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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, chlorate, 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 B4^T (= DSM 11900^T), the type strain of *A. parvum* is strain B6^T (= DSM 11968^T) and the type strain of *A. commune* is strain B8^T (= DSM 11901^T).

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 assimilatable 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

The GenBank accession numbers for the 16S rRNA sequences reported in this paper are AF035047–AF035054 (strains B1–B8, respectively).

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 B4^T, B6^T and B8^T, 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 citratiphilum* sp. nov. (type strain B4^T, DSM 11900^T), *Aquabacterium parvum* sp. nov. (type strain B6^T, DSM 11968^T) and *Aquabacterium commune* sp. nov. (type strain B8^T, DSM 11901^T). The type species of the new genus is *Aquabacterium commune*.

METHODS

Bacterial strains. Strains B1–B8^T 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 (Reasoner & Geldreich, 1985). The strains have been deposited with the DSMZ as DSM 11900^T (strain B4^T), DSM 11901^T (strain B8^T) and DSM 11968^T (strain B6^T).

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 l^{-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 phosphotungstate (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 2 × 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. 10^8 cells ml^{-1}) in modified R2A medium were observed on agar-slides (Pfennig & Wagener, 1986) by phase-contrast microscopy. Staining of polyalkanoate inclusion bodies with Nile blue A (Sigma) was performed as described by Ostle & Holt (1982). 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 $(NH_4)_2SO_4$, 1·36 g KH_2PO_4, 0·123 g $MgSO_4.7H_2O$, 0·017 g $CaSO_4.2H_2O$ and 0·006 g $FeSO_4$. 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 (OD_{588}) 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) H_2O_2 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 10^8 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 was combined with an equal volume of sterile skimmed milk) by observation of clear zones around the colonies. The incubation period for catalase production and hydrolysis of DNA, aesculin, urea, starch and casein was 5 d. 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* ($B4^T$), *A. parvum* ($B6^T$) and *A. commune* ($B8^T$)

Characteristic	$B4^T$	$B6^T$	$B8^T$
Oxidase test	+	+	+
Catalase reaction	−	−	−
Reduction of:			
NO_3^-	+	+	+
NO_2^-, Fe^{3+}, SO_4^{2-}, ClO_3^-	−	−	−
Hydrolysis of:			
Urea	+	+	−
Casein	−	−	+
Aesculin, DNA, starch, gelatin	−	−	−
Temperature range (°C)	10–36	14–34	6–34
NaCl concentration (% w/v)	0–1·8	0–0·8	0–0·4
pH range	5·5–10·0	6·5–10·0	6·5–9·5
Substrate utilization:*			
Propionate, pyruvate, bromosuccinate	+	−	+
Glycerol, lactate, citrate, γ-hydroxybutyrate	+	−	−
Benzoate, Casamino acids, glutamate	−	−	+

*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 $B4^T$, $B6^T$ or $B8^T$ included caprate, oxalate, formate, malonate, glutarate, phthalate, glyoxylate, tartrate, methanol, ethanol, D-glucose, L-arabinose, D-mannose, D-mannitol, D-maltose, D-fructose, D-xylose, D-ribose, L-rhamnose, D-lactose, D-galactose, D-trehalose, D-melibiose, D-ribulose, sucrose, *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) % KNO_3 or 0·02 % (w/v) KNO_2, 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 0·2 ml freshly grown liquid cultures in 10 ml liquefied and tempered (41 °C) modified R2A agar, containing 1·5 % (w/v) agar and as electron acceptors: (i) atmospheric oxygen, (ii) 0·1 % (w/v) KNO_3, (iii) 0·02 % (w/v) KNO_2, (iv) 10 mM $NaClO_3$, (v) 10 mM Na_2SO_4 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 N_2 atmosphere (99·99 %) for 14 d and examined for visible colonies by using stereomicroscopy at 4 × magnification.

