysis, strains cai42T and b45 grouped together with the cluster of type strains of the genus Defluviimonas and shared 95.5–96.3% 16S rRNA gene sequence similarity with them. Strains cai42T and b45 did not have photosynthetic pigments or the pufM gene, in contrast to the genus Rhodobacter. The DNA G+C contents of the two isolates were approximately 60.4–60.8 mol%, values much lower than for Haematobacter (65.0–65.5 mol%). Regarding fatty acids, strains cai42T and b45 lacked C18:0 3-OH and C19:0 cyclo ω8c components but contained trace amounts of C18:2ω6, 9c/anteiso-C18:0 compared with D. aeurii JCM 18630T and D. denitrificans DSM 18921T. The two new isolates contained particular polar lipid components, PL, GL, AL1 and two other unknown lipids, which were not found in D. aeurii JCM 18630T or D. denitrificans DSM 18921T. Furthermore, strains cai42T and b45 were negative for activity of alkaline phosphatase and naphthol-AS-BI-phosphoamidase and positive for utilization of gluconate and malic acid, which also differed from the two Defluviimonas strains. The phylogenetic, physiological and chemotaxonomic analyses showed that strains cai42T and b45 represented a species of the genus Defluviimonas that was clearly distinct from existing species. Therefore, strains cai42T and b45 are considered to represent a novel species of the genus Defluviimonas, for which the name Defluviimonas alba sp. nov. is proposed.
Table 1. Differential characteristics between strains cai42\textsuperscript{T} and b45 and the five reference type strains

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2*</th>
<th>3*</th>
<th>4*</th>
<th>5*</th>
<th>6*</th>
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<tbody>
<tr>
<td>Isolation source</td>
<td>Water</td>
<td>Aquaculture\textsuperscript{a}</td>
<td>Tidal flat\textsuperscript{b}</td>
<td>Clinical specimens\textsuperscript{c}</td>
<td>Clinical specimens\textsuperscript{c}</td>
<td>Freshwater\textsuperscript{c}</td>
</tr>
<tr>
<td>Colour of colonies</td>
<td>Creamy</td>
<td>Yellow</td>
<td>White</td>
<td>White</td>
<td>Yellow</td>
<td>Yellow brown</td>
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<td>Pilli</td>
<td>++</td>
<td>+\textsuperscript{a}</td>
<td>--\textsuperscript{b}</td>
<td>--\textsuperscript{c}</td>
<td>--\textsuperscript{c}</td>
<td>--\textsuperscript{c}</td>
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<tr>
<td>Optimum temperature (\degree C)</td>
<td>30</td>
<td>30–40\textsuperscript{a}</td>
<td>30\textsuperscript{b}</td>
<td>35\textsuperscript{d}</td>
<td>35\textsuperscript{d}</td>
<td>30–35\textsuperscript{e}</td>
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<tr>
<td>Optimum pH</td>
<td>8</td>
<td>6.5–7\textsuperscript{a}</td>
<td>7–7.5\textsuperscript{b}</td>
<td>7\textsuperscript{d}</td>
<td>7\textsuperscript{d}</td>
<td>7.5\textsuperscript{e}</td>
</tr>
<tr>
<td>Optimum NaCl (%)</td>
<td>1–3</td>
<td>0.5–2\textsuperscript{a}</td>
<td>1–1.5\textsuperscript{b}</td>
<td>1–2</td>
<td>1–2</td>
<td>Not required\textsuperscript{f}</td>
</tr>
<tr>
<td>Anaerobic growth</td>
<td>–</td>
<td>FA\textsuperscript{a}</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+\textsuperscript{g}</td>
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<tr>
<td>Pigment</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<td>API ZYM results:</td>
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<tr>
<td>Alkaline phosphatase</td>
<td>–</td>
<td>+</td>
<td>w</td>
<td>w</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Esterase lipase (C-8)</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
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<td>Leucine aminopeptidase</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>w</td>
<td>–</td>
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<tr>
<td>Valine aminopeptidase</td>
<td>–</td>
<td>w</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Acid phosphatase</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>w</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Naphthol-AS-BI-phosphoamidase</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>w</td>
<td>+</td>
<td>–</td>
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<tr>
<td>(\beta)-Galactosidase</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<td>(\alpha)-Glucosidase</td>
<td>–</td>
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<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Polar lipids\textsuperscript{f}</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
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<tr>
<td>DNA G + C content (mol%)</td>
<td>60.4–60.8</td>
<td>65.1\textsuperscript{a}</td>
<td>61.6\textsuperscript{b}</td>
<td>65.0\textsuperscript{d}</td>
<td>65.5\textsuperscript{d}</td>
<td>64.4–67.5\textsuperscript{e}</td>
</tr>
</tbody>
</table>

\*Data from: \textsuperscript{a} Foesel \textit{et al.} (2011); \textsuperscript{b} Math \textit{et al.} (2013); \textsuperscript{c} Helsel \textit{et al.} (2007); \textsuperscript{d} Wang \textit{et al.} (2014); \textsuperscript{e} Hansen & Imhoff (1985)

\*FA, facultatively aerobic.

\*PE, phosphatidyethanolamine, PG, phosphatidylglycerol, GL, glycolipid, PC, phosphatidylcholine, AL, unidentified aminolipid, PL, unidentified phospholipid, L, unidentified lipid.
Description of *Defluvimonas alba* sp. nov.

*Defluvimonas alba* (al’ba. L. fem. adj. alba white, the colour of colonies).

Cells are aerobic, Gram-stain-negative, rod-shaped, 1.0–1.5 μm long and 0.5–0.7 μm wide. After growth on LB agar (pH 8) for 48 h, colonies are creamy and circular (with a diameter of 0.5–1.0 mm). Growth occurs at 20–37 °C, at pH 7–9 and with 0–5 % (w/v) NaCl. Optimum growth occurs at 30 °C, at pH 8 and with 1–3 % (w/v) NaCl. Positive for activities of oxidase, catalase, esterase (C4), leucine aminopeptidase and β-glucosidase, weakly positive for starch hydrolysis, but negative for activities of alkaline phosphatase, esterase lipase (C8), lipase (C14), valine aminopeptidase, cystine aminopeptidase, trypsin, α-chymotrypsin, acid phosphatase, α-galactosidase, naphthol-AS-BI-phosphoamidase, β-galactosidase, N-acetyl-β-glucosaminidase, β-glucuronidase, β-glucosidase, α-mannosidase, β-fucosidase, nitrate reduction, indole reduction, D-glucose fermentation, arginine dihydrolase and urease activities, and hydrolysis of gelatin and aesculin. Utilizes D-glucose, arabinose, mannitol, maltose, potassium gluconate and malic acid, but not D-mannose, N-acetyl-D-glucosamine, capric acid, adipic acid, citrate or phenylacetic acid. Cannot use nitrate or nitrite as electron acceptors. The predominant ubiquinone is Q-10. The major cellular fatty acids are C10:0 3-OH, C16:0 and summed feature 8. The DNA G+C content is 60.4–60.8 mol%. The polar lipids are phosphatidylethanolamine (PE), phosphatidylglycerol (PG), glycolipid (GL), phosphatidylcholine (PC), two unidentified aminolipids (AL1 and 2), an unidentified phospholipid (PL2) and two unidentified lipids (L2 and 2).

The type strain, cai42T (=CGMCC 1.12518T =LMG 27406T), was isolated from oil-production water of Xinjiang Oilfield, China.

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References


