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Aquabacterium limnoticum sp. nov., isolated from a freshwater spring

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A Gram-negative, facultatively anaerobic, short-rod-shaped, non-motile and non-spore-forming bacterial strain, designated ABP-4^T, was isolated from a freshwater spring in Taiwan and was characterized using the polyphasic taxonomy approach. Growth occurred at 20–40 °C (optimum, 30–37 °C), at pH 7.0–10.0 (optimum, pH 7.0–9.0) and with 0–3 % NaCl (optimum, 0 %). Phylogenetic analyses based on 16S rRNA gene sequences showed that strain ABP-4^T, together with *Aquabacterium fontiphilum* CS-6^T (96.4 % sequence similarity), *Aquabacterium commune* B8^T (96.1 %), *Aquabacterium citratiphilum* B4^T (95.5 %) and *Aquabacterium parvum* B6^T (94.7 %), formed a deep line within the order *Burkholderiales*. Strain ABP-4^T contained summed feature 3 (comprising $C_{16:1}$ $\omega7c$ and/or $C_{16:1}$ $\omega6c$), $C_{18:1}$ $\omega7c$ and $C_{16:0}$ as predominant fatty acids. The major cellular hydroxy fatty acid was $C_{10:0}$ 3-OH. The major isoprenoid quinone was Q-8 and the DNA G+C content was 68.6 mol%. The polar lipid profile consisted of a mixture of phosphatidylethanolamine, phosphatidylglycerol, phosphatidylserine, diphosphatidylglycerol and several uncharacterized phospholipids. The DNA–DNA relatedness of strain ABP-4^T with respect to recognized species of the genus *Aquabacterium* was less than 70 %. On the basis of the genotypic, chemotaxonomic and phenotypic data, strain ABP-4^T represents a novel species in the genus *Aquabacterium,* for which the name *Aquabacterium limnoticum* sp. nov. is proposed. The type strain is ABP-4^T (=BCRC 80167^T=KCTC 23306^T).

The genus *Aquabacterium*, belonging to the *Sphaerotilus-Leptothrix* group within the order *Burkholderiales* of the class *Betaproteobacteria*, was proposed by Kalmbach *et al.* (1999). At the time of writing, this genus comprises four recognized species: *Aquabacterium citratiphilum*, *Aquabacterium parvum* and *Aquabacterium commune*, isolated from the Berlin drinking water system (Kalmbach *et al.*, 1999), and *Aquabacterium fontiphilum*, isolated from Taiwan spring water (Lin *et al.*, 2009). Members of the genus *Aquabacterium* are characterized as Gram-negative, facultatively anaerobic, rod-shaped, motile by means of monotrichous flagella, oxidase-positive and catalase-negative, and chemotaxonomically highly diverse having summed feature 3, C18 : 1 $\omega7c$ and C16 : 0 as predominant fatty acids and by having a DNA G+C content between 63.4 and 66.0 mol% (Kalmbach *et al.*, 1999; Lin *et al.*, 2009). The present study was carried out to clarify the taxonomic position of the *Aquabacterium*-like bacterial strain ABP-4^T by using a polyphasic taxonomy approach.

During the characterization of micro-organisms from a freshwater spring (GPS location: 24° 7′ 15.1″ N 120° 40′ 27.4″ E; pH 7.8, 28 °C) in Taichung County, Taiwan, a semi-transparent colony was isolated and selected for detailed analysis; this bacterial strain was designated ABP-4^T. Strain ABP-4^T was isolated on R2A agar (BD Difco) plates after incubating at 25 °C for 3 days. Subcultivation was performed on R2A agar at 25 °C for 48–72 h. On this medium, strain ABP-4^T was able to grow at 25–40 °C. It was preserved at

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of *Aquabacterium limnoticum* strain ABP-4^T is GU319965.

A supplementary figure, showing 2D TLC of polar lipids of strain ABP-4^T, and a supplementary table, showing GN2 microplate oxidation data, are available with the online version of this paper.

