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AQUABACTERIUM SILICAE SP. NOV., ISOLATED FROM A DEEP AQUIFER

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AQUABACTERIUM SILICAE
DEEP AQUIFER
BURKHOLDERIALES

ABSTRACT. – A novel mesophilic chemoheterotrophic bacterial strain designated LMB275^T, that grows under aerobic/microaerophilic conditions, was isolated from the thermal spring water of a deep aquifer in France. Cells were oxidase-positive and catalase-negative, with a polar flagellum. Phylogenetic analyses based on 16S rRNA gene sequences suggested that strain LMB275^T belongs to the genus *Aquabacterium*, and most closely related to *Aquabacterium parvum* B6^T (99.1 %), *Aquabacterium commune* B8^T (97.9 %) and *Aquabacterium citratiphilum* B4^T (97.0 %). The DNA-DNA relatedness between strain LMB275^T and *A. parvum* was below 70.0 %. The DNA G+C content was 66 mol %. The fatty acid composition differed from all other species of the genus with the dominance of $C_{18:1}$ ω9c. The polar lipids consisted of phosphatidylethanolamine as the major component, phosphatidylglycerol, diphosphatidylglycerol and several uncharacterized phospholipids. The strain was not able to assimilate all the carbohydrates tested and used different inorganic electron acceptors. Based on the physiological, chemotaxonomic and phylogenetic analyses, and the low level of DNA relatedness with the closest phylogenetic relative *A. parvum*, it can be concluded that the strain LMB275^T represents a new species of the genus *Aquabacterium*, for which the name *Aquabacterium silicae* sp. nov. is proposed. The type strain of *A. silicae* is LMB275^T (CNCM I-5858).

INTRODUCTION

The deep continental groundwaters harbour characteristic natural microbial communities with specific phylogenetic compositions and special physiological capabilities, and a large part corresponds to undescribed microorganisms that still await isolation (Konno *et al*. 2013, Ino *et al*. 2016, Lopez-Fernandez *et al*. 2018, Savio *et al*. 2018, Bourrain *et al*. 2020). In these oligotrophic ecosystems where they have to cope with the absence of light and easily available organic carbon, they display metabolic lifestyles organized around inorganic resources and small concentrations of refractory dissolved organic matter that surround them (Hoehler & Jørgensen 2013, Lesaulnier *et al*. 2017). Chemolithotrophic (autotrophic and heterotrophic) metabolisms using inorganic compounds as electron sources, inorganic carbon fixation as well as dissolved organic matter for carbon assimilation, are thus particularly relevant to develop or subsist in these habitats and were reported in numerous studies (Griebler & Lueders 2009, Fukuda *et al*. 2010, Wu *et al*. 2016, Kumar *et al*. 2018a). How chemolithoautotrophic and heterotrophic organisms live together and interplay with their physicochemical environment are still pending issues, and their isolation and the investigation of their physiological and metabolic capacities would be very helpful to answer these questions. This report describes the characterization of a novel bacterium affiliated to the genus *Aquabacterium*, LMB275^T (= CNCM I-5858), isolated from thermal spring water that has long been investigated for the singularity and the remarkable stability its microbial composition (Bourrain *et al*. 2012, 2020).

The genus *Aquabacterium*, within the order Burkholderiales in the Betaproteobacteria class, was proposed by Kalmbach *et al*. (1999) with the description of three species, *A. parvum*, *A. citratiphilum*, and *A. commune* as the type species. The description of the genus has been emended by Chen *et al*. (2012), and more recently by Hirose *et al*. (2020). It now contains ten species with the further description of *A. fontiphilum* (Lin *et al*. 2009), *A. limnoticum* (Chen *et al*. 2012), *A. olei* (Pham *et al*. 2015), *A. tepidiphilum* (Khan *et al*. 2019), *A. pictum* (Hirose *et al*. 2020), *A. lacunae* (Chen *et al*. 2020), and lastly, *A. terrae*, the most recently described species but not validated at the time of writing (Dahal *et al*. 2021). Most of the type strains were isolated from freshwater ecosystems, except for *A. olei* and *A. terrae,* which originate from soils. Members of the genus *Aquabacterium* are characterized as Gram-negative, oxidase-positive, and non-spore-forming rods, most of them are motile using a monotrichous flagellum, and the presence of polyalkanoate and polyphosphate inclusion bodies were reported for *A. parvum*, *A. commune*, *A. citratiphilum* and *A. tepidiphilum*. *Aquabacterium pictum* is the only bacteriochlorophyll *a*-containing *Aquabacterium* species to date and

could be classified among the aerobic anoxygenic phototrophic bacteria (Hirose *et al*. 2020).

