Aquipuribacter hungaricus gen. nov., sp. nov., an actinobacterium isolated from the ultrapure water system of a power plant

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A Gram-positive actinobacterium, strain IV-75^T, was isolated by using R2A agar from the ultrapure water system of a power plant in Hungary. The strain exhibited a rod–coccus cell cycle, and was strictly aerobic, non-motile, catalase-positive and oxidase-negative. 16S rRNA gene sequence analysis revealed that strain IV-75^T belonged to the suborder *Micrococcineae* and clustered with members of the family *Intrasporangiaceae*. Its closest phylogenetic neighbour was *Arsenicococcus bolidensis* CCUG 47306^T (94.3% 16S rRNA gene sequence similarity). The peptidoglycan of strain IV-75^T contained *meso*-diaminopimelic acid and MK-10(H₄) was the major menaquinone. The polar lipid pattern contained phosphatidylglycerol, two unidentified phospholipids, one glycolipid and several other lipid components. The major fatty acids were anteiso-C_{15:0}, C_{18:1ω9c} and C_{16:0}. Based on the moderate levels of 16S rRNA gene sequence similarity to all members of the family *Intrasporangiaceae* and the unique combination of chemotaxonomic characteristics, strain IV-75^T is considered to represent a novel species of a new genus, for which the name *Aquipuribacter hungaricus* gen. nov., sp. nov. is proposed. The type strain of *Aquipuribacter hungaricus* is IV-75^T (=DSM 21674^T=NCAIM B 02333^T).

Many industrial applications use ultrapure water (UPW) as a basic material. Despite the fact that UPW generally contains very low levels of organic (total organic carbon <3 mg) and inorganic (conductivity <1 µS m⁻¹) material, it is well known that oligotrophic bacteria can grow and proliferate in such nutrient-deficient environments (Patterson *et al.*, 1991; Poindexter, 1981; Soini *et al.*, 2002). Planktonic micro-organisms can be dispersed within the water body but many also attach to surfaces and form biofilms. Micro-organisms that colonize industrial cooling water systems can affect the efficiency of various operation units and their by-products may cause or speed up corrosion (Costerton *et al.*, 1987).

In 2007, the water purification system of a Hungarian power plant was studied by using cultivation and cultivation-independent methods (Bohus *et al.*, 2010). Among the isolated bacteria, one strain, designated IV-75^T, was found to be only distantly related to any recognized taxon based on its

16S rRNA gene sequence. The aim of the present study was to describe strain IV-75^T and determine its taxonomic position using a polyphasic approach.

Sampling was carried out in a power plant close to the town of Paks. Water for the plant is taken from the River Danube and is treated in a purification system consisting of mechanical filtration, lime softening, and scavenger, cationic, anionic and mixed-bed ion exchange units; the resulting UPW is stored in a tank before it is loaded into the circulation system (Bohus *et al.*, 2010). UPW for isolation of bacteria was sampled with extreme care under aseptic conditions, collected in sterilized screw-capped flasks and transferred to the laboratory at 4 °C. The oligotrophic medium R2A agar (Reasoner & Geldreich, 1985) was used for cultivation of bacteria by direct plating. All plates were incubated at 28 °C for 5–10 days. Later, bacterial colonies were purified and the strains were maintained on R2A agar slants.

Colony morphology was studied on R2A agar medium by direct and stereomicroscopic observations of single colonies. Cell size, shape, arrangement and motility were studied by using native preparations and by Gram staining according to Claus (1992). Endospore and acid-fast staining tests were carried out according to the method of Murray *et al.* (1994).

Abbreviation: UPW, ultrapure water.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain IV-75^T is FM179321.

Two supplementary figures are available with the online version of this paper.

Scanning electron microscopic investigations were based on techniques described by Makk & Ács (1996).