−80 °C in R2A broth with 20 % (v/v) glycerol or by lyophilization. *A. citratiphilum* B4^T, *A. parvum* B6^T and *A. commune* B8^T were obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ), and *A. fontiphilum* CS-6^T was obtained from the Bioresource Collection and Research Center (BCRC). All four type strains were used as reference strains for phenotypic and genotypic tests.

The bacterial cells were observed by phase-contrast microscopy (DM 2000; Leica) using cells grown on R2A agar at 30 °C for 6 h (lag phase), 18 h (exponential phase) and 36 h (stationary phase) to ascertain the morphology. Cellular motility was tested by using the hanging drop method. The Gram Stain Set S (BD Difco) kit and the Ryu non-staining KOH method (Powers, 1995) were used for testing the Gram reaction. Poly-β-hydroxybutyrate granule accumulation was observed under light microscopy after staining of the cells with Sudan black. Colony morphology was observed on R2A agar using a stereoscopic microscope (SMZ 800; Nikon).

The pH range for growth was determined by measuring the OD_{600} of R2A broth cultures. The pH was adjusted to pH 4–11 (at intervals of 1.0 pH unit) prior to sterilization using appropriate biological buffers (Breznak & Costilow, 1994): citrate/Na_2HPO_4 buffer, pH range 4.0–5.0; phosphate buffer, pH range 6.0–7.0; Tris buffer, pH range 8.0–9.0; no buffer, pH range 10.0–11.0. Verification of the pH values after autoclaving revealed only minor changes. The temperature range for growth was determined on R2A broth by culturing at 4–50 °C. To investigate the tolerance to NaCl, R2A broth was prepared according to the formula of the BD Difco medium with NaCl concentration adjusted to 0, 0.5 and 1.0–10.0 %, w/v (at intervals of 1.0 %). Growth under anaerobic conditions was determined after incubating strain ABP-4^T on R2A agar in the Oxoid AnaeroGen system. The potential utilization of various electron acceptors [KNO_3, KNO_2, $NaClO_3$, Na_2SO_4 or iron (III) citrate] was studied as described by Kalmbach *et al.* (1999). Strain ABP-4^T was examined for a broad range of phenotypic properties. Activities of catalase, oxidase, urease and lipase (corn oil), and hydrolysis of starch, casein, gelatin and Tweens 20, 40, 60 and 80 were determined using standard methods (Smibert & Krieg, 1994). Catalase production was demonstrated on a slide by the production of bubbles from a drop of a 3 % hydrogen peroxide solution. Heavy inoculation of standard Christensen urea agar medium was used to demonstrate urease production. The gelatin liquefaction test was performed by using R2A broth supplemented with 4 % gelatin and incubated at the optimum growth temperature. Additional biochemical tests were performed using API ZYM, and API 20NE kits (bioMérieux) and carbon source utilization was evaluated using the GN2 microplate (Biolog). All commercial phenotypic tests were performed according to the manufacturers' recommendations.

The sensitivity of strain ABP-4^T to antibiotics was tested by using the disc diffusion method after spreading cell suspensions (0.5 McFarland) on R2A agar (BD Difco) plates. The discs (Oxoid) contained the following antibiotics: ampicillin (10 μg), chloramphenicol (30 μg), gentamicin (10 μg), kanamycin (30 μg), nalidixic acid (30 μg), novobiocin (30 μg), rifampicin (5 μg), penicillin G (10 μg), streptomycin (10 μg), sulfamethoxazole (23.75 μg) plus trimethoprim (1.25 μg) and tetracycline (30 μg). The effect of antibiotics on cell growth was assessed after 2 days growth at 30 °C. The diameter of the antibiotic disc is 8 mm. The strain was considered susceptible when the diameter of the inhibition zone was >13 mm, intermediate at 10–12 mm and resistant at <10 mm, as described by Nokhal & Schlegel (1983).

