Transfer of *Defluviibacter lusatiensis* to the genus *Aquamicrobium* as *Aquamicrobium lusatiense* comb. nov. and description of *Aquamicrobium aerolatum* sp. nov.

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Analysis of the 16S rRNA gene sequences of the type strains of *Aquamicrobium defluvii* and *Defluviibacter lusatiensis* shows a similarity of 98.3 %. There is no evidence for clear phenotypic differences between these organisms that justifies assignment to different genera. Both have ubiquinone Q-10 as the dominant quinone type and very similar fatty acid profiles, with 18:1 ω 7c and 19:0 cyclo ω 8c predominating. A proposal is made to transfer *Defluviibacter lusatiensis* to the genus *Aquamicrobium* as *Aquamicrobium lusatiense* comb. nov. (type strain S1^T = CIP 106844^T = DSM 11099^T). Furthermore, a novel species within this genus, *Aquamicrobium aerolatum* sp. nov., is described, with strain Sa14^T (=CCUG 57044^T = DSM 21857^T), isolated from air in a duck shed, as the type strain.

The monospecific genus *Defluviibacter* was proposed by Fritsche *et al.* (1999a) for Gram-negative strains isolated from an industrial wastewater-treatment plant that were able to degrade 2,4-dichlorophenol, 4-chloro-2-methylphenol, 4-chlorophenol and phenol. Originally proposed as '*Defluviibacter lusatie*' (sic), the name was corrected to *Defluviibacter lusatiensis* on validation (Fritsche *et al.*, 1999b). Slightly earlier, the monospecific genus *Aquamicrobium* was proposed by Bambauer *et al.* (1998a) for a Gram-negative strain, also isolated from wastewater, that was able to degrade thiophene-2-carboxylate. The two genera were proposed independently. The name *Aquamicrobium defluvii* was validly published in 1998 (Bambauer *et al.*, 1998b).

A white-greyish strain Sa14^T was isolated from air collected in a duck shed on tryptone soy agar (TSA) at 26 °C. The building accommodated 2500 ducks (each 14 months old). Bioaerosol samples were collected by filtration on gelatin filters, with an air sampling rate of 30 l min⁻¹. Subsequently, the filters were dissolved and diluted in isotonic NaCl (up to 10⁻⁸). The concentration of strain Sa14^T, calculated from the number of colonies with an identical morphology in the same dilution step, was approximately 10⁴ c.f.u. (m³ air)⁻¹.

Subcultivation was done on nutrient agar (Oxoid) at 28 °C for 24 h. On this agar, Sa14^T was able to grow at 10–36 °C, but not at 4 or 45 °C. Growth at 30 °C was also observed

on TSA and R2A agar, but not on Salmonella–Shigella agar or MacConkey agar (all from Oxoid). The pH range (pH 4–10 at intervals of 1 pH unit) and requirement for NaCl (at 0, 1, 2, 3, 5 and 7 % w/v) were determined using R2A medium. Gram-staining was performed as described by Gerhardt *et al.* (1994). Cell morphology was observed under a Zeiss light microscope at $\times 1000$ magnification, using cells that had been grown for 24 h at 28 °C on nutrient agar (Oxoid). Oxidase activity was tested using oxidase reagent (bioMérieux) according to the instructions of the manufacturer.

The cells were Gram-negative, rod-shaped, non-spore-forming, non-fluorescent and oxidase-positive. Cell morphology and other details are given in the species description.

The 16S rRNA gene was analysed as described previously (Kämpfer *et al.*, 2003). Analysis of the sequence data, including multiple alignment, was performed by using the software package MEGA version 4.0 (Tamura *et al.*, 2007). A distance-matrix method (distance options according to Kimura's two-parameter model) using clustering with the neighbour-joining method (Fig. 1) as well as a discrete character-based maximum-parsimony method (data not shown) were used. In each case, bootstrap values were calculated based on 1000 replications. The 16S rRNA gene fragment obtained from strain Sa14^T was a continuous stretch of 1346 bp. Sequence similarity calculations indicated that strain Sa14^T showed the highest degree of similarity to the type strains of *D. lusatiensis* (96.9 %) and

The GenBank/EMBL/DBJ accession number for the 16S rRNA gene sequence of strain Sa14^T is FM210786.

