Reclassification of *Aquaspirillum itersonii* and *Aquaspirillum peregrinum* as *Novispirillum itersonii* gen. nov., comb. nov. and *Insolitispirillum peregrinum* gen. nov., comb. nov.

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Phylogenetic analysis based on 16S rRNA gene sequences showed that *Aquaspirillum itersonii* and *Aquaspirillum peregrinum* form distinct phylogenetic lineages within the Alphaproteobacteria, whereas *Aquaspirillum serpens*, the type species of the genus *Aquaspirillum*, belongs to the Betaproteobacteria. *A. itersonii* and *A. peregrinum* exhibited 16S rRNA gene sequence similarity values of 82.0–82.4% to the type strain of *A. serpens* and of 91.8–92.0% to each other. *A. itersonii* and *A. peregrinum* were clearly distinguishable from *A. serpens* by differences in ubiquinone types and fatty acid profiles. *A. itersonii* subsp. *itersonii* LMG 4337\(^\mathrm{T}\) and *A. itersonii* subsp. *nipponicum* LMG 7370\(^\mathrm{T}\) contained Q-10 as the predominant ubiquinone, and *A. peregrinum* subsp. *peregrinum* LMG 4340\(^\mathrm{T}\) and *A. peregrinum* subsp. *integrum* LMG 5407\(^\mathrm{T}\) contained Q-9 as the predominant ubiquinone, whereas *A. serpens* LMG 3734\(^\mathrm{T}\) had Q-8 as the predominant ubiquinone. *A. itersonii* and *A. peregrinum* were also distinguishable from *A. serpens* by some differences in the fatty acid composition, including major fatty acids and hydroxy fatty acids. On the basis of these data, *A. itersonii* and *A. peregrinum* should be reclassified into two novel genera and species, for which the names *Novispirillum itersonii* gen. nov., comb. nov. and *Insolitispirillum peregrinum* gen. nov., comb. nov., respectively, are proposed.

The genus *Aquaspirillum* was created by Hylemon *et al.* (1973) with the descriptions of 13 species and, subsequently, further *Aquaspirillum* species have been described (Kumar *et al.*, 1974; Strength *et al.*, 1976; Aragno & Schlegel, 1978; Maratea & Blakemore, 1981; Butler *et al.*, 1989). However, many *Aquaspirillum* species have been transferred to other genera or reclassified as members of novel genera (Schleifer *et al.*, 1991; Pot *et al.*, 1992; Cleenwerck *et al.*, 2003; Wauters *et al.*, 2003; Ding & Yokota, 2004; Spring *et al.*, 2004; Grabovich *et al.*, 2006). *Aquaspirillum itersonii* and *Aquaspirillum peregrinum* were also found to be phylogenetically related more closely to the Alphaproteobacteria than to the Betaproteobacteria, to which the type species of the genus *Aquaspirillum*, *Aquaspirillum serpens*, belongs. *A. itersonii* and *A. peregrinum* were described by Hylemon *et al.* (1973) as a result of the reclassification of *‘Spirillum itersonii’* (Giesberger, 1936) and *‘Spirillum peregrinum’* (Pretorius, 1963), respectively. *‘S. itersonii’ subsp. *nipponicum’* and *‘S. peregrinum’ subsp. *integrum’*, described by Terasaki (1973), were reclassified as *A. itersonii* subsp. *nipponicum* and *A. peregrinum* subsp. *integrum* by Terasaki (1979). *A. itersonii* and *A. peregrinum* were placed into the genus *Aquaspirillum* on the basis of morphological, physiological and nutritional characteristics and DNA base compositions (Hylemon *et al.*, 1973; Terasaki, 1979). Accordingly, the aim of the present work was to determine the exact taxonomic positions of *A. itersonii* and *A. peregrinum* by a polyphasic characterization that included the determination of phenotypic and chemotaxonomic properties and a detailed phylogenetic analysis based on newly determined 16S rRNA gene sequences.