The use of SO_4^{2-} or Fe^{3+} as electron acceptors was additionally examined under anaerobic conditions in a medium containing per litre distilled water: 1 g NaCl, 0·2 g K_2HPO_4, 0·27 g NH_4Cl, 0·41 g $MgCl_2.6H_2O$, 0·52 g KCl and 0·15 g $CaCl_2.2H_2O$. After autoclaving, the medium was completed by adding (l^{-1}): 2·5 g $NaHCO_3$, 50 mg vitamin B_{12}, 0·5 ml vitamin solution (Adrian *et al.*, 1998), 1 ml trace elements solution (Adrian *et al.*, 1998), 1 ml selenite/tungstate solution (Tschech & Pfennig, 1984), 0·5 mg resazurin as redox indicator and 0·4 g Na_2S as reducing agent. The medium was prepared under a N_2/CO_2 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. Na_2SO_4 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* ($DSM\ 6949^T$) 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 5·0 to 10·5 were determined spectroscopically (OD_{588}) 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 9·0–10·5; the pH was adjusted with HCl and NaOH. Growth at temperatures ranging from 4 to 40 °C was determined spectroscopically 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

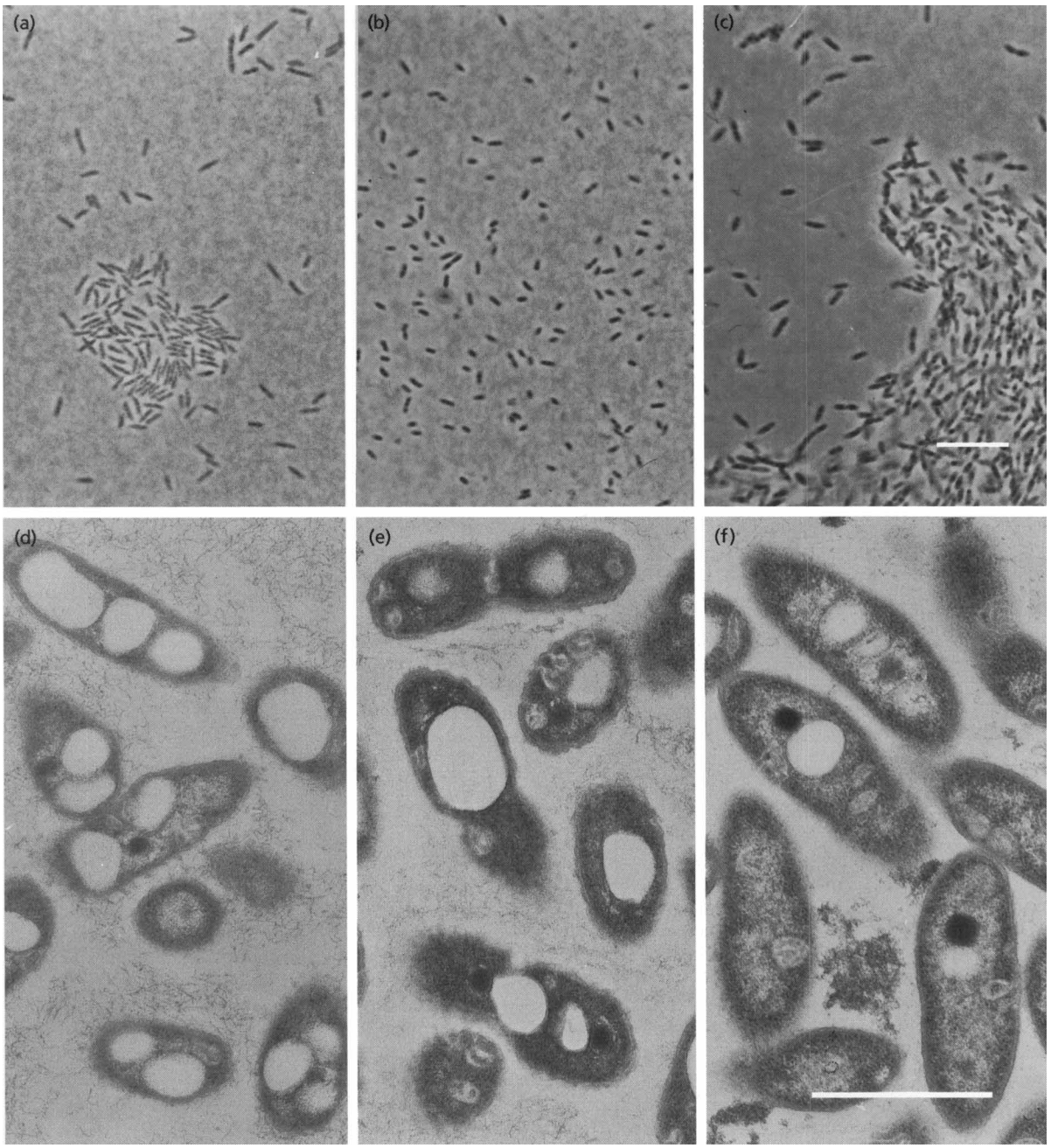

Fig. 1. (a)–(c) Phase-contrast micrographs of (a) *A. citratiphilum* (B4^T), (b) *A. parvum* (B6^T) and (c) *A. commune* (B8^T). Bar, 10 μm. (d)–(f) Transmission electron micrographs of (d) *A. citratiphilum*, (e) *A. parvum* and (f) *A. commune*. Typical cell morphologies of the three species, showing polyphosphate (black) and large polyalkanoate inclusion bodies (white). The cells are embedded in fibrillar matrix material. Bar, 1 μm.