Fig. 1. Phylogenetic analysis based on 16S rRNA gene sequences available from the EMBL database (accession numbers are given in parentheses). Bootstrap values based on 1000 replications are listed as percentages at branching points. Bar, 0.01 substitutions per nucleotide position.

A. defluvii (96.0 %). The 16S rRNA gene similarity between *D. lusatiensis* DSM 11099^T and *A. defluvii* DSM 11603^T was 98.3 %. Lower sequence similarities (<95.5 %) were found with members of the genera *Hoeflea* (Peix *et al.*, 2005), *Pseudaminobacter* (Kämpfer *et al.*, 1999) and *Nitrateductor* (Labbé *et al.*, 2004) of this lineage.

Chemotaxonomic analyses were performed as described by Collins (1985) (respiratory quinones) and Kämpfer & Kroppenstedt (1996) (fatty acids). Although respiratory quinones have low resolution within this group, the presence of Q-10 supports the affiliation of strain Sa14^T to this group, where all species investigated to date have Q-10 as the major quinone.

The fatty acid profile of strain Sa14^T revealed 18:1 ω 7c and 19:0 cyclo ω 8c as predominant fatty acids; 11-methyl 18:1 ω 7c and 16:0 were present in significant amounts and iso-15:0 3-OH was the major hydroxy fatty acid (Table 1). This is essentially in agreement with the fatty acid patterns of *A. defluvii* DSM 11603^T and *D. lusatiensis* DSM 11099^T, for which 18:1 ω 7c and 19:0 cyclo ω 8c were also the predominant fatty acids; however, it is obvious that latter two type strains were more similar to each other in their fatty acid profiles than either was to strain Sa14^T.

The ratio of the two dominant fatty acids (18:1 ω 7c and 19:0 cyclo ω 8c) differed considerably from the ratio originally reported for *D. lusatiensis* S1^T (Lechner *et al.*, 1995), probably because of different growth phases of the cells analysed. This phenomenon can be explained by the mechanism of cyclopropane ring formation in fatty acids, which has been described in detail by Grogan & Cronan (1997). Detailed fatty acid profiles are given in Table 1.

Results of the physiological characterization of Sa14^T are given in the species description, using methods that were described previously (Kämpfer *et al.*, 1991). Strain Sa14^T was not able to utilize carbohydrates, organic acids or amino acids under the chosen conditions. Few chromogenic substrates were hydrolysed (Table 2).

Table 1. Fatty acid profiles of the type strains of *Aquamicrobium* species

Strains: 1, *A. defluvii* DSM 11603^T; 2, *A. lusatiensis* comb. nov. DSM 11099^T; 3, *A. aerolatum* sp. nov. Sa14^T. Data were obtained in this study; values are percentages of total fatty acids. Strains were grown on TSA at 28 °C for 48 h prior to analysis. ND, Not detected.

Fatty acid	1	2	3
12:0 3-OH	0.5	0.4	ND
15:0	ND	ND	0.8
Summed feature 2*	ND	ND	0.8
Summed feature 3*	0.4	0.6	0.7
16:0	3.7	5.0	5.2
iso-15:0 3-OH	ND	ND	3.0
17:1 ω 8c	1.2	0.8	1.4
17:1 ω 6c	1.4	ND	1.5
17:0 cyclo	ND	1.8	ND
17:0	2.5	0.6	6.9
18:1 ω 7c	36.7	28.6	27.4
18:0	3.5	1.6	1.7
11-Methyl 18:1 ω 7c	0.7	1.7	10.5
19:0 cyclo ω 8c	46.8	53.7	33.9
20:2 ω 6,9c	0.6	1.2	0.9
20:1 ω 7c	2.1	2.1	ND

*Summed feature 2 contains 12:0 alde and/or an unknown fatty acid; summed feature 3 contains 16:1 ω 7c and/or iso-15:0 2-OH.