*A. itersonii* subsp. *itersonii* LMG 4337\(^\mathrm{T}\), *A. itersonii* subsp. *nipponicum* LMG 7370\(^\mathrm{T}\), *A. peregrinum* subsp. *peregrinum* LMG 4340\(^\mathrm{T}\), *A. peregrinum* subsp. *integrum* LMG 5407\(^\mathrm{T}\) and *A. serpens* LMG 3734\(^\mathrm{T}\) were obtained from the Laboratorium voor Microbiologie Universiteit Gent (LMG), Gent, Belgium. To investigate their physiological and biochemical characteristics, *A. itersonii* and *A. peregrinum* strains were cultivated routinely at 28 °C in the online version of this paper.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains LMG 4337\(^\mathrm{T}\), LMG 7370\(^\mathrm{T}\), LMG 4340\(^\mathrm{T}\) and LMG 5407\(^\mathrm{T}\) determined in this study are EF612765–EF612768, respectively.

Two-dimensional thin-layer chromatograms of polar lipids are available with the online version of this paper.
LMG medium no. 8, which contained (l distilled water)\(^{-1}\): 1 g succinic acid, 10 g peptone, 1 g (NH\(_4\))\(_2\)SO\(_4\), 1 g MgSO\(_4\) \(\cdot\) 7H\(_2\)O, 2 mg FeCl\(_3\) \(\cdot\) 6H\(_2\)O, 2 mg MnSO\(_4\) \(\cdot\) H\(_2\)O and 15 g agar, pH 7.0. Growth under anaerobic conditions was determined after incubation in a Forma anaerobic chamber on solid LMG medium no. 8 and on solid LMG medium no. 8 supplemented with potassium nitrate (0.1 %, w/v), both of which had been prepared under a nitrogen atmosphere. Growth at various temperatures (4–45°C) was measured on solid LMG medium no. 8.

Catalase and oxidase activities and hydrolysis of casein, gelatin, hypoxanthine, starch, Tweens 20, 40, 60 and 80, tyrosine, urea and xanthine were determined as described by Cowan & Steel (1965). Aesculin hydrolysis and nitrate reduction were studied as described by Lanyi (1987). Susceptibility to antibiotics was tested on solid LMG medium no. 8 supplemented with potassium nitrate (0.1 %, w/v), both of which had been prepared under a nitrogen atmosphere. Growth at various temperatures (4–45°C) was measured on solid LMG medium no. 8.

Cell biomass for DNA extraction and for analyses of isoprenoid quinones and polar lipids was obtained from cultivation in liquid LMG medium no. 8 at 28°C. Chromosomal DNA was isolated and purified according to the method described by Yoon et al. (1996), with the exception that RNase T1 was used in combination with RNase A to minimize RNA contamination. The 16S rRNA gene was amplified by PCR using two universal primers, 5\(^\prime\)-GAGTTTGATCCTGGCTCAG-3\(^\prime\) and 5\(^\prime\)-AGAAAGGAGGTGATCCAGCC-3\(^\prime\), as described previously (Yoon et al., 1998). Sequencing of the amplified 16S rRNA gene and phylogenetic analysis were performed as described previously (Yoon et al., 2003). Isoprenoid quinones were analysed by the method of Komagata & Suzuki (1987), using reversed-phase HPLC. For cellular fatty acid analysis,
Table 1. Percentage cellular fatty acid compositions of Aquaspirillum itersonii, Aquaspirillum peregrinum and Aquaspirillum serpens from this study

Strains: 1, A. itersonii subsp. itersonii LMG 4337T; 2, A. itersonii subsp. nipponicum LMG 7370T; 3, A. peregrinum subsp. peregrinum LMG 4340T; 4, A. peregrinum subsp. integrum LMG 5407T; 5, A. serpens LMG 3734T. Fatty acids that represented <0.5% in all strains were omitted.

<table>
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<tr>
<th>Fatty acid</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
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<td>Straight chain</td>
<td></td>
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<tr>
<td>C12:0</td>
<td>3.6</td>
<td>3.7</td>
<td>2.9</td>
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<td>4.4</td>
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<tr>
<td>C14:0</td>
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<td>0.8</td>
<td>0.9</td>
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<tr>
<td>C15:0</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td>C16:0</td>
<td>13.9</td>
<td>5.2</td>
<td>9.0</td>
<td>15.6</td>
<td>18.8</td>
</tr>
<tr>
<td>C17:0</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>0.6</td>
</tr>
<tr>
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<td>1.1</td>
<td>0.4</td>
<td>2.0</td>
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<td>8.0</td>
<td>–</td>
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<td>C15:1ω6c</td>
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<td>–</td>
<td>–</td>
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<td>–</td>
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<td>–</td>
<td>5.5</td>
<td>–</td>
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<tr>
<td>C16:0 3-OH</td>
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<td>2.6</td>
<td>2.5</td>
<td>2.3</td>
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<tr>
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<tr>
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<td>Summed features†</td>
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<tr>
<td>2</td>
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<td>3.0</td>
<td>3.0</td>
<td>2.8</td>
<td>–</td>
</tr>
<tr>
<td>3</td>
<td>16.7</td>
<td>7.9</td>
<td>14.5</td>
<td>15.2</td>
<td>51.4</td>
</tr>
</tbody>
</table>

