Aquabacterium gen. nov., with description of Aquabacterium citratiphilum sp. nov., Aquabacterium parvum sp. nov. and Aquabacterium commune sp. nov., three in situ dominant bacterial species from the Berlin drinking water system

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Three bacterial strains isolated from biofilms of the Berlin drinking water system were characterized with respect to their morphological and physiological properties and their taxonomic position. Phenotypically, the bacteria investigated were motile, Gram-negative rods, oxidase-positive and catalase-negative, and contained polyalkanoates and polyphosphate as storage polymers. They displayed a microaerophilic growth behaviour and used oxygen and nitrate as electron acceptors, but not nitrite, chloride, sulfate or ferric iron. The substrates metabolized included a broad range of organic acids but no carbohydrates at all. The three species can be distinguished from each other by their substrate utilization, ability to hydrolyse urea and casein, cellular protein patterns and growth on nutrient-rich media as well as their temperature, pH and NaCl tolerances. Phylogenetic analysis, based on 16S rRNA gene sequence comparison, revealed that the isolates are affiliated to the β1 subclass of Proteobacteria. The isolates constitute three new species with internal levels of DNA relatedness ranging from 44.9 to 51.3%. It is proposed that a new genus, Aquabacterium gen. nov., should be created, including Aquabacterium citratiphilum sp. nov., Aquabacterium parvum sp. nov. and Aquabacterium commune sp. nov. The type species of the new genus is Aquabacterium commune. The type strain of A. citratiphilum is strain B4T (= DSM 11900T), the type strain of A. parvum is strain B6T (= DSM 11968T) and the type strain of A. commune is strain B8T (= DSM 11901T).

Keywords: drinking water, biofilms, Aquabacterium gen. nov.

INTRODUCTION

Drinking water and the related distribution networks represent distinct oligotrophic systems in which bacterial growth is severely limited by low contents of assimilable organic carbon (LeChevallier et al., 1987; van der Kooij, 1992) and in some cases by phosphorus availability (Miettinen et al., 1996, 1997). In the past, the microbiology of drinking water systems has been studied almost exclusively from the point of view of public health. Most studies have focused on the detection of bacterial species causing infectious diseases, such as Legionella pneumophila (Rogers & Keevil, 1992; Rogers et al., 1994), opportunistic pathogens like Mycobacterium spp. (Schulze-Röbbecke & Fischeder, 1989) or Aeromonas spp. (Kühn et al., 1997) and coliform bacteria as indicator organisms for faecal contamination (Christian & Pipes, 1983; LeChevallier, 1990).

The autochthonous microbial population of this nutrient-deprived habitat, however, remains largely uncharacterized. A number of cultivation-based studies have attempted to describe the whole spectrum
of bacterial species present in drinking water, commonly yielding bacteria affiliated to the genera *Pseudomonas*, *Acinetobacter*, *Alcaligenes*, *Flavobacterium*, *Moraxella* and *Arthrobacter* (LeChevallier et al., 1987; Rogers et al., 1994; Tall et al., 1995; Ward et al., 1986; Olson & Nagy, 1984), but no information on the relevance and abundance of these organisms in their natural habitat could be gained. This is largely due to the lack of suitable cultivation approaches and, until recently, to the lack of appropriate methods to detect and monitor the in situ species composition of an ecosystem.

A number of bacterial strains isolated from biofilms of the Berlin drinking water distribution system were recently analysed by 16S rRNA gene sequencing and the development and application of highly specific oligonucleotide probes (Kalmbach et al., 1997). In situ analysis of drinking water biofilms enabled the retrieval of these bacteria within their natural habitat and showed that three of the isolated strains, designated strains B4T, B6T and B8T, dominated the biofilm population of the Berlin drinking water distribution system.

In the present study, these organisms are described by morphological and physiological characterization, DNA reassociation and 16S rRNA-based phylogenetic classification. On the basis of these data, we propose a new genus, *Aquabacterium* gen. nov., with the description of *Aquabacterium citratophilum* sp. nov. (type strain B4T, DSM 11900T), *Aquabacterium parvum* sp. nov. (type strain B6T, DSM 11968T) and *Aquabacterium commune* sp. nov. (type strain B8T, DSM 11901T). The type species of the new genus is *Aquabacterium commune*.