Growth under anaerobic conditions was examined using slant agar cultures on R2A agar medium incubated for 1 week in an anaerobic chamber (Forma Scientific) at 28 °C. Oxidase activity was tested by the method of Tarrand & Gröschel (1982). Catalase production and the Voges–Proskauer reaction were demonstrated by using the methods of Cowan & Steel (1974). Acid production from glucose was studied by the OF test according to Hugh & Leifson (1953). Growth at 4, 20, 28, 37, 45 and 60 °C was determined on R2A agar slants. NaCl tolerance (0, 2.5, 5, 10 %, w/v) and pH tolerance (3–11, steps of 1.0 pH unit) were checked in R2A broth. Urease activity, nitrate reduction, starch hydrolysis, indole production from tryptophan, caseinase, gelatinase, phosphatase activity, hydrolysis of Tween 80 and H₂S production from cysteine were studied according to Smibert & Krieg (1994). Acid production from different carbon sources as sole source of carbon and enzyme activities were tested by using the API 50CH and API ZYM systems (bioMérieux) according to the manufacturer's instructions.

The cell-wall diamino acid of strain IV-75^T was determined from whole-cell hydrolysates as described by Hasegawa *et al.* (1983). Isoprenoid quinones were extracted from cells cultivated in liquid Rich medium (Yamada & Komagata, 1972) according to the method of Collins *et al.* (1977) and were analysed by HPLC (Shimadzu LC 20A) and electron-impact MS [Singlequad 320 instrument equipped with a direct insertion device; Varian (Groth *et al.*, 1997)]. Cellular fatty acids were extracted from cells cultivated on R2A agar at 28 °C. Strain IV-75^T and the three strains of recognized *Arsenicococcus* species included in the fatty acid analyses showed similar growth behaviour and sufficient cells of comparable physiological age could be harvested from the third streak quadrant of the plates after cultivation under the conditions used. Fatty acid methyl esters were obtained following the method of Stead *et al.* (1992) and were analysed by GC via the Microbial Identification System (MIDI, Sherlock Version 6.1; TSBA40 database; Agilent Technologies model 6890N GC). Summed feature components were identified thereafter by GC/MS by using a Singlequad 320 instrument (Varian). Polar lipids were determined according to the method described by Minnikin *et al.* (1979) and were separated by two-dimensional TLC. To identify spots, ninhydrin reagent, Zinzadze reagent and molybdophosphoric acid were used (Embley & Wait, 1994). Genomic DNA for analysis of the G + C content was isolated after disruption of bacterial cells by using a French press (Thermo Spectronic). After purification on hydroxyapatite according to the procedure of Cashion *et al.* (1977), the DNA was degraded to nucleosides by using P1 nuclease and bovine intestinal mucosa alkaline phosphatase as described by Mesbah *et al.* (1989). The nucleosides were separated by reversed-phase HPLC (Shimadzu LC 20A) according to the method described by Tamaoka & Komagata (1984). The G + C content of the DNA was calculated from the ratio of deoxyguanosine to thymidine.

DNA extraction from strain IV-75^T and PCR-mediated amplification of the 16S rRNA gene were carried out by using the method of Rainey *et al.* (1996). The PCR product was purified with a Prep-A-Gene kit (Bio-Rad). Cycle sequencing was performed by using a Big Dye Terminator Cycle Sequencing kit (Applied Biosystems) according to the manufacturer's protocol. The PCR product was purified with the PCR-M Clean Up System (Viogene). Sequence analysis was performed by using an Applied Biosystems model 310 Genetic Analyzer.

The search for phylogenetically related type strains was initially carried out via the BLAST (Altschul *et al.*, 1997) and FASTA (Pearson & Lipman, 1988) programs as well as by using the EzTaxon Server (version 2.1; Chun *et al.*, 2007).