Genomic DNA was isolated by using a bacterial genomic kit and the 16S rRNA gene sequence was analysed as described by Chen *et al.* (2001). Primers FD1 (5′-AGAGTTTGATCCTGGCTCAG-3′) and RD1 (5′-AAGGAGGTGATCCAGCC-3′) were used for amplification of bacterial 16S rRNA genes by PCR. These primers correspond to nucleotide positions 8–27 and 1525–1541 of the *Escherichia coli* 16S rRNA gene, respectively, and can be used to amplify a nearly full-length 16S rRNA gene. The PCR product was purified, and direct sequencing was performed by using sequencing primers FD1/RD1 and 520F/800R (Anzai *et al.*, 1997) with a DNA sequencer (ABI Prism 3730; Applied Biosystems). An almost-complete 16S rRNA gene sequence (1443 nt) of strain ABP-4^T was compared against 16S rRNA gene sequences available from the EzTaxon server (Chun *et al.*, 2007), the Ribosomal Database Project (Maidak *et al.*, 2001) and GenBank database (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Analysis of the sequence data was performed by using the software package BioEdit (Hall, 1999) and MEGA, version 3.1 (Kumar *et al.*, 2004), after multiple alignments of the data by CLUSTAL_X (Thompson *et al.*, 1997). Distances (corrected according to Kimura's two-parameter model; Kimura, 1983) were calculated and clustering was performed with the neighbour-joining method (Saitou & Nei, 1987). The maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Kluge & Farris, 1969) trees were generated by using the treeing algorithms contained in the PHYLIP software package (Felsenstein, 1993). In each case, bootstrap values were calculated based on 1000 replications.

Phylogenetic analyses based on 16S rRNA gene sequence revealed that strain ABP-4^T was closely related to the species of the genera *Roseateles* (95.9–96.5 % sequence similarity), *Aquabacterium* (94.7–96.4 %), *Kinneretia* (96.2 %), *Aquincola* (96.1 %) and *Pelomonas* (95.0–96.0 %). Strain ABP-4^T formed a deep phyletic cluster with *A. fontiphilum* CS-6^T, *A. commune* B8^T, *A. citratiphilum* B4^T and *A. parvum* B6^T within the order *Burkholderiales* in the neighbour-joining tree (Fig. 1). The overall topologies of the phylogenetic trees obtained with the maximum-likelihood and maximum-parsimony methods were similar. Sequence similarity calculations (over 1400 bp) indicated that strain ABP-4^T was closely related to *A. fontiphilum* CS-6^T (96.4 % 16S rRNA gene sequence similarity), *A. commune* B8^T (96.1 %), *A. citratiphilum* B4^T (95.5 %) and *A. parvum* B6^T (94.7 %). In addition, 16S rRNA gene sequence similarity calculations indicated that

