Phylogeny of the ring-forming bacterium *Arcicella aquatica* gen. nov., sp. nov. (ex Nikitin *et al.* 1994), from a freshwater neuston biofilm

Denis I. Nikitin,¹ Carsten Strömpl,² M. S. Oranskaya¹
and Wolf-Rainer Abraham²

Correspondence
Wolf-Rainer Abraham
wab@gbf.de

¹Institute of Microbiology, Russian Academy of Sciences, Prospect 60-Letiya Otyabrya 7, korp. 2, Moscow 117811, Russia

²GBF – National Research Center for Biotechnology, Dept Environmental Microbiology, Mascheroder Weg 1, 38124 Braunschweig, Germany

Arcicella aquatica NO-502^T, obtained from a neuston film on a freshwater lake and belonging to the phylum *Bacteroidetes*, is characterized by ring-forming cells. The bacterium is a strict aerobe, with optimal growth between 28 and 30 °C. Carbohydrates, but no organic acids or amino acids, are used as substrates. The G + C content of strain NO-502^T is 34.5 mol%; its genome size is 2.9×10^9 Da. The genus *Arcicella* and its type species *Arcicella aquatica* (type strain NO-502^T = LMG 21963^T = CIP 107990^T) are proposed, and descriptions of this genus and species are given.

From a freshwater neuston film a slow-growing bacterial isolate, NO-502^T, was obtained which formed rings consisting of several bacterial cells. Such a morphology is also known from some other bacterial genera, including *Flectobacillus*. In 1994, Nikitin and colleagues described this isolate as '*Arcocella aquatica*' (Nikitin *et al.*, 1994); however, no type species was deposited in a public culture collection and the description remained invalid. The aim of this note is to describe the genus and species validly and to give further information on the phylogenetic position of the type strain, NO-502^T.

Strain NO-502^T was isolated on a medium containing 0.1 % peptone, yeast extract and glucose each at pH 7.0–7.2 from a neuston film of a freshwater lake near Moscow, Russia. Cells are polymorphic, showing vibrioid, curved and spiral-shaped cells.

The sequence of the 16S rRNA gene of strain NO-502^T was determined as described previously (Abraham *et al.*, 1999). It was found that an identical sequence was reported for isolate AH57 (accession no. AJ289964) from Lake Fuchskuhle, Germany (Glöckner *et al.*, 2000). The latter isolate together with the sequence of uncultured bacterium GKS2-216 (accession no. AJ290033) from the same study

and the clone sequence GWF23A (accession no. AJ011696) from a cave water ecosystem could be considered to fall within the range of a common genus and to represent one or more additional species. These three undescribed organisms came, like strain NO-502^T, from freshwater ecosystems. Next relatives of this genus are species of the genus *Flectobacillus*, as shown in Fig. 1. The 16S rDNA sequence similarity between strain NO-502^T and the most similar published sequence of an organism with a validly published name, *Flectobacillus major*, is 93.52 %. Bootstrap analyses of neighbour-joining trees supported the phylogenetic association of *Arcicella aquatica* with *Flectobacillus major* (data not shown). The wide distribution of *Flexibacter* species throughout the phylum *Bacteroidetes* (Fig. 1) was discussed by Nakagawa *et al.* (2002). Nikitin *et al.* (1994), however, listed further genomic data which, together with our data, require the separation of strain NO-502^T from the genus *Flectobacillus*. The G + C content of strain NO-502^T was found to be 34.5 %, while a G + C content of 39.5–40.3 mol% has been reported for *Flectobacillus major* (Gosink *et al.*, 1998).

Using tandem mass spectrometry (MS) the structures of many polar lipids of strain NO-502^T could be elucidated using collision-induced decay MS by electrospray ionization (ESI) recorded in the negative mode [(-)-ESI] (Abraham *et al.*, 1997). The phospholipids contained 2-*N*-(2'-*D*-hydroxyisopentadecanoyl)amino-3-hydroxyisooheptadeca-4(*E*)-ene-1-phosphate. The phosphate was detected as [M-1]⁻ ion at *m/z* 604 in (-)-ESI MS. The corresponding aminoethyl derivative was detected at *m/z* 647 and the

Published online ahead of print on 9 January 2004 as DOI 10.1099/ij.s.0.02896-0.

Abbreviations: ESI, electrospray ionization; MS, mass spectrometry.