Table 2. Differentiating characters of the type strains of *Aquamicrobium* species

Strains: 1, *A. defluvii* DSM 11603^T; 2, *A. lusatiensis* comb. nov. DSM 11099^T; 3, *A. aerolatum* sp. nov. Sa14^T. None of the substrates analysed by Kämpfer *et al.* (1991) were utilized. Tests were read after 48 h of incubation with the exception of hydrolysis of chromogenic substrates, which was read after 24 h. pNP, *p*-Nitrophenyl. All three strains hydrolysed L-alanine *p*-nitroanilide.

Test	1	2	3
Acid formation from:			
Glucose	+	+	–
D-Mannitol	+	–	–
myo-Inositol	–	+	–
Hydrolysis of:			
pNP phenylphosphonate	–	–	+
pNP phosphorylcholine	–	–	+
Assimilation of:			
N-Acetyl-D-galactosamine	–	+	–
Glucose	+	+	–
Adonitol	+	–	–
Glutarate	–	+	–
DL-3-Hydroxybutyrate	–	+	–
DL-Lactate	–	+	–

DNA–DNA hybridization experiments were performed between Sa14^T and *A. defluvii* DSM 11603^T and *D. lusatiensis* DSM 11099^T using the method described by Ziemke *et al.* (1998) except that, for nick translation, 2 µg DNA was labelled during a 3 h incubation at 15 °C. Strain Sa14^T showed relatively low DNA–DNA relatedness to *A. defluvii* DSM 11603^T (22.8 %) and *D. lusatiensis* DSM 11099^T (22.9 %).

From the results of 16S rRNA gene sequence comparison and DNA–DNA hybridization studies and from the chemotaxonomic and physiological characteristics, it is evident that strain Sa14^T is not a member of either of the species *A. defluvii* or *D. lusatiensis*.

All three strains show a remarkable congruence in phenotypic characters. They all produce Q-10 as the major menaquinone. The fatty acid profiles are very similar, being composed mainly of 18:1 ω 7c and 19:0 cyclo ω 8c (Table 1). Only a few differences in cell morphology and certain physiological tests are apparent (Table 2).

For these reasons, we propose to reclassify *Defluviobacter lusatiensis* to the genus *Aquamicrobium* as *Aquamicrobium lusatiense* comb. nov. A novel species, *Aquamicrobium aerolatum* sp. nov., is described to accommodate strain Sa14^T.

Description of *Aquamicrobium lusatiense* comb. nov.

Basonym: *Defluviobacter lusatiensis* Fritsche *et al.* 1999.

The description is as given by Fritsche *et al.* (1999a) with the following amendments. Ubiquinone 10 is the major quinone type. Predominant fatty acids are 18:1 ω 7c and 19:0 cyclo ω 8c. The type strain is strain S1^T (=CIP 106844^T =DSM 11099^T).

Description of *Aquamicrobium aerolatum* sp. nov.

Aquamicrobium aerolatum (ae.ro.la'tum. Gr. n. *aer* air; L. part. adj. *latus* -a -um carried; N.L. neut. part. adj. *aerolatum* airborne).

Cells are Gram-negative, aerobic, rod-shaped and motile, 0.3–0.5 µm long and 1.5–2 µm wide. Aerobic and oxidase-positive. Good growth after 48 h on nutrient agar and TSA. Colonies on nutrient agar are smooth, greyish white, translucent and shiny with entire edges. The type strain is unable to grow at 5 or 2 °C. Growth occurs at pH 5.5–10 and 0.9–7 % NaCl (w/v). The detailed fatty acid profile is given in Table 1. The type strain is not able to utilize carbohydrates, organic acids or amino acids under the conditions tested. Positive results for tests for alkaline phosphatase and L-alanine aminopeptidase activities.

The type strain is Sa14^T (=CCUG 57044^T =DSM 21857^T), isolated from air sampled in a duck shed.

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