**METHODS**

**Bacterial strains.** Strains B1–B8T were isolated in summer 1996 from biofilms grown on glass and polyethylene surfaces exposed in modified Robbins devices connected to a house installation system of the Berlin drinking water distribution network (Kalmbach et al., 1997). The temperature of the Berlin drinking water varied from 9.4 to 15.6 °C and the pH ranged from 7.2 to 7.7. The bacteria were isolated on R2A agar (Reaasoner & Geldreich, 1985). The strains have been deposited with the DSMZ as DSM 11900T (strain B4T), DSM 11901T (strain B8T) and DSM 11968T (strain B6T).

**Media and culture conditions.** For cultivation of strains, R2A medium was modified by replacing starch with 0.1% (v/v) Tween 80 (Sigma). Growth on nutrient-rich media was tested on nutrient agar, containing 3 g beef extract, 5 g peptone and 15 g agar 1° distilled water and on DEV nutrient agar (Anonymous, 1986). Oxidation of manganese was tested by growth on modified Rouf and Stokes medium (Wagner et al., 1994) as described by Spring et al. (1996). Unless specified otherwise, bacteria were grown in liquid medium with constant agitation (100 r.p.m.) at 20 °C.

**Transmission electron microscopy.** For negative-staining, samples of exponential-phase bacterial suspensions were dropped onto Piloform-coated copper grids. The material was negatively stained with a mixture of phosphotungstic acid (2%, w/v) and bacitracin (0.005%, w/v) according to Wolf et al. (1993) and examined with a Philips 400 electron microscope.

Thin sections of bacterial cells from exponential-phase cultures were prepared as described by Wolf et al. (1993). Fixation of the cells was carried out with 2.5% (v/v) glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2) for at least 16 h at 4 °C. After washing with cacodylate buffer, the cells were post-fixed with 1:5% (w/v) osmium tetroxide plus 1:65% (w/v) potassium dichromate in 0.1 M cacodylate buffer for 1 h at room temperature, dehydrated in a graded ethanol series and embedded in LR White (Science Services). Thin sections were cut with a Reichert OM U 3 ultramicrotome and post-stained for 6 min with 1% (w/v) lead citrate. Examination of the thin sections was performed with a Philips 400 electron microscope.

**SDS-PAGE.** Cells grown in liquid modified R2A medium were harvested by centrifugation at 10000 g, resuspended in 50 µl 2x SDS-gel-loading buffer [100 mM Tris/HCl, pH 6.8, 200 mM dithiothreitol, 4% (v/v) SDS, 0.2% (v/v) bromophenol blue, 20% (v/v) glycerol] and lysed by boiling for 5 min. The extracts were separated on a gel containing 12% (w/v) polyacrylamide and stained with Coomassie blue according to standard procedures (Sambrook et al., 1989).

**Morphological characteristics.** Wet mounts of cells grown to exponential phase (approx. 106 cells ml–1) in modified R2A medium were observed on agar-slides (Plennig & Wagener, 1986) by phase-contrast microscopy. Staining of polyalkanoate inclusion bodies with Nile blue A (Sigma) was performed as described by Ostle & Holt (1983). Gram-staining was performed by using the Gram-colour kit from Merck. Colony morphology was determined by stereomicroscopy of cultures grown on modified R2A agar after 10 d incubation at 20 °C in the dark.

**Biochemical characteristics.** The ability of the organisms to utilize different substrates was studied in mineral medium containing per litre distilled water: 0.66 g (NH4)2SO4, 1.36 g KH2PO4, 0.123 g MgSO4, 7H2O, 0.017 g CaSO4, 2H2O and 0.006 g FeSO4. The mineral medium was supplemented with autoclaved or filter-sterilized substrates (Table 1). Bacterial growth was determined for up to 7 d by measuring changes in the optical density (OD600) with a Beckman spectrophotometer.