Because preliminary searches revealed members of the suborder *Micrococcineae* to be the closest relatives of strain IV-75^T, the sequences of all type strains of type species of genera classified in this suborder (Schumann *et al.*, 2009; Euzéby, 1997; <http://www.bacterio.cict.fr/>) were chosen for comparison. The 16S rRNA gene sequence of strain IV-75^T was manually aligned with the program BioEdit (Hall, 1999) against sequences available from EMBL (<http://www.ncbi.nlm.nih.gov/>). The alignment was used to calculate

Table 1. Patterns of 16S rRNA gene signature nucleotides of strain IV-75^T and of those defining the families *Intrasporangiaceae*, *Dermatophilaceae* and *Dermacoccaceae*

Taxa: 1, strain IV-75^T; 2, *Intrasporangiaceae*; 3, *Dermatophilaceae*; 4, *Dermacoccaceae*. Nucleotides in lower-case letters are present in some but not all strains; Y, C or U; K, G or U; signature nucleotides different from those of strain IV-75^T are indicated in bold.

Position(s)	1	2*	3*	4*
120	A	A	A	A
131:231	A–G	A–G	Y–K	A–G
196	U	G	A	C
342:347	C–G	C–G	C–G	C–G
444:490	U–A	a–u	A–U	A–U
580:761	C–G	U–A	U–A	U–A
602:636	C–G	C–G	C–G	C–G
670:736	A–U	A–U	A–U	A–U
822:878	G–C	G–C	G–C	G–C
823:877	G–C	G–C	G–C	G–C
826:874	C–G	C–G	C–G	C–G
827	U	U	U	U
843	C	U	U	c
950:1231	U–A	U–A	U–A	U–A
1047:1210	G–C	G–C	G–C	G–C
1109	C	C	C	C
1145	G	G	G	G
1309:1328	G–C	G–C	G–C	G–C
1361	G	C	G	G
1383	C	C	C	C

*Data from Zhi *et al.* (2009).

a distance matrix based on the Kimura two-parameter correction (Kimura, 1980) and to construct a phylogenetic tree with the neighbour-joining method (Saitou & Nei, 1987) within the CLUSTAL X software package (Thompson *et al.*, 1997). Bootstrap analysis was based on 1000 resamplings (Felsenstein, 1985). Within the phylogenetic tree based on 16S rRNA gene sequences of the type strains of type species of the suborder *Micrococcineae*, strain IV-75^T fell within a clade comprising members of the families *Intrasporangiaceae*, *Dermacoccaceae* and *Dermatophilaceae* (see Supplementary Fig. S1 in IJSEM Online). The clustering of strain IV-75^T within the subcluster of *Arsenicococcus bolidensis* and *Ornithinimicrobium humiphilum* was supported by a moderate bootstrap value of 51%. The signature nucleotides of the 16S rRNA gene sequence of strain IV-75^T were determined and compared with those that define the families *Intrasporangiaceae*, *Dermacoccaceae* and *Dermatophilaceae* (Zhi *et al.*, 2009). Because the signature nucleotides of strain IV-75^T differed in three to five positions from those of these three families (Table 1), unambiguous affiliation at the level of families on the basis of signature nucleotides was not possible. The clustering of strain IV-75^T with the type strains of type species of the genera *Arsenicococcus* and

Ornithinimicrobium (both belonging to the family *Intrasporangiaceae*) in a phylogenetic tree representing the suborder *Micrococcineae* (Supplementary Fig. S1) supports the view that strain IV-75^T should be considered a member of the family *Intrasporangiaceae*. A phylogenetic tree was constructed by the methods described above based on 16S rRNA gene sequences of representatives of all genera classified within the family *Intrasporangiaceae* at the time of writing (Fig. 1; <http://www.bacterio.cict.fr/>). Strain IV-75^T fell within a subcluster encompassing members of the genera *Arsenicococcus*, *Ornithinimicrobium* and *Serinicoccus*. The clustering of strain IV-75^T with these three genera was confirmed by a maximum-likelihood analysis (Felsenstein, 1985) performed by using the program BioEdit (Hall, 1999; data not shown). Strain IV-75^T showed the following levels of 16S rRNA gene sequence similarity to those of its closest relatives calculated by using the EzTaxon server (Chun *et al.*, 2007; <http://www.eztaxon.org>): *Arsenicococcus bolidensis* CCUG 47306^T, 94.3%; *Arsenicococcus piscis* Kis4-19^T, 93.6%; *Ornithinimicrobium kibberense* K22-20^T, 94.0%; *Ornithinimicrobium humiphilum* DSM 12362^T, 93.2%; *Ornithinimicrobium pekingense* LW6^T, 92.7%; *Serinicoccus profundus* 0714S6-1^T, 92.1%; *Serinicoccus marinus*