The GenBank/EMBL/DBJ accession number for the 16S rRNA gene sequence of *Arcicella aquatica* NO-502^T is AJ535729.

Fig. 1. Unrooted dendrogram based on the 16S rDNA sequence of *Arcicella aquatica* NO-502^T within the context of the phylum *Bacteroidetes*. Sequence data of reference strains of other genera published in the GenBank/EMBL (Stoesser *et al.*, 2003) and/or RDP (Cole *et al.*, 2003) databases were obtained through the ARB database release June 2000 (<http://www.arb-home.de>). Sequence data were aligned with the FASTALIGNER of the ARB program and adjusted manually. Jukes–Cantor evolutionary distances were estimated using pair-wise sequence dissimilarities calculated with DNADIST, and the dendrogram was created with the FITCH program of the implemented PHYLIP package (Felsenstein, 1989). Bar, 10 nucleotide substitutions per 100 bases.

choline compound at *m/z* 689. In addition, homologues to these phospholipids were detected. 2-D-(2'-D-Hydroxyisopentadecanoyl)amino-3-D-hydroxyisoheptadecane-1-sulfonic acid was the only sulfolipid found in strain NO-502^T. The ion of a glycolipid was seen at *m/z* 729 and identified as 2-N-(2''-hydroxyisopentadecanoyl)amino-3-hydroxyisoheptadeca-4(*E*)-ene-1-hydroxycarbonyl-6-deoxy-6-amino-mannopyranoside (Table 1). The latter compound has already been described from this strain and from

Flectobacillus major (Batrakov *et al.*, 1999). Furthermore, three ions at *m/z* 977, 1181 and 1310 that belonged to unknown lipids were found in the lipid extract of strain NO-502^T.

In the lipid extract of *Flectobacillus major*, phosphatidyl-ethylamine was found but not fully characterized; hence, a comparison with the three different compounds of this phospholipid from strain NO-502^T was not possible. While

Table 1. Polar lipids of *Arcicella aquatica* NO-502^T as determined by negative ESI quadrupole time-of-flight (ESI-QTOF) MS

Mass (Da)	Lipid
591	2-D-(2'-D-Hydroxyisopentadecanoyl)amino-3-D-hydroxyisoheptadecane-1-sulfonic acid
605	2-N-(2'-Hydroxyisopentadecanoyl)amino-3-hydroxyisoheptadeca-4(<i>E</i>)-ene-1-phosphate
648	2-N-(2'-Hydroxyisopentadecanoyl)amino-3-hydroxyisoheptadeca-4(<i>E</i>)-ene-1-(2''-aminoethyl)-phosphate
662	2-N-(2'-Hydroxyisopentadecanoyl)amino-3-hydroxyoctadeca-4(<i>E</i>)-ene-1-(2''-aminoethyl)-phosphate
675	1-Pentadecanoyl-2-hexadecenoylphosphatidyl-2'-ethylamine
687	1,2-bis-Hexadecenoylphosphatidyl-2'-ethylamine
689	1-Hexadecanoyl-2-hexadecenoylphosphatidyl-2'-ethylamine
730	2'-N-(2''-Hydroxyisopentadecanoyl)amino-3'-hydroxyisoheptadeca-4'(<i>E</i>)-ene-1-hydroxycarbonyl-6-deoxy-6-amino-mannopyranoside
978	Unknown
1182	Unknown
1311	Unknown

2-D-(2'-D-hydroxyisopentadecanoyl)amino-3-D-hydroxyisopentadecane-1-sulfonic acid and 2-N-(2'-hydroxyisopentadecanoyl)amino-3-hydroxyisopentadeca-4(E)-ene-1-(2''-aminoethyl)-phosphate were detected both in *Flectobacillus major* and in strain NO-502^T, their corresponding 2'-deoxy derivatives, the lipoamino acid N-[3-D-(isoheptadecanoyloxy)isoheptadecanoyl]-glycine and the lipopeptide N-{N'-[3''-D-(isoheptadecanoyloxy)isoheptadecanoyl]glycyl}-L-serine were absent from strain NO-502^T but present in *Flectobacillus major* (Batrakov *et al.*, 2000). The homologue 2-N-(2'-hydroxyisopentadecanoyl)amino-3-hydroxyoctadeca-4(E)-ene-1-(2''-aminoethyl)-phosphate not known from *Flectobacillus major* is reported here for the first time.

