Aquaspirillum dispar Hylemon *et al.* 1973 and *Microvirgula aerodenitrificans* Patureau *et al.* 1998 are subjective synonyms

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The 16S rDNA sequences of [*Aquaspirillum*] *dispar* LMG 4329^T and *Microvirgula aerodenitrificans* SGLY2^T (= LMG 18919^T) were found to be very similar (> 99 %). DNA–DNA hybridizations between the two strains revealed a high level of DNA–DNA binding (84 %), showing that they represent a single species. *M. aerodenitrificans* and [*A.*] *dispar* were also phenotypically very similar. It is concluded that [*A.*] *dispar* and *M. aerodenitrificans* are subjective synonyms. As [*A.*] *dispar* was wrongly assigned to the genus *Aquaspirillum*, we propose that strains of [*A.*] *dispar* must be reclassified in the genus *Microvirgula*. The name *Microvirgula aerodenitrificans* must be retained for the unified taxon since it is the type of the genus *Microvirgula*.

The name *Aquaspirillum dispar* was proposed by Hylemon *et al.* (1973) for two aerobic freshwater spirilla with a G + C content of 63–65 mol% and the ability to reduce nitrate beyond the level of nitrite. Later studies on intra- and intergeneric relationships and chemotaxonomic characteristics of various species of *Aquaspirillum* showed that *A. dispar* should be removed from the genus *Aquaspirillum* and that a new genus should be created for organisms belonging to this species (Pot *et al.*, 1992; Hamana *et al.*, 1994; Sakane & Yokota, 1994). In this study, we determined the 16S rDNA sequence of [*A.*] *dispar* LMG 4329^T, compared it with all accessible 16S rDNA sequences in the EMBL database and found a high level of similarity (99.4 %) to the sequence of *Microvirgula aerodenitrificans* SGLY2^T (= LMG 18919^T). *M. aerodenitrificans* was created by Patureau *et al.* (1998) for an aerobic, denitrifying Gram-negative bacterium that could not be assigned at that time to any recognized genus on the basis of its 16S rDNA sequence. In the present note, we report on a polyphasic taxonomic study that demonstrates that [*A.*] *dispar* and *M. aerodenitrificans* belong in the same species.

[*A.*] *dispar* strains LMG 4329^T and LMG 4330 and *M. aerodenitrificans* LMG 18919^T were grown on medium M8 (Janssens *et al.*, 1998) or trypticase soy agar (TSA; BBL) and incubated at 28 °C. DNA for 16S rDNA sequence analysis was prepared by alkali extraction as described by Niemann *et al.* (1997). DNA for determination of the DNA base composition and hybridization analysis was prepared by the

method of Pitcher *et al.* (1989). To obtain DNA solutions free of RNA, an RNase treatment was performed, followed by a chloroform extraction. The 16S rDNA sequence of [*A.*] *dispar* LMG 4329^T was determined as described by Cleenwerck *et al.* (2002). The sequencing primers used were Gamma, *Gamma, PD, *PD, 3, *3 and *O (Coenye *et al.*, 1999). The 16S rDNA sequence of LMG 4329^T was compared with all accessible 16S rDNA sequences in the EMBL database and aligned with the 16S rDNA sequences of the phylogenetically closest species using the GeneCompar 2.1 software package (Applied Maths). Unknown bases were discarded from the calculations. The G + C content of the DNA was determined by HPLC according to the method of Mesbah *et al.* (1989) using a Waters Symmetry Shield RP₈ column thermostatted at 37 °C. DNA–DNA hybridizations were performed using a modification of the microplate method described by Ezaki *et al.* (1989) (Goris *et al.*, 1998; Cleenwerck *et al.*, 2002). The hybridization temperature was 45 °C. Gram staining and oxidase and catalase activity were determined as described by Cleenwerck *et al.* (2002). API 20 NE (bioMérieux) and Biolog GN2 analyses were performed according to the manufacturers' instructions. Cellular fatty acid methyl ester extraction and analyses from cells grown on TSA (BBL) for 24 h at 28 °C were performed according to the recommendations of the MIDI system (Microbial Identification System).

The 16S rDNA sequence of [*A.*] *dispar* LMG 4329^T (1490 bases) was 99.4 % similar to the 16S rDNA sequence of *M. aerodenitrificans* SGLY2^T (accession no. U89333). Sequence similarities to other published taxa belonging to the β -Proteobacteria were below 95 %. The closest taxa were *Laribacter hongkongensis* (accession no. AF389085; 94.3 %), *Vogesella indigofera* (accession no. AB021385; 91.8 %),

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The EMBL/GenBank/DBJ accession number for the 16S rDNA sequence of strain LMG 4329^T is AJ487013.

Table 1. Fatty acid compositions of the type strains of *[A.] dispar* and *M. aerodenitrificans*

Values are percentages of total fatty acids; values less than 1 % are not given.