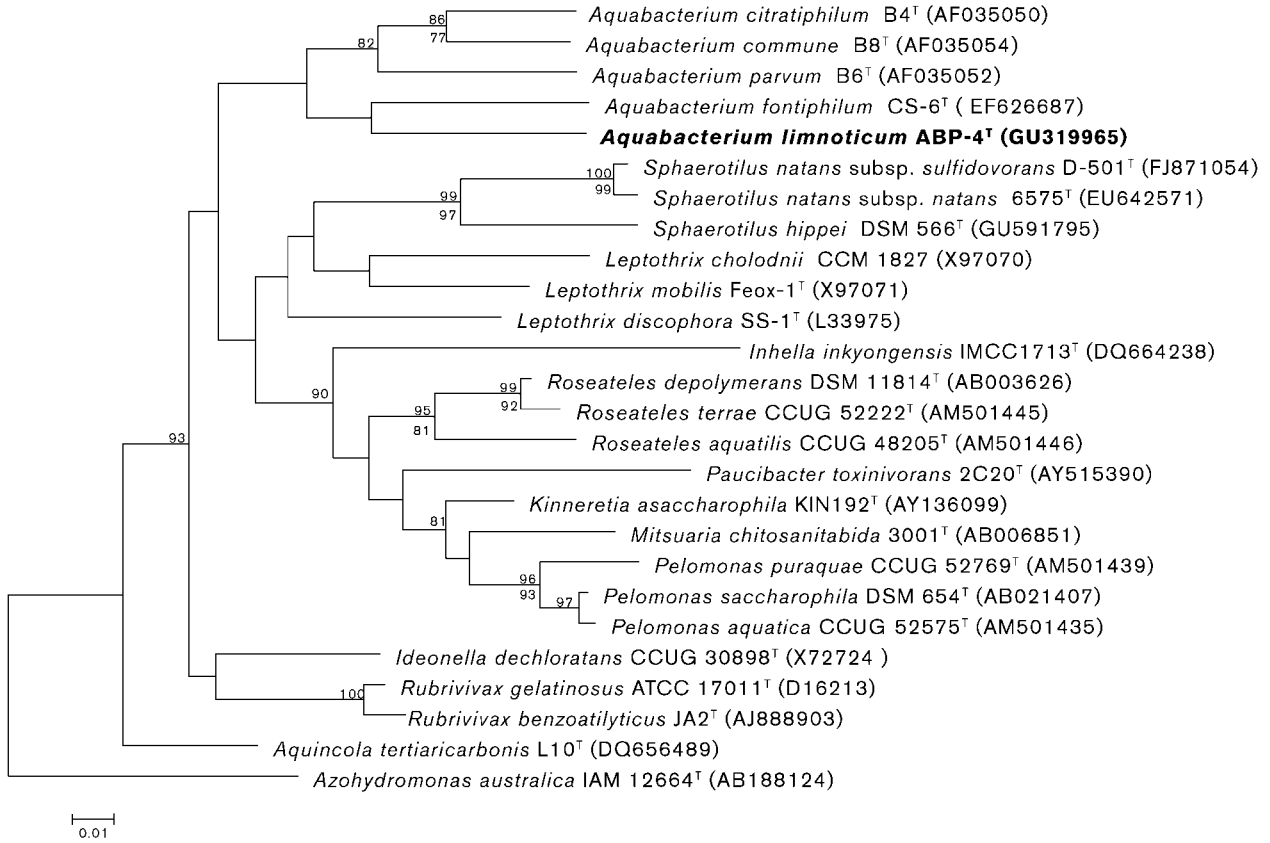

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the position of *A. limnoticum* ABP-4^T and related taxa in the order *Burkholderiales* of the class *Betaproteobacteria*. Numbers at nodes are bootstrap percentages >70 % based on the neighbour-joining (above nodes) and maximum-parsimony (below nodes) tree-making algorithms. *Azohydromonas australica* IAM 12664^T was used as an out-group. Bar, 0.01 substitutions per nucleotide position.

strain ABP-4^T is closely related to *Roseateles terrae* CCUG 52222^T and *Roseateles aquatilis* CCUG 48205^T (96.5 % 16S rRNA gene sequence similarity), *Kinneretia asaccharophila* KIN192^T (96.2 % 16S rRNA gene sequence similarity) and *Aquincola tertiaricarbonis* L10^T (96.1 % 16S rRNA gene sequence similarity). However, these four related strains exhibit distinctly deeper branches in the phylogenetic tree (Fig. 1).

DNA–DNA hybridization experiments were carried out by using the method of Ezaki *et al.* (1989). The level of DNA–DNA relatedness of strain ABP-4^T with *A. fontiphilum* CS-6^T, *A. commune* B8^T, *A. citratiphilum* B4^T and *A. parvum* B6^T was 31.0±1.2 %, 15.8±1.3 %, 16.5±1.3 % and 36.8±1.2 %, respectively. Since the recommended DNA–DNA relatedness threshold for the definition of a species is 70 % (Wayne *et al.*, 1987), these results indicate that strain ABP-4^T does not belong to any known species of the genus *Aquabacterium*.