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 B4^T, B6^T and B8^T was determined by HPLC at the DSMZ.

RESULTS

Cell and colony morphology

Individual cells of strains B4^T, B6^T and B8^T 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 B4^T, B6^T and B8^T 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 B4^T and B8^T were 2–4 μm long and about 0·5 μm in diameter and strain B6^T was 1–2 μm long and 0·5 μm in diameter. When streaked onto modified R2A agar plates and incubated for 10 d, strain B4^T formed cream-white, flat colonies with a smooth margin and a diameter of 2–3 mm. Strain B6^T 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 B8^T formed transparent, flat colonies that had a smooth margin and were 1·5–2 mm in diameter. When grown on modified Rouf and Stokes medium, colonies of strains B4^T, B6^T and B8^T were white, indicating that manganese oxidation did not occur.

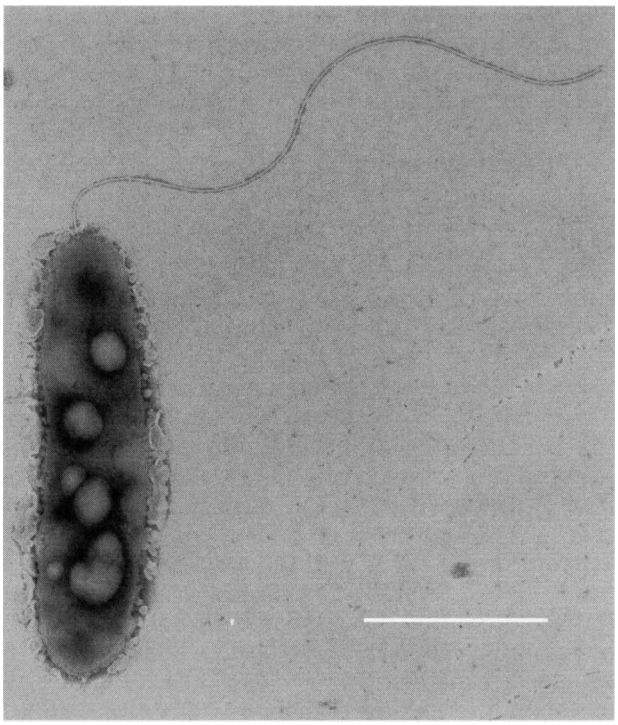

Fig. 2. Transmission electron micrograph of a negatively stained cell of *A. citratiphilum* (B4^T), showing the polar inserted flagellum and polyalkanoate inclusion bodies. Bar, 1 μm.

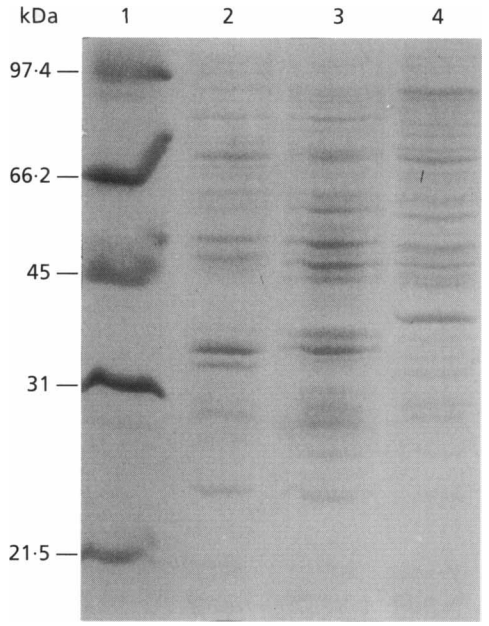

Fig. 3. Cellular protein patterns after SDS-PAGE of whole-cell extracts of *A. citratiphilum* (B4^T) (lane 2), *A. parvum* (B6^T) (lane 3) and *A. commune* (B8^T) (lane 4). Molecular masses of standards (lane 1) are indicated (in kDa) on the left.