Cytochrome oxidase activity was determined with the Bacident Oxidase assay (Merck) and catalase production was determined by using a 3% (v/v) H2O2 solution on colonies grown on modified R2A agar. DNA hydrolysis was examined by using commercially available DNase agar (Oxoid) and flooding of the plates with 1 M HCl after growth of the colonies. Aesculin hydrolysis was determined in liquid modified R2A medium supplemented with 0.01% (w/v) aesculin and 0.05% (w/v) iron(III) citrate. Hydrolysis of urea was determined in liquid modified R2A medium containing 2% (w/v) urea and 0.001% (w/v) phenol red, inoculated with approximately 104 cells. Hydrolysis of starch was determined on modified R2A agar containing 0.2% (w/v) soluble starch and no glucose by flooding of the plates with iodine solution (Merck). Hydrolysis of casein was tested on casein agar (double-strength, modified R2A agar containing 0.2% (w/v) soluble starch and no glucose by flooding of the plates with casein). Gelatin hydrolysis was determined by incubation for 6 weeks in modified liquid R2A medium supplemented with 12% (w/v) gelatin.
Table 1. Biochemical characteristics and substrate utilization of A. citratiphilum (B4T), A. parvum (B6T) and A. commune (B8T)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>B4T</th>
<th>B6T</th>
<th>B8T</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxidase test</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Catalase reaction</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Reduction of:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NO₃⁻</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>NO₂⁻, Fe²⁺, SO₄²⁻, ClO₃⁻</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Hydrolysis of:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urea</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Casein</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Aesculin, DNA, starch, gelatin</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Temperature range (°C)</td>
<td>10-36</td>
<td>14-34</td>
<td>6-34</td>
</tr>
<tr>
<td>NaCl concentration (% w/v)</td>
<td>0-18</td>
<td>0-08</td>
<td>0-04</td>
</tr>
<tr>
<td>pH range</td>
<td>5-5-10</td>
<td>6-5-10</td>
<td>6-5-9</td>
</tr>
<tr>
<td>Substrate utilization:*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Propionate, pyruvate, bromosuccinate</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Glycerol, lactate, citrate, β-hydroxybutyrate</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Benzoate, Casamino acids, glutamate</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

*Substrates that were utilized by all three strains included Tweens 20, 40, 60 and 80, acetate, butyrate, valerate, caproate, caprylate, succinate, adipate, pimelate, azelate, sebacate, fumarate, malate, butanol and β-hydroxybutyrate. Substrates that were not utilized by strains B4T, B6T or B8T included caprate, oxalate, formate, malonate, glutarate, phthalate, glyoxylate, tartrate, methanol, ethanol, D-glucose, L-arabinose, D-mannose, D-mannitol, D-malate, D-fructose, D-xylene, D-ribose, L-rhamnose, D-lactose, D-galactose, D-trehalose, D-melibiose, D-ribulose, succrose, N-acetylglucosamine, gluconate, galacturonate and ascorbate. Substrate concentrations were 5 mM except Casamino acids, which were used at 0.1% (w/v), and Tweens 20, 40, 60 and 80, butanol and glycerol, which were used at 0.1% (v/v).

Reduction of nitrate and nitrite was determined chemically by inoculating freshly grown cultures in modified R2A medium containing 0-17% agar supplemented with 0.1 (w/v)% KNO₃ or 0.02% (w/v) KNO₂, respectively. The cultures were grown for 5 d without agitation. The presence of nitrite was determined with equal volumes of 0-6% (w/v) N-(1-naphthyl)ethylenediamine dihydrochloride in 5 M acetic acid and 0.8% (w/v) sulfanilic acid in 5 M acetic acid, according to Neyra et al. (1977).

Electron acceptors. The potential use of different electron acceptors was studied in deep-agar cultures by inoculating freshly grown cultures in modified R2A medium containing 1% (w/v) agar and as electron acceptors: (i) atmospheric oxygen, (ii) 0.1% (w/v) KNO₃, (iii) 0.02% (w/v) KNO₂, (iv) 10 mM NaClO₄, (v) 10 mM Na₂SO₄ or (vi) 10 mM iron(III) citrate. Fermentation capacity was tested in deep-agar cultures with modified R2A medium and in mineral medium containing 5 mM β-hydroxybutyrate. All cultures were grown under atmospheric conditions as well as N₂ atmosphere (99-99%) for 14 d and examined for visible colonies by using stereomicroscopy at 4× magnification.

The use of SO₄²⁻ or Fe²⁺ as electron acceptor was additionally examined under anaerobic conditions in a medium containing per litre distilled water: 1 g NaCl, 0.2 g K₂HPO₄, 0.27 g NH₄Cl, 0.41 g MgCl₂, 6H₂O, 0.52 g KCl and 0.15 g CaCl₂. Solution pH was adjusted to 4.1 using acetic acid and 0.4 g Na₂SO₃ as a reducing agent. The medium was prepared under N₂/CO₂ atmosphere (80:20, v/v). Bacterial strains were inoculated in screw-cap bottles in an anaerobic medium supplemented with 0.1% Tween 80 as the sole carbon source. Na₂SO₄ or iron(III) citrate was added to a final concentration of 10 mM. The use of the electron acceptors was monitored by measuring cellular growth for a period of 14 d after incubation. Desulfovibrio desulfuricans (DSM 6949T) was used as a positive control for iron and sulfate reduction in modified R2A deep-agar cultures and in the anaerobic medium.