MATERIALS AND METHODS

Bacterial strains of the study: Strain LMB275^T was isolated from thermal spring water collected at the catchment point of a deep aquifer (180 meters deep) located in the north of the Hérault department in France at the eastern end of the "Montagne Noire" mountain. The water temperature at the catchment point is constant at 21° C and the chemical composition is controlled by the solubility of minerals (dolomite and quartz), which gives it weak mineralization (conductivity 475 µS cm^{-1}, pH 7.5), dominated by bicarbonate (271.5 mg l^{-1}), Ca^{2+} (51.3 mg l^{-1}) and Mg^{2+} (26.7 mg l^{-1}) (Lions 2020). The water sample was taken in July 2007 and was immediately filtered through a 0.22 µm pore size polycarbonate filter. The filter was laid on a R3A agar plate (Reasoner & Geldreich 1985) and incubated for 10 days at 25° C. A bacterial strain, named LMB275^T was isolated and purified from a colony that had developed and was stored at –80° C in R3A broth with 35 % (v/v) glycerol or 5 % DMSO. For detailed taxonomic description, the strain LMB275^T was sub-cultured in a modified R2A medium where starch was replaced by 0.1 % (v/v) Tween 80 (Sigma) to improve the growth as recommended by Kalmbach *et al*. (1999).

For biochemical capacities, growth, or genomic studies, the reference strains *Aquabacterium parvum* B6^T (DSM 11968), *Aquabacterium commune* B8^T (DSM 11901), and *Aquabacterium citratiphilum* B4^T (DSM 11900) were evaluated together with strain LMB275^T. These strains were obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ, Leibniz Institut, Germany).

Transmission electron microscopy: Transmission electron micrographs of negatively stained cells (Raguénès *et al*. 1997) were performed from exponential-phase cultures in modified R2A broth to determine the morphological characteristics of strain LMB275^T.

To investigate the capability to form polyalkanoate inclusion bodies, thin sections of cells from exponential phase cultures in R2A broth were prepared as described by Derelle *et al*. (2008) with some modifications: fixation was done for 16 h at 4° C with 2.5 % glutaraldehyde prepared in 0.1 M cacodylate buffer and post-fixation was carried out with 1 % OsO_4 in 0.1 M cacodylate. Thin sections were stained with uranyl acetate and lead citrate before examination on a 7500 Hitachi transmission electron microscope.

DNA analysis: Genomic DNA was extracted with the Maxwell® 16 MDx Instrument (AS3000, Promega) from a 2 ml liquid culture with the Maxwell® 16 LEV Blood DNA kit (Promega, Charbonnières-les-Bains, France) including an initial lysis step as described by Fagervold *et al*. (2020). PCR targeting the 16S rRNA gene was performed with universal bacterial primers 27Fmod (5'AGRGTTTGATCMTGGCTCAG-3') (Eiler & Bertilsson 2004) and 1492Rmod (5'-TACGGYTACCTTGTTAYGACTT-3') (Acinas *et al*. 2005). The thermal cycling program started at 94° C for 5 min, followed by 30 cycles of 94° C for 15 s, 50° C for 15 s and 72° C for 15 s, and by a final step of 72° C for 1.5 min. The PCR product was purified using a PCR clean up kit ExoSap IT (Applied Biosystems), and then sequenced using primers 907R (5'-CCGTCAATTCCTTTGAGTTT-3') and S8 (5'-GTAGCGGTGAAATGCGTAGA-3'), the BigDye Terminator Cycle Sequencing kit (Applied Biosystems) with an ABI Prism 3130xl automated DNA analyzer (Applied Biosystems).