The cellular fatty acids consisted mainly of even-numbered saturated fatty acids, while C_{16:1ω7/t}, C_{18:1ω6} and C_{18:1ω7} were the main unsaturated fatty acids (Table 2). Strain NO-502^T has higher proportions of C_{14:0} and C_{16:0} than *Flectobacillus major*, while C_{15:1} and C_{17:1}, present in *Flectobacillus major*, are missing from strain NO-502^T (Nikitin *et al.*, 1994). The cellular fatty acid composition of strain NO-502^T is distinct from those of many type strains of the family *Spirosomaceae*, as NO-502^T has low amounts of branched fatty acids (Urakami & Komagata, 1986). However, these branched fatty acids are part of the sphingolipids of this strain, making it a clearly distinct member of this family.

Enzyme activities were determined using the API ZYM test and the results are given in Table 3. High activities of amino-acid-hydrolysing enzymes were found, even though strain NO-502^T could not grow on amino acids; no lipase activity was observed. In the API ZYM test, catalase, β-galactosidase and β-glucosidase activities were observed, but no nitrate was reduced, no acids were produced by fermentation on glucose and no protease nor urease activity was found (Table 3). The nutritional requirements of strain NO-502^T and *Flectobacillus major* were determined on a mineral medium [0.01 g KH₂PO₄ l⁻¹, 0.1 g K₂HPO₄ l⁻¹, 0.2 g (NH₄)₂HPO₄ l⁻¹, 0.03 g MgSO₄ l⁻¹, 0.01 g NaCl

l⁻¹, 0.01 g CaCl₂ l⁻¹, trace amounts of MnSO₄ and FeSO₄, 0.005 % yeast extract; pH 7.2] with concentrations of 0.1 % of the carbon sources (Nikitin *et al.*, 1994). The substrates used differently by these two strains have been listed in Table 3 and compared with other type species of the family *Spirosomaceae*.

The 16S rRNA gene sequence similarity (93.5 % and below) to related genera, and the differences in the polar lipids, the cellular fatty acids and biochemical and physiological characteristics between *Flectobacillus major* and strain NO-502^T together with the differences in their G + C contents do not allow the placement of these species in the same genus. Therefore, for strain NO-502^T *Arcicella* gen. nov. is proposed, with this strain as the type strain of *Arcicella aquatica* sp. nov.

Description of the genus *Arcicella* gen. nov.

Arcicella (Ar.ci.cel'la. L. masc. n. *arcus* the arc; L. fem. *cella* cell; N.L. fem. n. *Arcicella* arc-shaped cell).

Table 3. Biochemical characteristics of *Arcicella aquatica* NO-502^T compared with related taxa

Taxa: 1, *Arcicella aquatica* NO-502^T; 2, *Flectobacillus major* (Gosink *et al.*, 1998); 3, *Flexibacter flexilis*; 4, *Runella slithyiformis*; 5, *Spirosoma linguale* (Raj & Maloy, 1990). Scale is from 0 (no reaction) to 5 (strong reaction); W, weak reaction; -, no reaction; +, growth; ++, abundant growth. NA, Not available. Strain NO-502^T was unable to grow on monomethylamine, ethanol, alanine, glycine, valine, leucine, arginine, lysine, proline, cystine or tryptophan, whereas *Flectobacillus major* could (Nikitin *et al.*, 1994); no data are available for these substrates for taxa 3–5.

Assay	1	2	3	4	5
Alkaline phosphatase	5	+	NA	+	W
Esterase (C4)	3	-	NA	-	-
Acid phosphatase	5	4+	NA	+	W
β-Galactosidase	5	+	NA	+	+
Oxidase	NA	+	+	+	+
Catalase	+	W	-	W	+
Urease	-	+	NA	-	-
Aesculin hydrolysis	+	+	NA	-	NA
Acid production from glucose	-	+	+	+	+
Growth on:					
Succinate	-	+	NA	-	+
Dulcitol	-	+	NA	-	-
Acetate	+	-	NA	-	-
Propionate	-	++	NA	-	-
Pyruvate	-	++	NA	-	+
Tyrosine	-	++	NA	NA	NA
Glutamate	-	+	NA	-	+
Aspartate	+	+	NA	NA	NA

*From Larkin & Borrall (1984).