*ECL, Equivalent chain length.
†Summed features represent groups of two or three fatty acids that could not be separated by GLC with the MIDI system. Summed feature 2 contained iso-C16:1ω and/or C14:0 3-OH. Summed feature 3 contained C16:1ω7c and/or iso-C15:0 2-OH.

cell biomass of the five strains was harvested from solid LMG medium no. 8 plates after incubation for 3 days at 28 °C. Fatty acids were extracted and fatty acid methyl esters were prepared according to the standard protocol of the MIDI/Hewlett Packard Microbial Identification system (Sasser, 1990). Polar lipids were extracted according to Minnikin et al. (1984) and identified by two-dimensional TLC after spraying with appropriate detection reagents (Minnikin et al., 1984; Komagata & Suzuki, 1987). The DNA G+C content was determined by the method of Tamaoka & Komagata (1984), with a modification that DNA was hydrolysed by using nuclease P1 (Sigma) and the resultant nucleotides were analysed by reversed-phase HPLC.

Almost-complete 16S rRNA gene sequences of A. itersonii subsp. itersonii LMG 4337T, A. itersonii subsp. nipponicum LMG 7370T, A. peregrinum subsp. peregrinum LMG 4340T and A. peregrinum subsp. integrum LMG 5407T determined in this study each comprised 1442 nt. There were five nucleotide differences in the sequences between A. itersonii subsp. itersonii LMG 4337T and A. itersonii subsp. nipponicum LMG 7370T. There were two nucleotide differences between A. peregrinum subsp. peregrinum LMG 4340T and A. peregrinum subsp. integrum LMG 5407T. In the phylogenetic tree, A. itersonii subsp. itersonii LMG 4337T, A. itersonii subsp. nipponicum LMG 7370T, A. peregrinum subsp. peregrinum LMG 4340T and A. peregrinum subsp. integrum LMG 5407T formed distinct phylogenetic lineages within the Alphaproteobacteria and were related distinctly to the clade comprising A. serpens, the type species of the genus Aquaspirillum, which belongs to the Betaproteobacteria (Fig. 1). A. itersonii and A. peregrinum showed low 16S rRNA gene sequence similarity values of 91.8–92.0% to each other, and similarity values of 82.2–82.4% and 82.0% to the type strain of A. serpens, respectively.

The predominant isoprenoid quinone detected in A. itersonii subsp. itersonii LMG 4337T and A. itersonii subsp. nipponicum LMG 7370T was ubiquinone-10 (Q-10) at a peak area ratio of approximately 90–92%. The predominant isoprenoid quinone detected in A. peregrinum subsp. peregrinum LMG 4340T and A. peregrinum subsp. integrum LMG 5407T was ubiquinone-9 (Q-9) at a peak area ratio of approximately 89–90%. A. serpens LMG 3734T had Q-8 as the predominant ubiquinone, at a peak area ratio of approximately 95%. The same results were obtained by Sakane & Yokota (1994). Accordingly, A. itersonii and A. peregrinum could be distinguished clearly from A. serpens and from each other by differences in the predominant ubiquinone types. The cellular fatty acid profiles also distinguish A. itersonii and A. peregrinum from A. serpens (Table 1). A. itersonii and A. peregrinum contain C18:1ω7c as the major fatty acid, whereas A. serpens contains C16:1ω7c and/or iso-C15:0 2-OH (Table 1). A. itersonii and A. peregrinum were also distinguishable from A. serpens by some differences in fatty acid composition, including hydroxy fatty acids (Table 1), as also shown by the study of Sakane & Yokota (1994). The phylogenetic and chemotaxonomic data suggest that A. itersonii and A. peregrinum should be placed in two different genera that are distinct from the genus Aquaspirillum. Therefore, we propose to reclassify A. itersonii and A. peregrinum as two novel genera and species, Novispirillum itersonii gen. nov., comb. nov. and Insolitispirillum peregrinum gen. nov., comb. nov., respectively.