DNA-DNA hybridization was carried out by the DSMZ Identification Service (Leibniz-Institut DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany) to determine the level of relatedness between the strain LMB275^T and the closest relative *A. parvum* DSM 11968^T. After DNA purification by chromatography on hydroxyapatite according to Cashion *et al*. (1977), DNA-DNA hybridization was carried out in duplicate according to the thermal denaturation and renaturation method (De Ley *et al*. 1970, Huss *et al*. 1983) using a Cary spectrophotometer equipped with a Peltier temperature controller with an *in-situ* temperature probe (Varian).

DNA G+C % content was estimated from melting temperatures using the procedure described by Gonzalez & Saiz-Gimenez (2002), with slight modifications. Briefly, melting curves were realized for LMB275^T, *A. commune* DSM 11901^T, *A. citratiphilum* DSM 11900^T and *A. parvum* DSM 11968^T in 4 × 20 µl replicates using 500 ng DNA, 1X Sybr Green I (Lonza) and 30 % formamide (Applied Biosystems). Total raw fluorescence at 4 channels was measured in a ramp from 25 to 100° C at 1° C.min^{-1} using a StepOne plus (Thermo Fisher) real time PCR instrument, and Tm estimated as the temperature where [fluorescence (T °C) – fluorescence (T °C – 1°C)] was maximal. Since the equation from Gonzalez & Saiz-Gimenez (2002) overestimated the values experimentally measured by Kalmbach *et al*. (1999), we estimated G+C % from a regression between measured Tm and G+C reported by Kalmbach *et al*. (1999) for *A. commune* DSM 11901^T, *A. citratiphilum* DSM 11900^T and *A. parvum* DSM 11968^T. The equation used was G+C = 0.2008 × Tm + 51.977.

Phylogenetic positioning: The 16S rDNA gene sequence of strain LMB275^T was compared to those in EzBioCloud (Yoon *et al*. 2017). Multiple sequence alignment was performed using the BioEdit software version 7.2.5 (Hall 1999). Phylogenetic trees were reconstructed using Bayesian inference (BI) and Maximum-likelihood (ML) methods, using a General Time Reversible model with a heterogeneous substitution rate parameter (Gamma) and a proportion of invariable sites, chosen with jModelTest (Darriba *et al*. 2012). Bayesian analysis was performed using MrBayes version 3.2.6 (Ronquist *et al*. 2012) and ML analyses were carried out using the Mega X software (Kumar *et al*. 2018b), validated with a bootstrap procedure based on 1000 replications. As the tree topologies from Bayesian and ML analyses were similar, only the BI tree is shown.

Media and culture conditions: The capacity of strain LMB275^T to grow in different conditions was tested and compared to that of *Aquabacterium parvum* B6^T, *Aquabacterium commune* B8^T, and *Aquabacterium citratiphilum* B4^T in the same experiment. The temperature range for growth was assessed on a series of modified R2A plates by replacing starch with 0.1 % (v/v) tween 80. The plates were incubated to temperature varying from 4° C to 45° C. NaCl tolerance was investigated using modified R2A broth with various NaCl concentrations (0 to 5 %, w/v). The pH range for growth (pH 5.0 to 10.0 with intervals of 1.0 pH units) was determined in modified R2A broth that was buffered with the appropriate quantity of MES (2- (N-Morpholino) ethanesulfonic acid, 4-Morpholineethanesulfonic acid) for pH 5.0, HEPES (N- (2-Hydroxyethyl) piperazine-N'-(2-ethanesulfonic acid) for pH 6.0 to 8.0, or AMPSO (N- (1,1-Dimethyl-2-hydroxyethyl)-3-amino-2-hydroxypropanesulfonic acid) for pH 9.0 and 10.0 (Sigma). The concentrations of bacterial cells in liquid cultures were regularly assessed with an ODScanner (System-c bioprocess) during seven days.

The capacity of strains to grow at 30° C on nutrient-rich media was tested on Trypticase Soy Agar (TSA, Sigma) and on nutrient agar containing 3 g beef extract, 5 g peptone, 5 g NaCl, and 15 g agar per litre.

Growth under aerobic, anaerobic, microaerophilic, and under 5 % CO_2 conditions was determined on modified R2A agar using GENbag anaer, GENbag microaer, and GENbag CO_2 systems, respectively (BioMérieux) after incubating the strains for 2 days at 30° C.