The fatty acid profiles of strain ABP-4^T, *A. fontiphilum* CS-6^T, *A. commune* B8^T, *A. citratiphilum* B4^T and *A. parvum* B6^T were determined using cells grown on R2A agar at 30 °C for 2 days. The fatty acid methyl esters were prepared, separated and identified by using the Instant FAME method of the Microbial Identification System (MIDI) version 6.0B and the RTSBA6 6.00 database. All five strains had straight-chain fatty acids, unsaturated fatty acids and hydroxyl fatty acids. The fatty acid profile of strain ABP-4^T was similar to those of the other four *Aquabacterium* species, although there were differences in the proportions of some components (Table 1). The major fatty acids (>10.0 %) of strain ABP-4^T were summed feature 3 (comprising $C_{16:1}$ $\omega7c$ and/or $C_{16:1}$ $\omega6c$; 33.8 %), $C_{18:1}$ $\omega7c$ (19.2 %) and $C_{16:0}$ (18.3 %). The major cellular hydroxy fatty acid in strain ABP-4^T was $C_{10:0}$ 3-OH (2.4 %). Isoprenoid quinones were extracted and purified according to the method of Collins (1985) and were analysed by HPLC. The respiratory quinones of strain ABP-4^T were Q-8 (81.3 %) and Q-7 (18.7 %). The respiratory quinones of *A. commune* B8^T were Q-8 (76.2 %) and Q-7 (23.8 %). The DNA G+C content of strain ABP-4^T, determined by HPLC according to Mesbah *et al.* (1989), was 68.6±1.0 mol%.

Polar lipids were extracted and analysed by 2D TLC according to Embley & Wait (1994). Strain ABP-4^T exhibited

Table 1. Cellular fatty acid composition of *Aquabacterium* species

All data are from this study. All strains were grown on R2A agar at 30 °C for 2 days. Values are percentages of the total fatty acids; fatty acids that make up <1 % of the total are not shown or indicated by −. For unsaturated fatty acids, the position of the double bond is located by counting from the methyl (ω) end of the carbon chain. *cis* isomer is indicated by the suffix *c*.

Fatty acid	*A. limnoticum* ABP-4^T	*A. fontiphilum* CS-6^T	*A. commune* B8^T	*A. citratiphilum* B4^T	*A. parvum* B6^T
$C_{10:0}$	−	1.0	−	2.3	2.0
$C_{12:0}$	4.9	7.8	5.6	3.4	3.3
$C_{14:0}$	2.9	−	1.9	3.9	4.1
$C_{16:0}$	18.3	20.6	31.0	19.0	21.3
$C_{18:0}$	7.5	1.7	1.6	2.0	7.8
$C_{10:0}$ 3-OH	2.4	6.6	5.8	7.8	6.8
$C_{12:0}$ 2-OH	−	−	1.5	2.7	2.4
$C_{18:1}$ ω7*c*	19.2	38.6	8.8	20.1	18.2
$C_{18:1}$ ω9*c*	−	1.8	−	1.2	1.4
Summed feature 3*	33.8	18.5	42.4	35.0	30.4

*Summed features are groups of two or three fatty acids that cannot be separated by GLC using the MIDI system. Summed feature 3 comprises $C_{16:1}$ ω7*c* and/or $C_{16:1}$ ω6*c*.