Fig. 1. Neighbour-joining tree constructed from 16S rRNA gene sequences of strain IV-75^T and representatives of genera classified in the family *Intrasporangiaceae*. *Arthrobacter globiformis* DSM 20124^T was used as outgroup. Bootstrap values (expressed as percentages of 1000 replications) are given at branch points; only values >70% are shown. Bar, 0.01 substitutions per nucleotide position.

Table 2. Differential characteristics of strain IV-75^T and related genera

Taxa: 1, strain IV-75^T (data from this study); 2, *Arsenicococcus* [data from Collins *et al.* (2004) and Hamada *et al.* (2009)]; 3, *Ornithinimicrobium* [data from Groth *et al.* (2001), Mayilraj *et al.* (2006) and Liu *et al.* (2008)]; 4, *Serinicoccus* [data from Yi *et al.* (2004), Traiwan *et al.* (2011) and Xiao *et al.* (2011)]. ND, No data available. Data in square brackets were reported for only a few species.

Characteristic	1	2	3	4
Cell morphology	Rod–coccus cell cycle	Cocci	Irregular rods and cocci	Coccoid
Peptidoglycan diamino acid*	<i>meso</i> -Dpm	LL-Dpm	L-Orn [L-Lys]	L-Orn [<i>meso</i> -Dpm]
DNA G + C content (mol%)	75	71–72	69–71	72.0–73.5
Major menaquinone†	MK-10(H ₄)	MK-8(H ₄)	MK-8(H ₄)	MK-8(H ₄)
Polar lipids‡	PG, PLs, GLs	ND	PI, PG, DPG, PLs, GLs	PI, PG, DPG, GL [PC]
Peptidoglycan type§	A1γ (A31)	A3γ (A41.1)	ND	ND [A1γ (A31)]

*Dpm, 2,6-Diaminopimelic acid; Lys, lysine; Orn, ornithine.

†Example for isoprenoid quinones: MK-8(H₄), partially saturated menaquinone with two of eight isoprene units hydrogenated.

‡DPG, Diphosphatidylglycerol; GL(s), unidentified glycolipid(s); PC, phosphatidylcholine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PL(s), unidentified phospholipid(s).

§Data given in parentheses are according to Schleifer & Kandler (1972) and DSMZ (<http://www.dsmz.de/>).

JC1078^T, 91.9%; and *Serinicoccus chungangensis* CAU 9536^T, 91.9%.

Differential characteristics of strain IV-75^T and related genera are shown in Table 2. Because strain IV-75^T showed highest 16S rRNA gene sequence similarity to members of the genus *Arsenicococcus*, its physiological and biochemical characteristics as well as its fatty acid profile were compared with those of strains of this genus (Tables 3 and 4) side by side. To represent the phenotypic diversity of the genus *Arsenicococcus*, *Arsenicococcus bolidensis* DSM 15745^T, *Arsenicococcus piscis* DSM 22760^T and *Arsenicococcus bolidensis* DSM 30750 (isolate FR2_MS13 from a spacecraft-associated clean room, Stieglmeier *et al.*, 2009; sharing 94.1% 16S rRNA gene sequence similarity with strain IV-75^T) were included in this study.

Strain IV-75^T differed from all representatives of the genus *Arsenicococcus* based on its morphological cell cycle (Fig. 2), in being negative in the nitrate reduction test, in its ability to use L-arabinose and potassium-5-ketogluconate as sole source of carbon, and in its inability to utilize a series of D-isomer sugars (Table 3). In contrast to members of the genus *Arsenicococcus*, strain IV-75^T showed valine arylamidase and N-acetylglucosaminidase activity but no naphthol-AS-BI-phosphohydrolase activity (Table 3).