Growth conditions

Strain B4^T grew well in liquid as well as on solid R2A medium, whereas strains B6^T and B8^T 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 B4^T was the only strain that grew well on nutrient-rich media. Strain B6^T produced tiny colonies (0·1 mm diameter), whereas strain B8^T 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 B4^T, B6^T and B8^T are summarized in Table 1. Strains B4^T, B6^T and B8^T are aerobic, oxidase-positive and catalase-negative bacteria. When grown in deep-agar cultures, strains B4^T, B6^T and B8^T 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 B6^T and B8^T, nitrate reduction could be

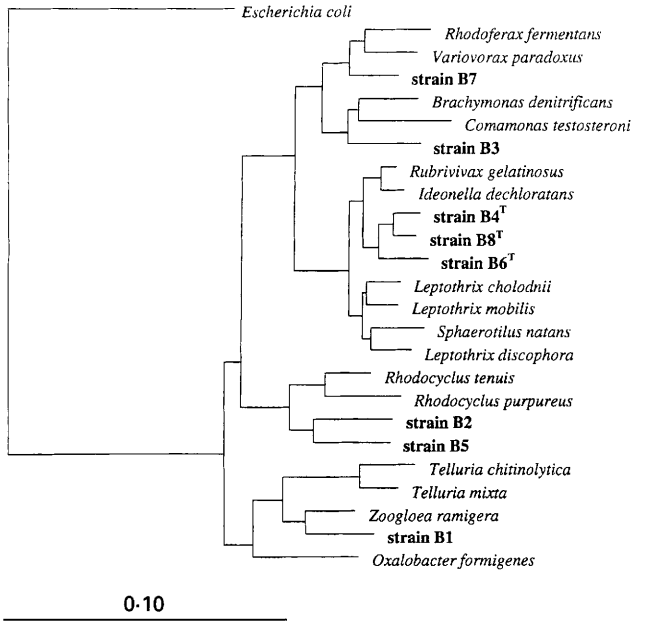

Fig. 4. Phylogenetic tree inferred from 16S rRNA gene sequences (at least 1300 bases), reflecting the relationships of *A. citratiphilum* (B4^T, DSM 11900^T; accession no. AF035050), *A. parvum* (B6^T, DSM 11968^T; AF035052) and *A. commune* (B8^T, DSM 11901^T; AF035054) to their closest known relatives and to the drinking water biofilm isolates B1 (AF035047), B2 (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 N_2 atmosphere. Strain B4^T 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(III) utilized as electron acceptors in deep-agar cultures or liquid medium.

Starch, aesculin, gelatin and DNA were not hydrolysed by strains B4^T, B6^T and B8^T. Strain B8^T 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 B4^T and B6^T but not by strain B8^T. The substrates utilized by strains B4^T, B6^T and B8^T 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 B4^T, B6^T and B8^T after separation by SDS-PAGE and staining with Coomassie blue are shown in Fig. 3. Although strains B4^T, B6^T and B8^T 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 B4^T and B6^T share one common protein of about 35 kDa, which is not present in strain B8^T, 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* (B4^T), *A. parvum* (B6^T) and *A. commune* (B8^T)

Taxon	rRNA sequence identity (%)		
	B4^T	B6^T	B8^T
Aquabacterium citratiphilum (B4^T)	–	–	–
Aquabacterium parvum (B6^T)	96·5	–	–
Aquabacterium commune (B8^T)	98·2	97·2	–
Ideonella dechloratans (CCUG 30898^T)	96·4	96·7	96·6
Leptothrix cholodnii (CCM 1827 = LMG 7171)	96·7	95·3	96·5
Rubrivivax gelatinosus (ATCC 17011^T)	95·7	96·4	95·7
Leptothrix mobilis (LMG 17066^T)	95·5	95·9	96·1
Leptothrix discophora (ATCC 43182^T)	94·4	94·6	94·4
Sphaerotilus natans (accession no. Z18534)	94·9	93·7	94·6