Fatty acid	<i>[A.] dispar</i> LMG 4329 ^T	<i>M. aerodenitrificans</i> LMG 18919 ^T
12:0	7.1	7.0
12:0 3-OH	9.0	9.6
14:0	1.4	—
15:0	1.3	1.1
16:0	24.2	21.6
17:1ω6c	2.0	1.9
18:1ω7c	14.6	17.4
Summed feature 3*	38.5	38.3

*Summed feature 3 comprises 16:1ω7c and/or 15:0 iso 2-OH.

Table 2. Phenotypic characteristics that differentiate *M. aerodenitrificans* from its closest phenotypic and phylogenetic relatives

Species: 1, *M. aerodenitrificans*; 2, *Comamonas testosteroni* [data from Patureau *et al.* (1998) unless stated]; 3, *Pseudomonas alcaligenes* [Yuen *et al.* (2001) unless stated]; 4, *Laribacter hongkongensis* (Yuen *et al.*, 2001). *Comamonas testosteroni* and *P. alcaligenes* are the closest phenotypic relatives of *M. aerodenitrificans* and *L. hongkongensis* is its closest phylogenetic relative. Characters are scored as: +, positive; V, variable; —, negative; ND, not determined.

Characteristic	1	2	3	4
Motility	+	+	+	—
Denitrification:				
Nitrate→nitrite	+	+ ^{a*}	V ^c	+
Nitrite→N ₂	+	— ^a	— ^c	ND
Arginine dehydrolase	+	—	—	+
Urease	—	— ^b	—	+

*Data taken from: a, Willems *et al.* (1991); b, Yuen *et al.* (2001); c, Yumoto *et al.* (2001).

Zoogloea ramigera (accession no. X74913; 90.4 %) and *Chromobacterium violaceum* (accession no. AJ247211; 86.3 %). The DNA G+C contents of *[A.] dispar* LMG 4329^T and *M. aerodenitrificans* LMG 18919^T were respectively 64.3 and 64.5 mol%. The DNA–DNA binding value between LMG 4329^T and LMG 18919^T was 84 %, showing that the two strains belong to the same species. In addition, the target site of a specific probe developed by Patureau *et al.* (1998) for *M. aerodenitrificans* LMG 18919^T, within the V4 variable region of the 16S rRNA (positions 636–653; *Escherichia coli* numbering), was also present in the 16S rDNA sequence of *[A.] dispar* LMG 4329^T.

Comparison of the morphological, physiological and biochemical characteristics of *M. aerodenitrificans* LMG

18919^T and *[A.] dispar* strains LMG 4329^T and LMG 4330 (the latter two strains showing 100 % DNA–DNA relatedness; Aragno *et al.*, 1978) showed that these strains were phenotypically highly similar. Cells were Gram-negative, motile, curved to rod-shaped, occurring singly or in pairs. All three strains were oxidase- and catalase-positive. In API 20 NE tests, the three strains gave identical reactions. They were able to denitrify, assimilate caprate and malate and hydrolyse arginine. According to the API 20 NE identification system, the strains are phenotypically highly similar to *Comamonas testosteroni* and *Pseudomonas alcaligenes*, with only arginine dehydrolase as a differentiating feature. The three strains shared nearly identical carbon source oxidation patterns, as determined by the Biolog identification system. All strains were able to oxidize a large spectrum of carbon sources, except sugars. Some minor quantitative differences were found in the oxidation of glycogen, alaninamide, the organic acids *cis*-aconitic acid and α-ketovaleric acid and the amino acids hydroxy-L-proline, D-serine and L-threonine. For *M. aerodenitrificans* LMG 18919^T, the Biolog results were similar to those obtained by Patureau *et al.* (1998) except that α-ketoglutaric acid, malonic acid, D-alanine and L-threonine were oxidized, while sebacic acid, L-leucine and glycerol were not. The cellular fatty acid profiles of LMG 4329^T and LMG 18919^T were very similar, with summed feature 3, 16:0 and 18:1 as the major non-polar fatty acid compounds and 12:0 3-OH as the only 3-hydroxy fatty acid (Table 1). Analogous results were obtained by Sakane & Yokota (1994) for *[A.] dispar* and, in their study, it was shown that this profile was not found in other *Aquaspirillum* species. Phenotypic characteristics that enable the differentiation of LMG 4329^T, LMG 4330 and LMG 18919^T from the phylogenetically and phenotypically most related species are given in Table 2.

The genotypic and phenotypic data given above clearly demonstrate that *[A.] dispar* and *M. aerodenitrificans* belong in the same species. Since *[A.] dispar* is not a genuine member of the genus *Aquaspirillum*, its members should be transferred to the genus *Microvirgula*. According to Rule 15 of the International Code of Nomenclature of Bacteria (Lapage *et al.*, 1992), the name *Microvirgula aerodenitrificans* must be retained for the unified taxon, since it is the type of the genus *Microvirgula*.

Emended description of *Microvirgula aerodenitrificans* Patureau *et al.* 1998

The description of the species is that given by Patureau *et al.* (1998), emended by the additional features described in the current paper. The species includes strains previously classified as *Aquaspirillum dispar*. The type strain is strain SGLY2^T (=DSM 15089^T=LMG 18919^T).

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