†From Nikitin *et al.* (1994).

‡From Raj & Maloy (1990).

Table 2. Polar lipid fatty acids (PL) and cellular fatty acids (TL) of strain NO-502^T

Values are given as a percentage of total fatty acids.

Fatty acid	PL	TL
C _{12:0}	-	7.2
C _{14:0}	-	14.7
C _{15:0}	6.0	5.3
C _{16:1ω7}	44.5	11.1
C _{16:1ω7t}	19.1	7.7
C _{16:0}	17.3	33.6
C _{18:1ω6}	13.1	7.2
C _{18:1ω7}	-	5.1
C _{18:0}	-	8.0

The description is based on the one published by Nikitin *et al.* (1994). Cells are vibrioid, measure 2.5–3.0 by 0.5–0.75 µm, and are stretched spirals or S-shaped. Colonies on solid media are mucous and light-orange pigmented. Growth occurs between 4 and 40 °C; the optimal growth temperature is 28–30 °C; optimal pH is around 7. Grows with NaCl concentrations between 5 and 60 g l⁻¹; above these concentrations and without NaCl no growth is observed. Aerobic, do not grow on C₁ compounds, amino acids or organic acids, but do grow on a wide range of carbohydrates. Biopolymers are not hydrolysed. Nitrate is not reduced. Main polar lipids are 2-*N*-(2'-hydroxyisopentadecanoyl)amino-3-hydroxyisooheptadeca-4(*E*)-ene-1-(2''-aminoethyl)-phosphate and its 3-hydroxyoctadeca-4(*E*)-ene homologues, phosphatidyl ethylamine, 2-*D*-(2'-*D*-hydroxyisopentadecanoyl)amino-3-*D*-hydroxyisooheptadecane-1-sulfonic acid and 2-*N*-(2''-hydroxyisopentadecanoyl)amino-3-hydroxyisooheptadeca-4(*E*)-ene-1-hydroxycarbonyl-6-deoxy-6-amino-mannopyranoside. Main fatty acids are C_{16:0}, C_{14:0}, C_{18:1}ω6 and C_{18:0}. G + C content is 34 mol%. Lives in freshwater habitats.

Type species is *Arcicella aquatica*.

Description of *Arcicella aquatica* sp. nov.

Arcicella aquatica (a.qua.ti'ca. L. adj. *aquatica* aquatic; *Arcicella* species from water).

The description is the same as given for the genus with the following additions. Main phospholipids are 2-*N*-(2'-hydroxyisopentadecanoyl)amino-3-hydroxyisooheptadeca-4(*E*)-ene-1-(2''-aminoethyl)-phosphate, 2-*N*-(2'-hydroxyisopentadecanoyl)amino-3-hydroxyoctadeca-4(*E*)-ene-1-(2''-aminoethyl)-phosphate, 1-pentadecanoyl-2-hexadecenoyl-phosphatidyl-2'-ethylamine, 1,2-bis-hexadecenoyl-phosphatidyl-2'-ethylamine and 1-hexadecanoyl-2-hexadecenoyl-phosphatidyl-2'-ethylamine. Cells display high activities of alkaline phosphatase, leucine and valine arylamidase, cystine arylamidase, trypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, β-galactosidase, α-glucosidase, catalase and *N*-acetyl-β-glucosaminidase, weak activities of esterase lipase (C8), α-chymotrypsin, α-galactosidase, β-glucosidase and α-fucosidase, and no activities of lipase (C₁₄), α-glucuronidase or α-mannosidase. Nitrate is not reduced to nitrite or nitrogen. Grows well on glucose, fructose, lactose, maltose, rhamnose, galactose, arabinose, ribose, sucrose, cellobiose and inulin; shows some growth on mannitol, sorbitol, acetate and aspartate, but no growth on sorbose, dulcitol, ethanol, methanol, formate, propionate, pyruvate, monomethylamine, citrate, oxalate, succinate, malate or amino acids, with the exception of aspartate.

Type strain is NO-502^T (=LMG 21963^T=CIP 107990^T). G + C content is 34.5 mol%. Genome size is 2.9 × 10⁹ Da. Isolated from a neuston film in the central part of Lake Trostenskoe near Zvenigorod in the Moscow region.