Physiological characteristics. NaCl, pH and temperature tolerance ranges were determined in modified R2A medium. Growth at various NaCl concentrations ranging from 0.2 to 2% (w/v) and pH values ranging from 5 to 10.5 were determined spectrophotometrically (OD₅₅₀) over a period of 5 d. To determine growth at different pH values, the modified R2A medium was supplemented with 10 mM Bis-Tris buffer for pH 5-6.5 and with 10 mM CAPS buffer for pH 9-10.5. The pH was adjusted with HCl and NaOH. Growth at temperatures ranging from 4 to 40°C was determined spectrophotometrically in modified R2A medium after 4, 7 and 14 d incubation of static cultures.

16S rRNA-based phylogenetic analysis. Extraction of genomic DNA and amplification and sequencing of the 16S rRNA genes from the strains investigated was performed as described previously (Kalmbach et al., 1997). The 16S rRNA sequences (Escherichia coli positions 50-1350) were aligned...
using the Aligner tool of the ARB software package (Strunk & Ludwig, 1995) and corrected manually according to primary and secondary structure similarity. Distance matrices were calculated from the completely aligned sequences and corrected for multiple base changes at single positions by the method of Jukes & Cantor (1969). Phylogenetic trees were constructed by the neighbour-joining method of Saitou & Nei (1987).

**DNA-DNA hybridization.** Isolation of genomic DNA and DNA-DNA hybridization was performed at the DSMZ. DNA was isolated and purified by chromatography on hydroxyapatite (Cashion et al., 1977) and DNA hybridization was performed as described by De Ley et al. (1970), by using a Gilford System 2600 spectrophotometer equipped with a Gilford 2527-R thermal programmer and plotter.

**DNA base composition.** The G + C content of strains B4T, B6T and B8T was determined by HPLC at the DSMZ.
Aquabacterium gen. nov.

RESULTS

Cell and colony morphology

Individual cells of strains B4T, B6T and B8T were motile, Gram-negative rods. They occurred as single cells, but tended to form cell aggregates. The cells of all three strains contained dark inclusion bodies visible by phase-contrast microscopy (Fig. 1), which could be identified as polyalkanoate storage polymers by staining with the fluorescent dye Nile blue A. Electron micrographs of thin sections of strains B4T, B6T and B8T demonstrated the presence of polyalkanoate and polyphosphate inclusion bodies and mesosome-like structures as well as fibrillar matrix material surrounding the cells (Fig. 1). The cells were motile by means of a single polar flagellum, which was one to two times the length of the bacterium (Fig. 2). Strains B4T and B8T were 2–4 μm long and about 0.5 μm in diameter and strain B6T was 1–2 μm long and 0.5 μm in diameter. When streaked onto modified R2A agar plates and incubated for 10 d, strain B4T formed cream-white, flat colonies with a smooth margin and a diameter of 2–3 mm. Strain B6T formed flat colonies with a smooth margin and a diameter of 1.5–2 mm, which were white in the centre and transparent at the edges. Strain B8T formed transparent, flat colonies that had a smooth margin and were 1.5–2 mm in diameter. When grown on modified Roux and Stokes medium, colonies of strains B4T, B6T and B8T were white, indicating that manganese oxidation did not occur.

Growth conditions

Strain B4T grew well in liquid as well as on solid R2A medium, whereas strains B6T and B8T produced small colonies on R2A agar but did not grow well in liquid R2A medium. Based on the results of initial substrate utilization tests, R2A medium was modified by replacing starch with 0.1% (v/v) Tween 80, resulting in improved growth of all strains investigated on solid as well as liquid medium. Strain B4T was the only strain that grew well on nutrient-rich media. Strain B6T produced tiny colonies (0.1 mm diameter), whereas strain B8T did not grow at all after 5 d incubation on nutrient-rich media. The temperature, pH and NaCl range for growth of the strains investigated is given in Table 1.