Electron acceptors: The ability of strain LMB275^T to reduce different inorganic electron acceptors in anaerobic condition (anaerobic chamber) was assessed in mineral deep agar medium (0.66 g $(NH4)_2SO_4$; 1.36 g KH_2PO_4 ; 0.123 g Mg SO_4.7 H_2O; 0.031 g $CaCl_2$ and 0.018 g Fe Cl_2 per litre) supplemented with 5 mM sodium acetate and one of the following compounds: 0.1 % (w/v) KNO_3, 0.2 % (w/v) KNO_2, 10 mM Na_2SO_4, 10 mM Na ClO_3 or 10 mM Fe(III)-citrate as electron acceptors (Kalmbach *et al*. 1999). Briefly, 0.2 ml of fresh liquid culture were inoculated in 10 ml in liquefied and tempered medium.

Biochemical characteristics: Cytochrome oxidase activity was determined with the Oxidase Reagent kit (BioMerieux, France) and catalase production was determined by using a 3 % (v/v) H_2O_2 solution on colonies grown on modified R2A agar. Biochemical tests and assimilation of various substrates were investigated in triplicate using GEN III Microplates (Biolog Inc., CA, USA) and API 20NE kit (BioMérieux, France) according to the manufacturer's instructions. The cultures were grown for 24 h at 30° C in a modified R2A medium. For GEN III testing, cells were centrifuged and added to the inoculum medium until the OD of the culture corresponds to 45 % transmittance. The values 50 % above the negative control for the three experiments were regarded as positive. Enzyme activities were tested using API ZYM test kit according to the instructions of the manufacturer (BioMérieux, France).

Chemotaxonomic characterization: The chemotaxonomic characterization of the strain LMB275^T was carried out by the Identification Service, Leibniz-Institut DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (Braunschweig, Germany) using cells grown on modified R2A medium at 25° C and harvested after 1 day of growth by centrifugation. Whole-cell fatty acid composition was determined using Sherlock Microbial Identification System (MIDI; Sherlock version 6.1; TSBA 40 peak-naming table database). Peaks were automatically integrated and fatty acid names and percentages calculated by the MIS Standard Software (Microbial ID).

Polar lipids were extracted from freeze-dried cell material using a choroform: methanol:0.3 % aqueous NaCl mixture (modified after Bligh & Dyer 1959) and were analyzed as described by Tindall *et al*. (2007).

Sensitivity to antibiotics: The sensitivity of bacterial strains to antibiotics was tested by the disc diffusion method after spreading cell suspensions (8.0 McFarland) on modified R2A agar plates. The antibiotics and their concentration used on filter-paper discs for the susceptibility assay are listed in the species definition. The effect of antibiotics on cell growth was assessed after 48 and 72 h at 30° C.

RESULTS

Cell size of strain LMB275^T is approximately 2.6 ± 0.2 µm in length and 0.6 ± 0.1 µm in width. They are motile straight rods, that mainly occurred as single cells, but can form cell aggregates as described for *A. parvum*, *A. commune*, and *A. citratiphilum* (Kalmbach *et al*. 1999). They possess a single polar flagellum as shown in Fig. 1A.

The presence of polyalkanoate and polyphosphate inclusion bodies was observed in bacterial cytoplasm as white and black globular structures respectively as well as extracellular fibrillar matrix material as has been already reported in many other species of the genus (Fig. 1B).

A nearly complete 16S rRNA gene sequence (1460 bp) of strain LMB275^T was obtained (Genbank accession N°ON107272). Initial comparison of 16S rRNA gene sequence within the EzBioCloud online database assigned strain LMB275^T into the genus *Aquabacterium* showed the highest sequence similarity with *A. parvum* DSM 11968^T (99.1 %). The following closest phylogenetic neighbors of strain LMB275^T were *A. commune* DSM 11901^T and *A. citratiphilum* DSM 11900^T with 97.9 and 97.0 % similarities respectively. The relationship of strain LMB275^T with all members of the genus *Aquabacterium* was also supported by the phylogenetic analysis based on 16S rRNA sequence and including gene sequences of all validly published *Aquabacterium* species. This analysis confirmed *A. parvum* as the closest phylogenetic neighbor of LMB275^T and showed that they formed together a unique lineage strongly supported by bootstrap value and