Acknowledgements

We thank Ina Buchholz and Jennifer Skerra for their skilful technical assistance. This work was supported by the grant 'Soil functions' of the HGF strategic funds.

References

- Abraham, W.-R., Meyer, H., Lindholm, S., Vancanneyt, M. & Smit, J. (1997). Phospho- and sulfolipids as biomarkers of *Caulobacter sensu lato*, *Brevundimonas*, and *Hyphomonas*. *Syst Appl Microbiol* **20**, 522–539.
- Abraham, W.-R., Strömpl, C., Meyer, H. & 8 other authors (1999). Phylogeny and polyphasic taxonomy of *Caulobacter* species. Proposal of *Maricaulis* gen. nov. with *Maricaulis maris* (Poindexter) comb. nov. as the type species, and emended description of the genera *Brevundimonas* and *Caulobacter*. *Int J Syst Bacteriol* **49**, 1053–1073.
- Batrakov, S. G., Sheichenko, V. I. & Nikitin, D. I. (1999). A novel glycosphingolipid from Gram-negative aquatic bacteria. *Biochim Biophys Acta* **1440**, 163–175.
- Batrakov, S. G., Mosezhnyi, A. E., Ruzhitsky, A. O., Sheichenko, V. I. & Nikitin, D. I. (2000). The polar-lipid composition of the sphingolipid-producing bacterium *Flectobacillus major*. *Biochim Biophys Acta* **1484**, 225–240.
- Cole, J. R., Chai, B., Marsh, T. L. & 8 other authors (2003). The Ribosomal Database Project (RDP-II): previewing a new autoaligner that allows regular updates and the new prokaryotic taxonomy. *Nucleic Acids Res* **31**, 442–443.
- Felsenstein, J. (1989). PHYLIP – Phylogeny inference package (version 3.2). *Cladistics* **5**, 164–166.
- Glöckner, F. O., Zaichikov, E., Belkova, N., Denissova, L., Pernthaler, J., Pernthaler, A. & Amann, R. (2000). Comparative 16S rRNA analysis of lake bacterioplankton reveals globally distributed phylogenetic clusters including an abundant group of actinobacteria. *Appl Environ Microbiol* **66**, 5053–5065.
- Gosink, J. J., Woese, C. R. & Staley, J. T. (1998). *Polaribacter* gen. nov., with three new species, *P. irgensii* sp. nov., *P. franzmannii* sp. nov. and *P. filamentus* sp. nov., gas vacuolate polar marine bacteria of the *Cytophaga-Flavobacterium-Bacteroides* group and reclassification of '*Flectobacillus glomeratus*' as *Polaribacter glomeratus* comb. nov. *Int J Syst Bacteriol* **48**, 223–235.
- Larkin, J. M. & Borral, R. (1984). Genus III. *Flectobacillus* Larkin, Williams and Taylor 1977, 152^{AL}. In *Bergey's Manual of Systematic Bacteriology*, vol. 1, pp. 129–132. Edited by N. R. Krieg & J. G. Holt. Baltimore: Williams & Wilkins.
- Nakagawa, Y., Sakane, T., Suzuki, M. & Hatano, K. (2002). Phylogenetic structure of the genera *Flexibacter*, *Flexithrix*, and *Microscilla* deduced from 16S rRNA sequence analysis. *J Gen Appl Microbiol* **48**, 155–165.
- Nikitin, D. I., Oranskaya, M. S., Pitryuk, I. A., Chernykh, N. A. & Lysenko, A. M. (1994). A new ring-forming bacterium *Arcocella aquatica* gen. et sp. nov. *Mikrobiologiya* **63**, 152–159 (in Russian).
- Raj, H. D. & Maloy, S. R. (1990). Proposal of *Cyclobacterium marinus* gen. nov., comb. nov., for a marine bacterium previously assigned to the genus *Flectobacillus*. *Int J Syst Bacteriol* **40**, 337–347.
- Stoesser, G., Baker, W., van den Broek, A. & 13 other authors (2003). The EMBL Nucleotide Sequence Database: major new developments. *Nucleic Acids Res* **31**, 17–22.
- Urakami, T. & Komagata, K. (1986). Methanol-utilizing *Ancylobacter* strains and comparison of their cellular fatty acid compositions and quinone systems with those of *Spirosoma*, *Flectobacillus*, and *Runella* species. *Int J Syst Bacteriol* **36**, 415–421.