Metabolic properties

The physiological properties of strains B4T, B6T and B8T are summarized in Table 1. Strains B4T, B6T and B8T are aerobic, oxidase-positive and catalase-negative bacteria. When grown in deep-agar cultures, strains B4T, B6T and B8T displayed a microaerophilic growth behaviour, indicated by growth of the organisms in the form of a disc, typically located 2 mm below the agar surface. None of the strains could grow by fermentation in modified R2A agar or in mineral medium with β-hydroxybutyrate as the sole carbon source. For strains B6T and B8T, nitrate reduction could be
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**Fig. 4.** Phylogenetic tree inferred from 16S rRNA gene sequences (at least 1300 bases), reflecting the relationships of *A. citratiphilum* (B4T, DSM 11900T; accession no. AF035050), *A. parvum* (B6T, DSM 11968T; AF035052) and *A. commune* (B8T, DSM 11901T; AF035054) to their closest known relatives and to the drinking water biofilm isolates B1 (AF035047), 82 (AF035048), B3 (AF035049), B5 (AF035051) and B7 (AF035053). Distance matrices were constructed from the aligned sequences and corrected for multiple base changes at single positions by the method of Jukes & Cantor (1969) and a phylogenetic tree was constructed by the neighbour-joining method of Saitou & Nei (1987) by using the ARB software package (Strunk & Ludwig, 1995). Bar represents 10 nucleotide substitutions per 100 nucleotides.

Demonstrated chemically as well as by growth in deep-agar cultures, both under atmospheric conditions as well as an N₂ atmosphere. Strain B4T displayed only weak production of nitrite from nitrate in the chemical assay but was able to produce visible growth in deep-agar cultures with nitrate as the sole electron acceptor. None of the strains reduced nitrite or chlorate, nor were sulfate or iron(II) utilized as electron acceptors in deep-agar cultures or liquid medium.

Starch, aesculin, gelatin and DNA were not hydrolysed by strains B4T, B6T and B8T. Strain B8T was the only strain able to hydrolyse casein and to grow with amino acids from casein hydrolysis or with glutamate as the sole carbon source (Table 1). Urea was hydrolysed by strains B4T and B6T but not by strain B8T. The substrates utilized by strains B4T, B6T and B8T are summarized in Table 1. All three strains utilized fatty acids, carboxylic acids and alcohols but did not grow on any of the carbohydrates tested.

**Cellular protein analysis**

Protein patterns of strains B4T, B6T and B8T after separation by SDS-PAGE and staining with Coomassie blue are shown in Fig. 3. Although strains B4T, B6T and B8T had some proteins in common, e.g. in the molecular mass range of 45 to 66 kDa, they exhibited a considerable heterogeneity in the range 31 to 45 kDa. Strains B4T and B6T share one common protein of about 35 kDa, which is not present in strain B8T, but each strain also displayed at least one distinct protein not present in either of the other two strains.

**Table 2. 16S rRNA gene sequence identity matrix for A. citratiphilum (B4T), A. parvum (B6T) and A. commune (B8T)**

<table>
<thead>
<tr>
<th>Taxon</th>
<th>B4T</th>
<th>B6T</th>
<th>B8T</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aquabacterium citratiphilum</em> (B4T)</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><em>Aquabacterium parvum</em> (B6T)</td>
<td>96.5</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><em>Aquabacterium commune</em> (B8T)</td>
<td>98.2</td>
<td>97.2</td>
<td>–</td>
</tr>
<tr>
<td><em>Ideonella dechloratans</em> (CCUG 30898*)</td>
<td>96.4</td>
<td>96.7</td>
<td>96.6</td>
</tr>
<tr>
<td><em>Leptothrix cholodnii</em> (CCM 1827 = LMG 7171)</td>
<td>96.7</td>
<td>96.5</td>
<td>96.5</td>
</tr>
<tr>
<td><em>Rubrivivax gelatinosus</em> (ATCC 17011*)</td>
<td>95.7</td>
<td>96.4</td>
<td>95.7</td>
</tr>
<tr>
<td><em>Leptothrix mobilis</em> (LMG 17066*)</td>
<td>95.5</td>
<td>96.6</td>
<td>96.1</td>
</tr>
<tr>
<td><em>Leptothrix discophora</em> (ATCC 43182*)</td>
<td>94.4</td>
<td>94.6</td>
<td>94.4</td>
</tr>
<tr>
<td><em>Sphaerotilus natans</em> (accession no. Z18534)</td>
<td>94.9</td>
<td>93.7</td>
<td>94.6</td>
</tr>
</tbody>
</table>