a complex polar lipid profile consisting of phosphatidylethanolamine (PE), phosphatidylglycerol (PG), phosphatidylserine (PS), diphosphatidylglycerol (DPG) and four uncharacterized phospholipids (PL1–PL4) (see Supplementary Fig. S1, available in IJSEM Online). Like its closest relatives *A. fontiphilum* CS-6^T, *A. commune* B8^T, *A. citratiphilum* B4^T and *A. parvum* B6^T, strain ABP-4^T exhibited a very similar polar lipid profile, and they all had PE, PG, PS, DPG and PL3. However, PL1 was absent in *A. parvum* B6^T, but present in strain ABP-4^T, *A. fontiphilum* CS-6^T, *A. commune* B8^T and *A. citratiphilum* B4^T. PL2 was detected in strain ABP-4^T, *A. commune* B8^T and *A. parvum* B6^T but not detected in *A. fontiphilum* CS-6^T and *A. citratiphilum* B4^T. PL4 was present in strain ABP-4^T, *A. citratiphilum* B4^T and *A. parvum* B6^T but absent in *A. fontiphilum* CS-6^T and *A. commune* B8^T. In addition, PL5 was detected in *A. commune* B8^T and *A. parvum* B6^T. These results suggested that there are some differences in the polar lipid profiles among them, although they belong to the same genus and have very similar profiles.

The physiological, biochemical and morphological characteristics of strain ABP-4^T are given in the species description, Table 2 and Supplementary Table S1 (available in IJSEM Online). Phenotypic examination revealed many common traits between the novel strain and its closest relatives, *A. fontiphilum* CS-6^T, *A. commune* B8^T, *A. citratiphilum* B4^T and *A. parvum* B6^T. However, strain ABP-4^T could be clearly differentiated from these four species by the absence of motility, by the utilization of chlorate as electron acceptor but not nitrate, by the inability to hydrolyse aesculin, by the absence of C8 esterase lipase activity and by the presence of α-glucosidase activity (Table 2), and by its ability to utilize glycogen, p-hydroxyphenylacetic acid, α-keto butyric acid, α-keto glutaric acid and L-asparagine as sole carbon sources (Supplementary Table S1).

Strain ABP-4^T is Gram-negative, rod-shaped, facultatively anaerobic and oxidase-positive. Growth is chemo-heterotrophic and occurs under mesophilic and neutrophilic conditions. Predominant fatty acids contain summed feature 3 (comprising $C_{16:1}$ ω7*c* and/or $C_{16:1}$ ω6*c*), $C_{18:1}$ ω7*c* and $C_{16:0}$. The DNA G+C content is 68.6 mol%. These characteristics of strain ABP-4^T are consistent with the description of the genus *Aquabacterium* (Kalmbach *et al.*, 1999; Lin *et al.*, 2009).

In addition, the data obtained from 16S rRNA gene sequence comparisons showed that the novel strain has a close phylogenetic relationship (>96.0 % sequence similarity) to the genera *Roseateles*, *Kinneretia* and *Aquincola*. However, the novel strain could be clearly differentiated from *Roseateles* species (Suyama *et al.*, 1999; Gomila *et al.*, 2008) by the absence of bacteriochlorophyll *a* production, by the utilization of nitrate or chlorate as an alternative electron acceptor, by the facultatively anaerobic growth, and by the high levels of $C_{12:0}$ and the absence of $C_{15:0}$. The properties such as the presence of catalase activity, and the absence of $C_{8:0}$ 3-OH and the presence of $C_{10:0}$ 3-OH, allow good discrimination of the novel strain from the species of the genus *Kinneretia* (Gomila *et al.*, 2010). Some features of the novel strain, such as the ability to utilize chlorate as an alternative electron acceptor, the high levels of $C_{18:1}$ ω7*c* and the absence of $C_{15:1}$, $C_{17:0}$ and $C_{17:0}$ cyclo, help to separate the novel strain from *Aquincola* species (Lechner *et al.*, 2007). These results suggest that strain ABP-4^T belongs to a genus which is evenly distant from the genera *Roseateles*, *Kinneretia* and *Aquincola*.

On the basis of the data obtained from 16S rRNA gene sequence comparisons, strain ABP-4^T occupies a distinct position within the genus *Aquabacterium*. The phylogenetic insight is supported by the unique combination of chemotaxonomic and biochemical characteristics of the novel strain.