The cellular fatty acid profile of strain IV-75^T contained anteiso-C_{15:0}, C_{18:1}ω9c and C_{16:0} as major components and differed by a lower content of iso-C_{15:0} and higher amounts of anteiso-C_{15:0} and C_{18:1}ω9c compared with all three reference *Arsenicococcus* strains (Table 4). Strain IV-75^T could be distinguished from *Arsenicococcus bolidensis* by a lower C_{16:1}ω7c content and from *Arsenicococcus piscis* by a much higher C_{18:1}ω9c content.

Strain IV-75^T contained MK-10(H₄) as the major menaquinone, in contrast to members of the genera *Arsenicococcus*, *Ornithinimicrobium* and *Serinicoccus*, which all contain menaquinone MK-8(H₄). The diamino acid *meso*-diaminopimelic

acid found in strain IV-75^T was detected only in one species of the genus *Serinicoccus*, *S. chungangensis* (Traiwan *et al.*, 2011), whereas other members of the genus *Serinicoccus* contain ornithine. Species of the genus *Arsenicococcus* display LL-diaminopimelic acid and species of the genus *Ornithinimicrobium* contain ornithine (*O. pekingense* additionally contains lysine; Liu *et al.*, 2008) (Table 2). The polar lipid profile of strain IV-75^T contained mainly unidentified components (see Supplementary Fig. S2 available in IJSEM Online). Three phospholipids (including phosphatidylglycerol), one glycolipid and several other lipid components but no aminolipids were detected.

Strain IV-75^T fell within the clade encompassing the suborder *Micrococineae* but showed less than 94.5% 16S rRNA gene sequence similarity to its members. Its major menaquinone, MK-10(H₄), has been found so far only in the distantly related genus *Actinotalea* (Schumann *et al.*, 2009); strain IV-75^T shared 92.6% 16S rRNA gene sequence similarity with the type strain of *Actinotalea fermentans*, the single recognized species of the genus. We suggest classifying strain IV-75^T tentatively within the family *Intrasporangiaceae* despite differences in 16S rRNA gene signature nucleotides because of its phylogenetic association with the genera *Arsenicococcus*, *Ornithinimicrobium* and *Serinicoccus*. Based on moderate 16S rRNA gene sequence similarity to members of these genera and its unique combination of chemotaxonomic characteristics and differences in phenotypic characteristics from both recognized species of the genus *Arsenicococcus* (Table 3), we suggest that strain IV-75^T represents a novel species of a new genus, for which the name *Aquipuribacter hungaricus* gen. nov., sp. nov. is proposed.

Description of *Aquipuribacter* gen. nov.

Aquipuribacter (A. qui. pu. ri. bac' ter. L. n. *aqua* water; L. adj. *purus* clean, pure; N.L. masc. n. *bacter* a rod; N.L. masc. n. *Aquipuribacter* a rod isolated from pure water).

Table 3. Differential physiological and biochemical characteristics of strain IV-75^T and strains of recognized *Arsenicococcus* species

Strains: 1, IV-75^T; 2, *Arsenicococcus bolidensis* DSM 15745^T; 3, *Arsenicococcus bolidensis* DSM 30750; 4, *Arsenicococcus piscis* DSM 22760^T. All results are from this study. All strains were positive for catalase, α -chymotrypsin, acid phosphatase, β -galactosidase and α -glucosidase activities and aesculin hydrolysis. All strains were negative for oxidase, urease, casease, gelatinase, phosphatase, lipase (C14), β -glucuronidase, α -mannosidase and α -fucosidase activities and utilization of glycerol, erythritol, D-arabinose, D-ribose, L-xylose, D-adonitol, methyl β -xylopyranoside, D-mannose, L-sorbose, L-rhamnose, dulcitol, inositol, D-sorbitol, methyl α -D-mannopyranoside, methyl α -D-glucopyranoside, amygdalin, inulin, melezitose, starch, xylitol, turanose, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, potassium gluconate and potassium 2-ketogluconate. All strains were negative for H₂S and indole production and for the Voges–Proskauer test. None of the strains was able to grow at pH 3, 4, 10 or 11 or with 10% NaCl.