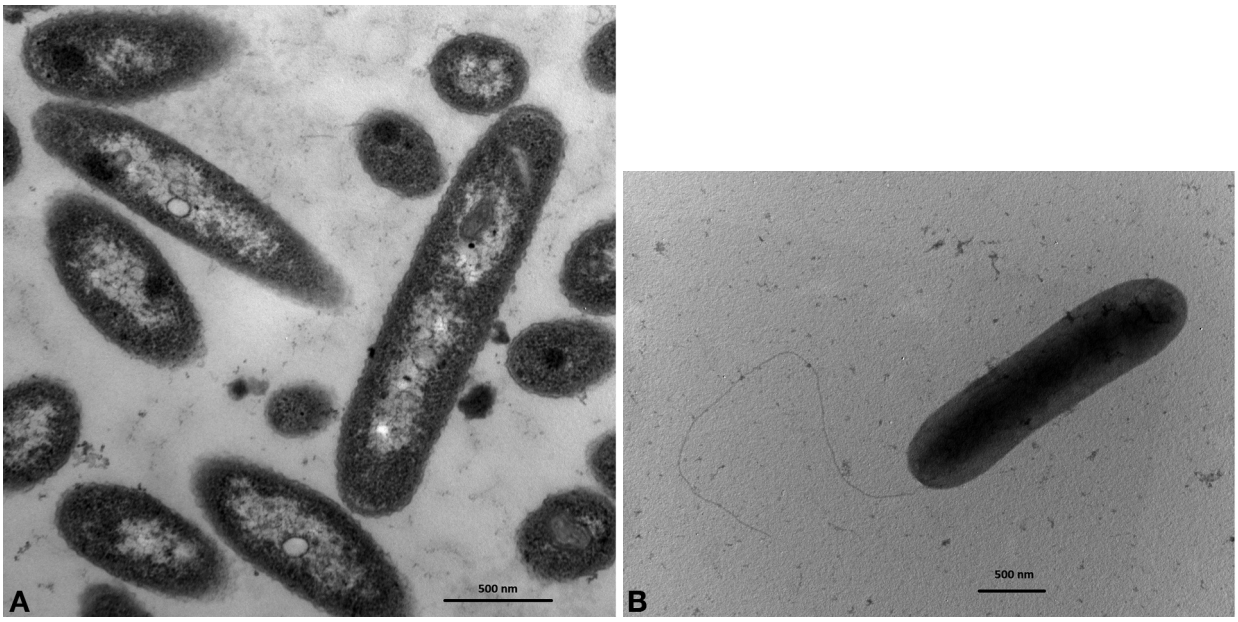

Fig. 1. – Transmission electron micrograph (**A**) of negatively stained cells of strain LMB275^T, showing the polar flagellum, (**B**) of a thin section of cell showing polyalkanoate (white) and polyphosphate (black) inclusion bodies.