Table 2. Differential characteristics of *Aquabacterium* species

All data are from this study except the G+C content of *A. fontiphilum* CS-6^T (Lin *et al.*, 2009) and *A. commune* B8^T, *A. citratiphilum* B4^T and *A. parvum* B6^T (Kalmbach *et al.*, 1999). +, Positive reaction; −, negative reaction. All strains are Gram-negative, mesophilic, neutrophilic, non-spore-forming, and positive for oxidase, catalase and leucine arylamidase activities. All strains are negative for DNase, trypsin, α-galactosidase, β-galactosidase, β-glucuronidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase activities, hydrolysis of starch, indole production and glucose acidification.

Characteristic	*A. limnoticum* ABP-4^T	*A. fontiphilum* CS-6^T	*A. commune* B8^T	*A. citratiphilum* B4^T	*A. parvum* B6^T
Motility	−	+	+	+	+
Reduction of:					
NO_3^-	−	+	+	+	+
ClO_3^-	+	−	−	−	−
Hydrolysis of:					
Urea	−	+	−	+	+
Casein	−	−	+	−	−
Gelatin	−	+	−	+	−
Aesculin	−	+	+	+	+
Enzymic activities:					
Arginine dihydrolase	−	−	+	+	−
Alkaline phosphatase	+	+	+	+	−
C8 esterase lipase	−	+	+	+	+
C14 lipase	−	+	−	+	−
Valine arylamidase	−	+	−	−	−
Cystine arylamidase	−	+	+	−	−
α-Chymotrypsin	−	−	−	+	−
Acid phosphatase	−	−	−	+	−
Naphthol-AS-BI-phosphohydrolase	+	+	−	−	+
α-Glucosidase	+	−	−	−	−
Assimilation of:					
Glucose	−	+	−	+	−
Arabinose	−	+	−	+	−
Mannose	−	+	−	−	−
Mannitol	−	+	−	+	−
N-Acetylglucosamine	−	+	−	+	−
Maltose	−	+	−	+	−
Gluconate	−	+	−	+	−
Caprate	−	−	−	+	−
Adipate	+	+	−	−	−
Malate	+	+	−	+	+
Citrate	−	+	−	−	−
Phenyl-acetate	−	+	−	−	−
DNA G+C content (mol%)	68.6	63.4	66	66	65

It is clear from the phylogenetic and phenotypic data that the strain ABP-4^T constitutes a novel member of the genus *Aquabacterium*. However, strain ABP-4^T could be differentiated from *A. fontiphilum* CS-6^T, *A. commune* B8^T, *A. citratiphilum* B4^T and *A. parvum* B6^T by a combination of physiological and biochemical characteristics (Table 2 and Supplementary Table S1). Hence, strain ABP-4^T constitutes a novel member of the genus *Aquabacterium*. The name *Aquabacterium limnoticum* sp. nov. is proposed for this taxon.

In this study, the two characteristics of absence of motility and the utilization of chlorate as electron acceptor were not observed for *Aquabacterium* species as they were in previous publications (Kalmbach *et al.*, 1999; Lin *et al.*, 2009). Differences were also noted in the catalase production of *Aquabacterium* species from the earlier reported data (Kalmbach *et al.*, 1999). Additionally, the chemotaxonomic characteristics of *Aquabacterium* species such as the major fatty acids containing summed feature 3 (comprising $C_{16:1}$ ω7*c* and/or $C_{16:1}$ ω6*c*), $C_{18:1}$ ω7*c* and $C_{16:0}$, and the presence of hydroxy fatty acid C10 : 0 3-OH may be helpful for distinguishing *Aquabacterium* from the other genera in the class *Betaproteobacteria*.