The almost complete 16S rRNA gene sequences (comprising 1429 nucleotides, *E. coli* positions 29–1458) of strains B4T, B6T and B8T have been determined in a previous study (Kalmbach et al., 1997). The phylogenetic tree shown in Fig. 4 reflects the relationships of strains B4T, B6T and B8T to their closest relatives and to additional bacterial strains (strains B1, B2, B3, B5 and B7) isolated from the same habitat. The three strains B4T, B6T and B8T form a monophyletic group within the βI-subclass of Proteobacteria. Levels of 16S rDNA sequence identity range from 98.2% between strains B4T and B8T to 97.2% between strains B6T and B8T and 96.5% between strains B6T and B4T. The closest validated closely related proteins of strains B4T, B6T and B8T are represented by *Ideonella dechloratans* (CCUG 30898*) and by *Leptothrix cholodnii* (CCM 1827 = LMG 7171); the corresponding identity values are given in Table 2. Other close phylogenetic relationships were represented by 16S rDNA sequences of *Rubrivivax gelatinosus* (ATCC 17011*), *Leptothrix mobilis* (GenBank accession no. X97071), *Leptothrix disco-
DNA–DNA reassociation

DNA–DNA hybridization studies revealed levels of relatedness between the investigated strains ranging from 44.9% (strains B4T and B8T) to 45.4% for strains B4T and B6T and 51.3% for strains B6T and B8T.

DNA base composition

The G+C content of the DNA of strain B4T was 66 mol%, that of strain B6T was 65 mol% and that of strain B8T was 66 mol%.

DISCUSSION

Three bacterial strains, termed B4T, B6T and B8T, were recently shown to represent the predominant, frequent in situ bacterial species in biofilms obtained from the Berlin drinking water distribution system (Kalmbach et al., 1997). In the present study, the morphological and physiological properties as well as the phylogenetic affiliation of these strains were investigated. An analysis of phylogenetic relationships based on 16S rDNA sequence comparisons revealed that they constitute a cluster of bacteria affiliated to the β1-subclass of Proteobacteria, located in the Rubrivivax–Leptothrix sub-branch of this taxonomic unit. DNA–DNA reassociations clearly showed that strains B4T, B6T and B8T represent three distinct bacterial species. The DNA base composition of strains B4T, B6T and B8T, ranging from 65 to 66 mol% G+C, was similar to those of the closest phylogenetic relatives, Leptothrix cholodnii (68–70 mol%) and Ideonella dechloratans (68 mol%).

From a physiological perspective, the β-subclass of Proteobacteria is an extremely heterogeneous superfamily with few common features (De Ley, 1992). This is illustrated well by the closest validly described relatives of strains B4T, B6T and B8T: the phototrophic species Rubrivivax gelatinosus, the sheathed iron- and manganese-depositing genera Leptothrix and Sphaerotilus and the aerobic, chlorate-reducing bacterium Ideonella dechloratans. On the other hand, several of the phenotypic characteristics of strains B4T, B6T and B8T are typical of members of the β-subclass of Proteobacteria. In common with the majority of species of the β-Proteobacteria, the three strains investigated are aerobic bacteria that do not ferment. Furthermore, all three strains were unable to catabolize carbohydrates but grew well on fatty acids and carboxylic acids (Table 1). Additionally, the accumulation of polyalkanoate storage bodies is a widespread feature among members of the β-Proteobacteria and has been reported for the closely related genera Leptothrix and Sphaerotilus (Mulder & Deinema, 1992).

Ideonella dechloratans and Leptothrix cholodnii, the closest phylogenetic neighbours of strains B4T, B6T and B8T (Table 2), are physiologically quite distinct from this cluster. Ideonella dechloratans is an aerobic, motile, chlorate-reducing bacterium isolated from activated sludge (Malnvist et al., 1994). It clearly differs from strains B4T, B6T and B8T in its ability to reduce chlorate, to produce catalase, to metabolize carbohydrates and to grow at up to 42°C. Moreover, Ideonella dechloratans possesses two or more polar flagella. Leptothrix cholodnii is a manganese-oxidizing bacterium, characterized by the formation of dark-brown colonies on modified Ruff and Stokes medium, whereas strains B4T, B6T and B8T were unable to oxidize manganese. In contrast to strains B4T, B6T and B8T, Leptothrix cholodnii is unable to metabolize malate, succinate, butyrate or fumarate but is able to metabolize carbohydrates (Spring et al., 1996).