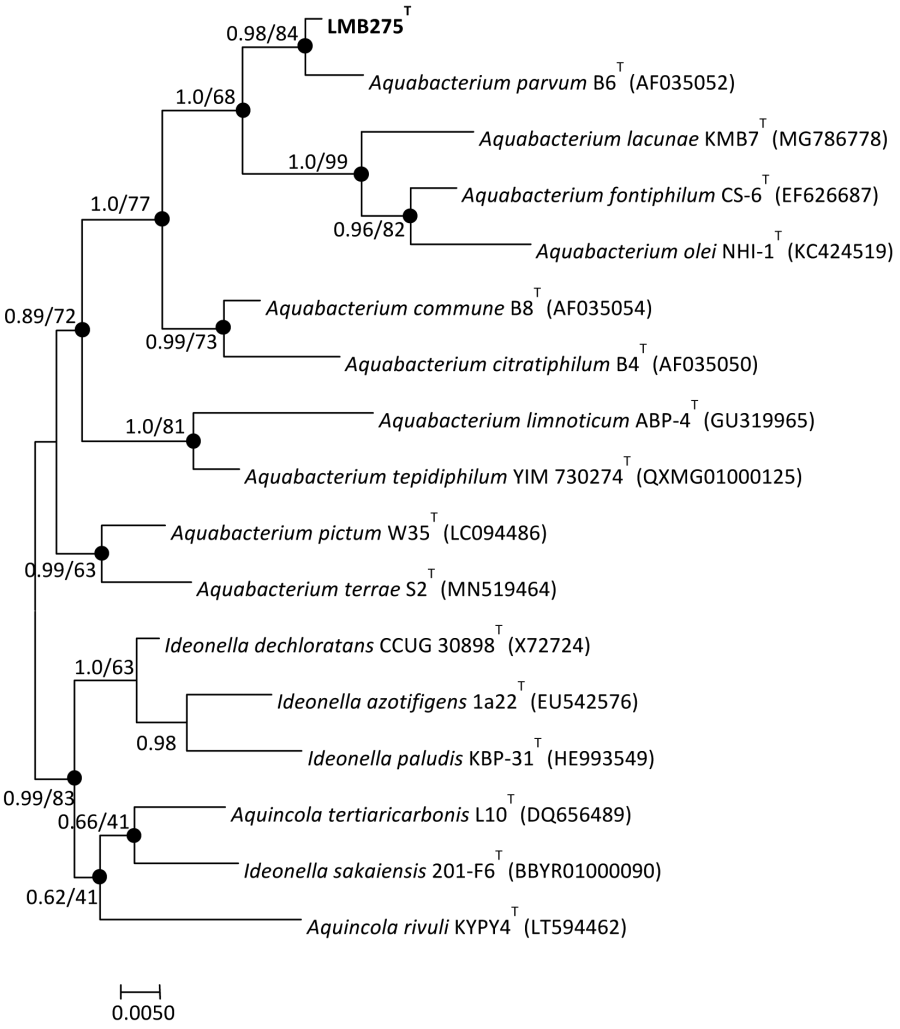

Fig. 2. – Phylogenetic tree based on the Bayesian analysis of 16S rRNA gene sequences showing the relationships of strain LMB275^T with the related *Aquabacterium* species. Genbank accession numbers are indicated in parentheses. Numbers are posterior probabilities / bootstrap support values in %. Bar: 0.005 substitution per nucleotide position.

Table I. – Phenotypic and genotypic characteristics that can be used to distinguish strain LMB275^T and members of phylogenetically related genera. Strains: 1, strain LMB275^T; 2, *A. parvum* DSM 11968^T; 3, *A. commune* DSM 11901^T ; 4, *A. citratiphilum* DSM 11900^T. * Data from Kalmbach *et al*. (1999). All strains are positive for reduction of nitrate, β-glucosidase activity, and assimilation of methyl pyruvate, tween 40, and β-hydroxy-D, L-butyric acid. All strains are negative for indole production, glucose fermentation, arginine dihydrolase, protease, and β-galactosidase activities, assimilation of dextrin, D-Maltose, D-trehalose, D-cellobiose, gentiobiose, sucrose, D-turanose, stachyose, D-raffinose, α-D-lactose, D-melibiose, β-methyl-D-glucoside, D-salicin, N-acetyl-D-glucosamine, N-acetyl-β-D-mannosamine, N-acetyl-D-galactosamine, N-acetyl neuraminic acid, α-D-glucose, D-mannose, D-fructose, D-galactose, 3-methyl glucose, L-fucose, L-rhamnose, inosine, D-sorbitol, D-mannitol, D-arabitol, myo-inositol, D-glucose-6-PO4, D-fructose-6-PO4, D-aspartic acid, D-serine, gelatin, glycyl-L-proline, L-alanine, L-arginine, L-aspartic acid, L-glutamic acid, L-histidine, L-pyroglutamic acid, L-serine, pectin, D-galacturonic acid, L-galactonic acid lactone, D-gluconic acid, D-glucuronic acid, mucic acid, quinic acid, D-saccharic acid, p-hydroxy-phenylacetic acid, D-lactic acid methyl ester, α-keto-glutaric acid, D-malic acid, γ-amino-butyric acid, α-Hydroxy-butyric acid, α-keto-butyric acid, and formic acid.

	1	2	3	4
Isolation source	Deep aquifer	Drinking water system	Drinking water system	Drinking water system
Accumulation of PHB	+	+	+	+
Growth at 45 °C	+	–	–	–
Growth at pH 6.0	+	–	–	+
Growth at pH 10.0	+	+	–	+
NaCl concentration (%,w/v)	0	0–1	0–4	0–4
Colony on TSA	+	+	–	+
Colony on Nutrient Agar	+	–	+	–
Assimilation of:				
D-fucose	–	–	+	–
Glycerol	–	–	–	+
Glucuronamide	+	–	+	–
L-Lactic acid	–	+	–	+
Citric acid	–	–	–	+
L-Malic acid	+	–	+	+
Bromo-Succinic acid	+	–	–	+
Acetoacetic acid	+	–	+	–
Propionic acid	+	–	–	+
DNA G+C content (mol%)	66	65*	66*	66*

that distinguished them from other members of the *Aquabacterium* genus (Fig. 2). The same result was obtained in all the implemented phylogenetic algorithms.