Description of Novispirillum gen. nov.

Novispirillum (No’vi.spi.ri.l’um. L. adj. novus new; N.L. dim. neut. n. spirillum a small spiral; N.L. neut. n. Novispirillum a new small spiral).

Cells are Gram-negative and helical. Catalase- and oxidase-positive. Nitrate is reduced to nitrogen gas. The predominant ubiquinone is Q-10. The major fatty acid is C18:1ω7c. The type species is Novispirillum itersonii (Giesberger 1936).
**Description of Novispirillum itersonii** (Giesberger 1936) **comb. nov.**

*Novispirillum itersonii* (i.ter.so’ni.i. N.L. gen. n. *itersonii* named after G. Van Iterson, a Dutch bacteriologist).

Basonym: *Aquaspirillum itersonii* (Giesberger 1936).

The description is as that given by Hylemon *et al.* (1973) and Terasaki (1979). Characteristics of the type strain determined in this study are given in Table 2.

**Description of Novispirillum itersonii subsp. itersonii** (Giesberger 1936) **comb. nov.**

*Novispirillum itersonii* subsp. *itersonii* (i.ter.so’ni.i. N.L. gen. n. *itersonii* named after G. Van Iterson, a Dutch bacteriologist).


The description is as that given by Hylemon *et al.* (1973). Characteristics of the type strain determined in this study
are given in Table 2. The type strain is ATCC 12639<sup>T</sup>=LMG 4337<sup>T</sup>=CCUG 49447<sup>T</sup>.

**Description of Novispirillum itersonii subsp. nipponicum** (Terasaki 1973) comb. nov.

Novispirillum itersonii subsp. nipponicum (nip.po’ni.cum. N.L. neut. adj. nipponicum pertaining to the country of Japan).


The description is as that given by Terasaki (1979). Characteristics of the type strain determined in this study are given in Table 2. The type strain is ATCC 33334<sup>T</sup>=LMG 5407<sup>T</sup>=CCUG 49449<sup>T</sup>=DSM 11589<sup>T</sup>.

**Description of Insolitispirillum gen. nov.**

*Insolitispirillum* (In.so.li’ti.spi.ri.l’lum. L. adj. insolitus unaccustomed; N.L. dim. neut. *n. spirillum* a small spiral; N.L. neut. *n*. *Insolitispirillum* an unaccustomed small spiral).

Cells are Gram-negative and helical. Catalase- and oxidase-positive. Nitrate is not reduced. The predominant ubiquinone is Q-9. The major fatty acid is C<sub>18</sub>:1ω7c. The type species is *Insolitispirillum peregrinum* (Pretorius 1963).

**Description of Insolitispirillum peregrinum** (Pretorius 1963) comb. nov.

*Insolitispirillum peregrinum* (pe.re.gri’num. L. neut. adj. *peregrinum* strange, foreign).


The description is as that given by Hylemon et al. (1973) and Terasaki (1979). Characteristics of the type strain determined in this study are given in Table 2.

**Description of Insolitispirillum peregrinum subsp. peregrinum** (Pretorius 1963) comb. nov.


The description is as that given by Hylemon et al. (1973). Characteristics of the type strain determined in this study are given in Table 2. The type strain is ATCC 15387<sup>T</sup>=LMG 4340<sup>T</sup>=CCUG 13795<sup>T</sup>=DSM 1839<sup>T</sup>.

**Description of Insolitispirillum peregrinum subsp. integrum** (Terasaki 1973) comb. nov.


The description is as that given by Terasaki (1979). Characteristics of the type strain determined in this study are given in Table 2. The type strain is ATCC 33334<sup>T</sup>=LMG 5407<sup>T</sup>=CCUG 49449<sup>T</sup>=DSM 11589<sup>T</sup>.

**Acknowledgements**

This work was supported by the 21C Frontier Program of Microbial Genomics and Applications (grant MG05-0401-2-0) from the Ministry of Science and Technology (MOST) of the Republic of Korea.

**References**