As a common trait, strains B4T, B6T and B8T displayed a microaerophilic growth behaviour. Growth at low oxygen levels is also described for the Leptothrix–Sphaerotilus group, conferring a competitive advantage on these organisms in ecosystems such as sewage or activated sludge (Mulder & Deinema, 1992). The microaerophilic growth behaviour is well suited for the anoxic conditions present in the groundwater used as the raw water source for the Berlin drinking water, where bacteria restricted to aerobic metabolism might not be able to proliferate. Although nitrate is not present in large amounts in the anoxic groundwater (typical nitrate concentrations ranged from 0.04 to 1.33 mg l⁻¹), it presumably plays a role as an alternative electron acceptor. The predominance of strain B8T throughout the drinking water distribution system (Kalmbach et al., 1997) might be supported by its broader temperature range compared to the temperature range of strains B4T and B6T, as well as by its greater nutritional versatility compared to that of strain B8T. With regard to their temperature requirements, all three strains grew at lower growth limit.

We propose the description of the new genus, Aquabacterium gen. nov., containing three species, Aquabacterium citratophilum sp. nov., Aquabacterium parvum sp. nov. and Aquabacterium commune sp. nov. A. commune is the type species of the new genus Aquabacterium.

Description of Aquabacterium gen. nov.

Aquabacterium (A.qua.bac'te.ri.um. L. n. aqua water; Gr. n. bakterion rod; Aquabacterium a rod-shaped bacterium, isolated from drinking water biofilms).

The three proposed new species, A. citratophilum, A. parvum and A. commune, were isolated from a drinking water biofilm in a house installation system at the Technische Universität Berlin. All members of the genus are Gram-negative, rod-shaped bacteria and are motile by means of monotrichous flagella. They contain polyalkanoate and polyphosphate inclusion bodies and produce fibrillar matrix material. They are oxidase-positive, catalase-negative, display a micro-
Description of *Aquabacterium commune* sp. nov.

*Aquabacterium commune* (com′mu.ne. L. adj. commune, -e common, referring to the predominance of the species in drinking water biofilms of the Berlin distribution system).

Cells are rods (0-5 × 2-4 μm) and possess a single, polar flagellum. Flat, transparent colonies with a smooth margin (1-5-2 mm diameter) are present after 10 d incubation at 20 °C on modified R2A agar. The temperature range for growth is 6-34 °C. Growth occurs between pH 6.5 and 9.5 and in the presence of NaCl at concentrations as high as 0.4%. In addition to the substrates used by all members of the genus, *A. commune* utilizes propionate, bromosuccinate, pyruvate, benzoate, Casamino acids and glutamate. *A. commune* is able to hydrolyse casein but not urea. The G+C content of the DNA is 66 mol%. Isolated from a drinking water house installation system. The type strain is strain B6T (= DSM 11901T).

Description of *Aquabacterium citratiphilum* sp. nov.

*Aquabacterium citratiphilum* (ci.tra.ti′phi.lum. L. n. citr us lemon tree; L. n. acidum acid; L. n. adj. acid um citri citric acid; Gr. adj. philos loving; M.L. n. adj. citratiphilum citrate-loving, referring to the preferred utilization of citrate as carbon and energy source).

Cells are rods (0-5 × 2-4 μm) and possess a single, polar flagellum. Flat, cream-white colonies with a smooth margin (2-3 mm diameter) are present after 10 d incubation at 20 °C on modified R2A agar. The temperature range for growth is 14-34 °C. Growth occurs between pH 5.5 and 9.5 and in the presence of NaCl up to 1.8%. The type species is *Aquabacterium commune*, since it is the most widespread species in drinking water system biofilms.

Cells are rods (0.5 × 1-2 μm) and possess a single, polar flagellum. Flat colonies with a smooth margin (1.5-2 mm diameter), which are white in the centre of the colony and transparent at the edges, are present after 10 d incubation at 20 °C on modified R2A agar. The temperature range for growth is 14-34 °C. Growth occurs between pH 6.5 and 10.0 and in the presence of NaCl up to 0.8%. *A. parvum* shows the substrate utilization typical for the genus *Aquabacterium*. *A. parvum* is able to hydrolyse urea. The G+C content of the DNA is 65 mol%. Isolated from a drinking water house installation system. The type strain is strain B6T (= DSM 11968T).

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