16S rRNA sequence analysis

The almost complete 16S rRNA gene sequences (comprising 1429 nucleotides, *E. coli* positions 29–1458) of strains B4^T, B6^T and B8^T have been determined in a previous study (Kalmbach *et al.*, 1997). The phylogenetic tree shown in Fig. 4 reflects the relationships of strains B4^T, B6^T and B8^T to their closest relatives and to additional bacterial strains (strains B1, B2, B3, B5 and B7) isolated from the same habitat. The three strains B4^T, B6^T and B8^T form a monophyletic group within the β1-subclass of *Proteobacteria*. Levels of 16S rDNA sequence identity range from 98·2% between strains B4^T and B8^T to 97·2% between strains B6^T and B8^T and 96·5% between strains B6^T and B4^T. The closest validly described relatives of strains B4^T, B6^T and B8^T are represented by *Ideonella dechloratans* (CCUG 30898^T) 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^T), *Leptothrix mobilis* (GenBank accession no. X97071), *Leptothrix disco-*

phora (ATCC 43182^T) and *Sphaerotilus natans* (GenBank accession no. Z18534).

DNA–DNA reassociation

DNA–DNA hybridization studies revealed levels of relatedness between the investigated strains ranging from 44·9 % (strains B4^T and B8^T) to 45·4 % for strains B4^T and B6^T and 51·3 % for strains B6^T and B8^T.

DNA base composition

The G+C content of the DNA of strain B4^T was 66 mol %, that of strain B6^T was 65 mol % and that of strain B8^T was 66 mol %.

DISCUSSION

Three bacterial strains, termed B4^T, B6^T and B8^T, 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 B4^T, B6^T and B8^T represent three distinct bacterial species. The DNA base composition of strains B4^T, B6^T and B8^T, 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 B4^T, B6^T and B8^T: 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 B4^T, B6^T and B8^T 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 B4^T, B6^T and B8^T (Table 2), are physiologically quite distinct from this cluster. *Ideonella dechloratans* is an aerobic, motile, chlorate-reducing bacterium isolated from activated sludge (Malmqvist *et al.*, 1994). It clearly differs from strains B4^T, B6^T and B8^T 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 Rouf and Stokes medium, whereas strains B4^T, B6^T and B8^T were unable to oxidize manganese. In contrast to strains B4^T, B6^T and B8^T, *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 B4^T, B6^T and B8^T 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 B8^T 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 B4^T and B6^T, as well as by its greater nutritional versatility compared to that of strain B6^T. With regard to their temperature requirements, all three strains grew at their lower growth limit.

We propose the description of the new genus, *Aquabacterium* gen. nov., containing three species, *Aquabacterium citratiphilum* 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. citratiphilum*, *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-

aerophilic growth behaviour and cannot grow by fermentation. Nitrate serves as an alternative electron acceptor, but not nitrite, chlorate, sulfate or iron(III). Manganese is not oxidized. All three species utilize Tweens 20, 40, 60 and 80, acetate, butyrate, valerate, capronate, caprylate, succinate, adipate, pimelate, azelate, sebacate, fumarate, β-hydroxybutyrate, malate and butanol. None of them utilizes any of the carbohydrates tested, nor is starch, aesculin, gelatin or DNA hydrolysed. The G+C content of the DNA ranges from 65 to 66 mol%. The type species is *Aquabacterium commune*, since it is the most widespread species in drinking water system biofilms.

Description of *Aquabacterium commune* sp. nov.

Aquabacterium commune (com'mu.ne. L. adj. *communis*, *-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 B8^T (= DSM 11901^T).

Description of *Aquabacterium citratiphilum* sp. nov.

Aquabacterium citratiphilum (ci.tra.ti'phi.lum. L. n. *citrus* lemon tree; L. n. *acidum* acid; L. n. adj. *acidum 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 10–36 °C. Growth occurs between pH 5·5 and 10·0 and in the presence of NaCl up to 1·8%. In addition to the substrates used by all members of the genus, *A. citratiphilum* utilizes propionate, bromosuccinate, pyruvate, lactate, citrate, γ-hydroxybutyrate and glycerol. *A. citratiphilum* is able to hydrolyse urea. The G+C content of the DNA is 66 mol%. Isolated from a drinking water house installation system. The type strain is strain B4^T (=DSM 11900^T).

Description of *Aquabacterium parvum* sp. nov.

Aquabacterium parvum (par'vum. L. adj. *parvus* small). 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 B6^T (=DSM 11968^T).

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