Characteristic	1	2	3	4
Salinity range for growth (% NaCl)	0–2.5	0–5	0–5	0–2.5
pH range for growth	6–9	5–9	5–9	6–8
Nitrate reduction to nitrite	–	+	+	+
Hydrolysis of:				
Starch	+	+	+	–
Tween 80	–	+	+	–
Acid production from:				
L-Arabinose	+	–	–	–
D-Xylose	–	+	+	–
D-Fructose	–	+	+	+
D-Galactose	–	+	+	–
D-Glucose	–	+	+	+
D-Mannitol	–	+	+	–
Arbutin	–	+	+	–
N-Acetylglucosamine	–	+	–	–
Salicin	–	+	+	–
Cellobiose	–	+	+	–
Maltose	–	+	+	+
Lactose	–	+	+	–
Melibiose	–	+	+	–
Sucrose	–	+	+	+
Trehalose	–	+	+	–
Raffinose	–	+	+	+
D-Xylose	–	+	+	–
Gentiobiose	–	+	+	–
Glycogen	–	+	–	+
Potassium 5-ketogluconate	+	–	–	–
Alkaline phosphatase	+	+	+	–
Esterase (C4)	+	+	+	–
Esterase lipase (C8)	+	+	+	–
Leucine arylamidase	+	+	+	–
Valine arylamidase	+	–	–	–
Cystine arylamidase	+	–	–	–
Trypsin	–	–	–	+
Naphthol-AS-BI-phosphohydrolase	–	+	+	+
α -Galactosidase	+	+	–	–
β -Glucosidase	+	+	–	+
N-Acetyl- β -glucosaminidase	+	–	–	–

Table 4. Cellular fatty acid composition of strain IV-75^T and strains of recognized *Arsenicococcus* species

Strains: 1, IV-75^T; 2, *Arsenicococcus bolidensis* DSM 15745^T; 3, *Arsenicococcus bolidensis* DSM 30750; 4, *Arsenicococcus piscis* DSM 22760^T. All strains were cultivated on R2A agar at 28 °C for 72 h and fatty acids were analysed in parallel in the present study. –, Not detected.

Fatty acid	1	2	3	4
Saturated straight-chain				
C _{13:0}	–	0.1	–	–
C _{14:0}	1.3	2.3	1.6	–
C _{15:0}	0.7	2.2	1.3	0.8
C _{16:0}	9.2	11.1	5.3	1.6
C _{17:0}	3.9	4.6	2.2	4.2
C _{18:0}	7.3	4.2	1.3	1.9
C _{19:0}	–	0.1	–	–
C _{16:0} N alcohol	1.2	–	–	–
Unsaturated straight-chain				
C _{15:1ω6c}	–	0.4	0.5	–
C _{16:1ω7c*}	1.4	12.9	14.1	1.5
C _{17:1ω8c}	0.8	4.2	5.7	4.3
C _{18:1ω9c}	17.5	11.2	12.8	1.4
C _{18:3ω6c} (6, 9, 12)	7.8	–	–	–
Summed feature 6†	–	0.2	0.3	–
Saturated branched-chain				
iso-C _{13:0}	0.3	2.9	0.9	1.1
iso-C _{14:0}	2.2	13.7	10.1	5.5
iso-C _{15:0}	5.3	14.8	21.6	37.4
iso-C _{16:0}	3.8	4.1	8.7	11.8
iso-C _{17:0}	3.0	0.6	1.3	2.5
anteiso-C _{13:0}	–	1.2	0.3	–
anteiso-C _{15:0}	29.8	7.3	6.3	10.2
anteiso-C _{17:0}	4.6	1.2	2.1	5.9
10-Methyl-C _{17:0}	–	–	0.4	1.0
10-Methyl-C _{18:0}	–	–	0.2	–
Unsaturated branched-chain				
iso-C _{15:1ω9c}	–	–	0.1	1.4
iso-C _{16:1}	–	–	0.6	2.2
iso-C _{17:1ω9c}	–	0.2	1.3	2.5
anteiso-C _{17:1ω9c}	–	–	0.8	2.8