The DNA-DNA hybridization value was 47.3 % DNA-DNA similarity (39.7 % for duplicate), far below the threshold value of 70 % recommended for the definition of bacterial species and thus clearly indicating that strain LMB275^T does not belong to the closest phylogenetic relative species *A. parvum*. The estimated G+C % for LMB275^T was 66 % (Table I).

The strain grew optimally at 30-37° C, pH 7.0 and with no NaCl but was able to grow between 10 to 45° C (but not 4° C), between 6.0 to 10.0 pH units, and was not able to grow in all other NaCl concentrations tested. Colonies of the strain LMB275^T formed onto Trypticase Soy Agar and nutrient agar but were not observed on nutrient agar for *A. parvum* B6^T and *A. citratiphilum* B4^T, and on TSA for *A. commune* B8^T. Weak growth was observed with 5 % CO_2, and no growth occurred under anaerobic conditions. Strain LMB275^T grew using nitrate, chlorate, sulfate and iron (III), but no reduction of nitrite was observed.

The physiological and genomic characteristics of strain LMB275^T compared to its closest phylogenetic relatives *A. parvum*, *A. commune* and *A. citratiphilum* are summarized in Table I.

The predominant cellular fatty acid was $C_{18:1}$ ω9c (33.9 %), $C_{16:0}$ (21.7 %), and summed feature 3 (16.9 %) comprised of 16:1 ω7c and/or $C_{16:1}$ ω6c (Table II). The profile strongly differs from those reported for the closest phylogenetic relatives *A. parvum*, *A. commune* and *A. citratiphilum* as $C_{18:1}$ ω9c represented a minor part when detected in these strains. The polar lipid profile consisted of a mixture of phosphatidylethanolamine as the major component, phosphatidylglycerol, diphosphatidylglycerol and several uncharacterized phospholipids.

In summary, strain LMB275^T distinguished from the related species of the genus *Aquabacterium* by its physiological and biochemical characteristics as well as by its phylogenetic position and DNA-DNA relatedness. Therefore, we suggest that strain LMB275^T represents a novel species of the genus *Aquabacterium*, for which we propose the name *Aquabacterium silicae* sp. nov.

Table II. – Fatty acid composition (%) of LMB275^T and members of the closest phylogenetically related species of the genus *Aquabacterium*. Strains: 1, strain LMB275^T; 2, *A. parvum* DSM 11968^T; 3, *A. commune* DSM 11901^T ; 4, *A. citratiphilum* DSM 11900^T. Values are percentage of the total fatty acids. Fatty acids with values of less than 1.0% are indicated by "–". Data from Chen *et al*. (2012). ** Summed Feature are groups of two or three fatty acids that cannot be separated by GLC using the MIDI system. Summed Feature 3 comprises C16:1 ω7c and/or C16:1 ω6c. Summed Feature 8 comprises C18:1 ω7c and/or C18:1 ω6c.

	1	**2***	**3***	**4***
$C_{10:0}$	–	2.0	–	2.3
$C_{12:0}$	4.3	3.3	5.6	3.4
$C_{14:0}$	–	4.1	1.9	3.9
$C_{16:0}$	21.7	21.3	31.0	19.0
$C_{18:0}$	–	7.8	1.6	2.0
$C_{10:0}$ 3-OH	4.8	6.8	5.8	7.8
$C_{12:0}$ 2-OH	1.9	2.4	1.5	2.7
$C_{16:1}$ ω9c	7.5	–	–	–
$C_{18:1}$ ω7c	6.8	18.2	8.8	20.1
$C_{18:1}$ ω9c	**33.9**	1.4	–	1.2
**Summed In Feature 3	16.9	30.4	42.4	35.0
**Summed In Feature 8	6.8	–	–	–

Description of* Aquabacterium silicae *sp. nov.