Emended description of the genus *Aquabacterium* Kalmbach *et al.* 1999

The formal description given by Kalmbach *et al.* (1999) remains correct except that some species are non-motile, catalase-positive, and use nitrate or chlorate as an alternative

electron acceptor. The major cellular fatty acids are summed feature 3 (comprising $C_{16:1}$ $\omega7c$ and/or $C_{16:1}$ $\omega6c$), $C_{18:1}$ $\omega7c$ and $C_{16:0}$, and the major hydroxy fatty acid is $C_{10:0}$ 3-OH except that some species also contain $C_{12:0}$ 2-OH.

Description of *Aquabacterium limnoticum* sp. nov.

Aquabacterium limnoticum (lim.no′ti.cum. Gr. n. *limnê*, pool of standing water, lake; L. neut. suff. *-ticum*, suff. denoting made of or belonging to; N. L. neut. adj. *limnoticum*, from or belonging to a lake).

Cells are Gram-negative, facultatively anaerobic, non-spore-forming, non-motile, short-rod-shaped and chemoheterotrophic. Poly-β-hydroxybutyrate accumulation is not observed. After 48 h of incubation on R2A agar at 30 °C, the mean cell size is approximately 0.8–1.0 μm in diameter and 1.3–2.0 μm in length. Colonies are semi-transparent, convex, round and smooth with entire edges. The colony size is approximately 1.0–1.7 mm in diameter on R2A agar after 72 h incubation at 25 °C. Growth occurs at 20–40 °C (optimum, 30–37 °C), at pH 7.0–10.0 (optimum, pH 7.0–9.0) and with 0–3 % NaCl (optimum, 0 %). Positive for oxidase activity and weakly positive for catalase activity. Positive for hydrolysis of corn oil. Negative for hydrolysis of urea, starch, gelatin, casein, Tweens 20, 40, 60 and 80. Chlorate serves as an alternative electron acceptor, but nitrate, nitrite, sulfate or iron (III) do not. In API 20NE tests, positive reactions for assimilation of adipate and malate, and negative reactions for nitrate reduction, indole production, glucose acidification, aesculin and gelatin hydrolysis, arginine dihydrolase, urease and β-galactosidase activities and assimilation of glucose, arabinose, mannose, mannitol, *N*-acetylglucosamine, maltose, gluconate, caprate, citrate and phenylacetate. In the API ZYM kit, alkaline phosphatase, leucine arylamidase, naphthol-AS-BI-phosphohydrolase and α-glucosidase activities are present and C4 esterase, C8 esterase lipase, C14 lipase, valine arylamidase, cysteine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, α-galactosidase, β-galactosidase, β-glucuronidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase activities are absent. The following compounds are utilized as sole carbon sources in the GN2 microplate: glycogen, Tween 80, pyruvic acid methyl ester, β-hydroxybutyric acid, p-hydroxyphenylacetic acid, α-keto butyric acid, α-keto glutaric acid, DL-lactic acid, succinic acid, bromosuccinic acid, succinamic acid, L-alanine, L-asparagine and L-glutamic acid. All other substrates in the GN2 microplate are not utilized. Resistant to rifampicin and sensitive to penicillin G, ampicillin, chloramphenicol, gentamicin, kanamycin, tetracycline, novobiocin, streptomycin, sulfamethoxazole plus trimethoprim and nalidixic acid. The major fatty acids are summed feature 3 (comprising $C_{16:1}$ $\omega7c$ and/or $C_{16:1}$ $\omega6c$), $C_{18:1}$ $\omega7c$ and $C_{16:0}$. The major hydroxy fatty acid is C10 : 0 3-OH. The predominant polar lipids were phosphatidylethanolamine, phosphatidylglycerol, phosphatidylserine, diphosphatidylglycerol and four uncharacterized phospholipids. The DNA G+C content is 68.6 mol%. The major respiratory quinone is Q-8.

The type strain is ABP-4^T (=BCRC 80167^T=KCTC 23306^T) isolated from a freshwater spring, Taichung County, Taiwan.

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