*Identified as part of summed feature 3 (C_{16:1 ω 7c} and/or iso-C_{15:2-OH}) but identified as C_{16:1 ω 7c} by GC/MS.

†Summed feature 6 comprised C_{19:1 ω 11c} and/or C_{19:1 ω 9c}.

Cells exhibit a rod–coccus cycle, are aerobic and non-endospore-forming, and stain Gram-positive. The peptidoglycan type is A1 γ based on directly cross-linked *meso*-diaminopimelic acid. The predominant menaquinone is MK-10(H₄) and the major polar lipids are phosphatidylglycerol, two unidentified phospholipids and one glycolipid. The major cellular fatty acids are anteiso-C_{15:0}, C_{18:1 ω 9c} and C_{16:0}. The G + C value of the genomic DNA is approximately 75 mol%. Phylogenetically, the genus belongs to the family *Intrasporangiaceae*, suborder *Micrococcineae*, within the order *Actinomycetales*. The type species is *Aquipuribacter hungaricus*.

Fig. 2. Scanning electron micrographs demonstrating the typical cell cycle of strain IV-75^T grown in R2A broth. In the first 44 h of growth, cells occur mainly as single cocci (a). After 44 h, cells start to elongate to become regular rods, reaching a maximum length after 76–84 h cultivation (b). After approximately 90 h, the rods constrict to give 4–5 coccoid bodies per cell. Some of the cells show a tendency to branch (c). After 96 h of cultivation, cells revert to a coccoid morphology (d). Bars, 5 µm.

Description of *Aquipuribacter hungaricus* sp. nov.

Aquipuribacter hungaricus (hun.ga'ri.cus. M.L. masc. adj. *hungaricus* of or belonging to Hungary, where the type strain was isolated).

Displays the following characteristics in addition to those given for the genus. Rods are 0.9–1.1 µm by 3.8–6.2 µm; cocci are 0.9–1.4 µm in diameter. Cells are non-motile. Colonies are pale orange, smooth, convex and circular on R2A agar medium after 7 days of incubation. Catalase-positive and oxidase-negative. Growth occurs with up to 2.5% NaCl. The temperature range for growth on R2A agar is 20–37 °C with an optimal temperature between 20 and 28 °C. The pH optimum is 7–8. Does not reduce nitrate to nitrite. L-Arabinose and potassium-5-ketoglucuronate are utilized but the following carbon sources are not: D-xylose, D-fructose, D-galactose, D-glucose, D-mannitol, arbutin, cellobiose, maltose, lactose, melibiose, sucrose, trehalose, raffinose, D-xylose, gentiobiose, N-acetylglucosamine and salicin. Aesculin is hydrolysed. H₂S and indole are not produced. Alkaline phosphatase, acid phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, α-chymotrypsin, valine arylamidase, N-acetylglucosaminidase, α-galactosidase, β-galactosidase, α-glucosidase and β-glucosidase are present but naphthol-AS-BI-phosphohydrolase and trypsin are absent. The peptidoglycan contains *meso*-diaminopimelic acid and the major menaquinone is MK-10(H₄). The major cellular fatty acids are anteiso-C_{15:0}, C_{18:1}ω9*c* and C_{16:0}.

The type strain, IV-75^T (=DSM 21674^T=NCAIM B 02333^T), was isolated from UPW of a Hungarian power plant. The DNA G+C content of the type strain is 75.0 mol% (HPLC).

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