Aquabacterium silicae (si.li'cae. L. gen. n. of silex, as the type strain was isolated from a siliceous environment).

Cells are rod-shaped, aerobic and microaerophile, chemo-heterotrophic, motile using a single polar flagellum. Cells are approximately 2.6 ± 0.2 µm long and 0.6 ± 0.1 µm wide. Cells grown on modified R2A medium form 0.5 mm diameter colonies, white in the center and transparent at the edge. Accumulation of poly-β-hydroxybutyrate and polyphosphate is observed. Grows well on modified R2A, TSA and Nutrient Agar. On modified R2A media, colonies are circular, convex, and 0.5 mm in diameter after 48 h of aerobic incubation at 30° C. Growth occurs in the temperature range 10-45° C, with no NaCl and at a pH ranging from 6 to 10. Optimum growth occurs at 30-37° C, pH 7 with no NaCl. The bacterial strain exhibits positive oxidase and no catalase activities. Nitrate, chlorate, sulfate and iron (III) can be used as the electron acceptor for anaerobic respiration, but not nitrite.

In API 20NE tests, reduction of nitrate, urea hydrolysis, β-glucosidase activity, and assimilation of malate are positive. Negative for indole production, glucose fermentation, arginine dihydrolase, protease, β-galactosidase activities, and assimilation of D-glucose, L-arabinose, D-mannose, D-mannitol, N-acetyl-glucosamine, D-maltose, gluconic acid, capric acid, adipic acid, citric acid and phenyl-acetic acid. Utilization of carbon sources using Biolog GEN III Microplate test panels shows positive reactions for glucuronamide, methyl pyruvate, L-malic acid, bromo-succinic acid, tween 40, β-hydroxy-D,L-butyric acid, acetoacetic acid, propionic acid, acetic acid. It is negative for dextrin, D-Maltose, D-trehalose, D-cellobiose, gentiobiose, sucrose, D-turanose, stachyose, D-raffinose, α-D-lactose, D-melibiose, β-methyl-D-glucoside, D-salicin, N-acetyl-D-glucosamine, N-acetyl-β-D-mannosamine, N-acetyl-D-galactosamine, N-acetyl neuraminic acid, α-D-glucose, D-mannose, D-fructose, D-galactose, 3-methyl glucose, D-fucose, L-fucose, L-rhamnose, inosine, D-sorbitol, D-mannitol, D-arabitol, myo-inositol, glycerol, D-glucose-6-PO_4, D-fructose-6-PO_4, D-aspartic acid, D-serine, gelatin, glycyl-L-proline, L-alanine, L-arginine, L-aspartic acid, L-glutamic acid, L-histidine, L-pyroglutamic acid, L-serine, pectin, D-galacturonic acid, L-galactonic acid lactone, D-gluconic acid, D-glucuronic acid, mucic acid, quinic acid, D-saccharic acid, p-hydroxy-phenylacetic acid, D-lactic acid methyl ester, L-lactic acid, citric acid, α-keto-glutaric acid, D-malic acid, γ-amino-butyric acid, α-hydroxy-butyric acid, α-keto-butyric acid, formic acid. In API ZYM assays, positive for alkaline phosphatase, esterase, esterase lipase, leucine arylamidase, acid phosphatase and naphthol-AS-BI-phosphatase. Negative for lipase, valine arylamidase, cysteine arylamidase, trypsin and α-chymotrypsin.

Resistant to cefotaxim (30 µg), polymixin B (300 UI), ampicillin (10 µg), penicillin (10 UI) and amoxicillin (30 µg) and sensitive to chloramphenicol (30 µg), ciprofloxacin (5 µg), vancomycin (30 µg), rifampicin (30 µg), imipenem (10 µg), ofloxacin (5 µg), kanamycin (30 µg), tetracyclin (30 µg), erythromycin (15 µg), streptomycin (10 µg).

The major components of cellular fatty acids are $C_{18:1}$ ω9c, $C_{16:0}$, and summed feature 3 (16:1 ω7c and/or $C_{16:1}$ ω6c). The major polar lipid is phosphatidylethanolamine. The G+C content of genomic DNA is 66 %.

The type strain is LMB275^T (= CNCM I-5858), which was isolated from a deep aquifer located in the Cévennes mountains (France).

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