Aquitalea magnusonii gen. nov., sp. nov., a novel Gram-negative bacterium isolated from a humic lake

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A Gram-negative, rod-shaped, non-spore-forming betaproteobacterium (TRO-001DR8^T) was isolated from humic-lake samples collected from northern Wisconsin, USA. On the basis of 16S rRNA gene sequence analysis, strain TRO-001DR8^T belonged to the family *Neisseriaceae*, and the phylogenetic distance from its closest relative, *Chromobacterium violaceum*, was 95 %. Strain TRO-001DR8^T lacked the violet pigmentation of *C. violaceum* and shared only 26 % DNA–DNA relatedness with *C. violaceum*. The DNA G+C content of strain TRO-001DR8^T was 59 mol%. The predominant fatty acids were $C_{16:1}\omega7c$ + $C_{16:1}\omega7c$ 2-OH iso (52·5 %), $C_{16:0}$ (21·7 %), $C_{18:1}\omega7c$ (8·0 %) and $C_{12:0}$ (5·1 %). Strain TRO-001DR8^T grew optimally at 35 °C and pH 6·0, did not utilize sucrose, but did use glucose, some organic acids and most protein amino acids. Biochemical, physiological, chemotaxonomic and phylogenetic analyses showed that strain TRO-001DR8^T could not be assigned to any known genus of the *Betaproteobacteria*. Therefore, the isolate represents a novel genus and species, for which the name *Aquitalea magnusonii* gen. nov., sp. nov. is proposed. The type strain is TRO-001DR8^T (=ATCC BAA-1216^T=BCCM/LMG 23054^T).

As part of an analysis of microbial diversity in lakes, we have been culturing novel organisms from humic and oligotrophic lakes. This work describes the isolation and characterization of a novel betaproteobacterium, strain TRO-001DR8^T, from Trout Bog Lake in northern Wisconsin, USA (46·03° N 89·69° W). This facultatively anaerobic strain was isolated by dilution to extinction on dilute R2A medium (Difco). A sample (1 l) of surface water was collected in June 2004 and filtered through a 100 μm mesh filter and shipped to Gainesville, Florida, by express mail. Upon arrival in Gainesville, the sample was filtered through a sterile 0·2 μm filter 25 mm in diameter to concentrate bacterial cells. One-quarter of this filter was placed in 1 ml R2A medium diluted 1 : 10 with sterile distilled water. Cells on the filter piece were suspended in the test tube by vortexing. The resulting suspension of cells was serially diluted, with 100 μl each dilution spread on plates containing dilute (1 : 10) R2A medium solidified with 1·5 % agar.

Phylogenetic assignment of the 16S rRNA gene of strain TRO-001DR8^T placed this organism among the *Neisseriaceae*. DNA isolation prior to 16S rRNA gene amplification was done as described previously (Borneman *et al.*, 1996). Sequencing of the 16S rRNA gene was performed as described previously (Chelius & Triplett, 2000), except that the 8f primer (*Escherichia coli* numbering) was used for the amplification of the gene from strain TRO-001DR8^T DNA. DNA sequencing was performed at the Interdisciplinary Center for Biotechnology Research at the University of Florida. Using BLASTN from NCBI, the closest cultured relative of strain TRO-001DR8^T is *Chromobacterium violaceum*, with 95 % similarity over 1500 bases between the 16S rRNA gene sequences of these two organisms. *C. violaceum* inhabits soil and water and produces a characteristic violet pigment on agar media (Dessaux *et al.*, 2004). Strain TRO-001DR8^T does not utilize sucrose but will use glucose, some organic acids and most protein amino acids. It is not pigmented and differs metabolically from *Chromobacterium*.

The 16S rRNA gene sequences of two uncultured relatives present in the databases showed 95–98 % similarity with the 16S rRNA gene from strain TRO-001DR8^T. One of the uncultured relatives (AB089102) was discovered in DNA isolated from the gut of the termite *Reticulitremes speratus* (Hongoh *et al.*, 2003). The other clone (AB076875) was discovered following PCR amplification of DNA from an activated sludge (Khan *et al.*, 2002).

The 16S rRNA gene sequences from 11 genera within the *Neisseriaceae* were compared to determine the mean

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain TRO-001DR8^T is DQ018117.

A table showing some genotypic, phenotypic and nutritional characteristics that distinguish strain TRO-001DR8^T from related betaproteobacteria is available as supplementary material in IJSEM Online.

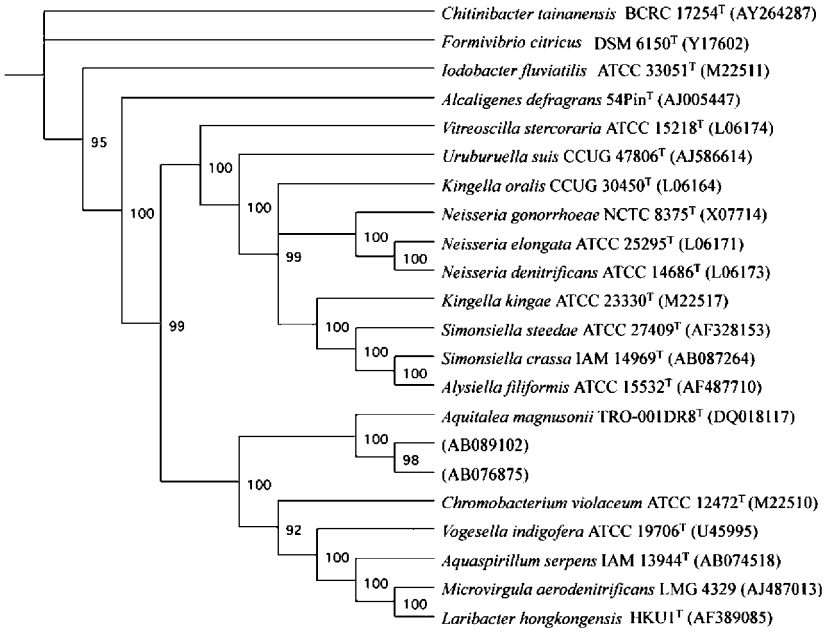

Fig. 1. Phylogenetic tree, based on the alignment of 16S rRNA genes, comparing strain TRO-001DR8^T (*Aquitalea magnusonii*) with other selected betaproteobacteria within the *Neisseriaceae*. A Bayesian estimate of phylogeny was used to infer a posterior probability distribution of trees using Markov chain Monte Carlo searches (Ronquist & Huelsenbeck, 2003). A consensus tree was developed on the basis of the 10 000 trees developed by using the Markov chain Monte Carlo method. The consensus tree was generated using TREEVIEW (Page, 1996).

distance between the most closely related pairs of genera: this was found to be 94 %, with a range of 90–98 %. The distance between *Chromobacterium* and the proposed *Aquitalea* genus is similar to the mean distance between any two genera in this family.

The 16S rRNA gene sequences were aligned using the web-based CLUSTAL W program at the Biology Work Bench (http://workbench.sdsc.edu/). The 16S rRNA gene of strain TRO-001DR8^T (GenBank accession no. DQ018117) was aligned against 19 reference strains (Fig. 1). The 16S rRNA genes of the two most closely related uncultured organisms were also included in this analysis (Fig. 1). A Bayesian estimate of phylogeny was determined using MrBayes 3, which infers a posterior probability distribution of trees using Markov chain Monte Carlo searches (Ronquist & Huelsenbeck, 2003). These searches were run with four chains for 1 000 000 generations with trees sampled every 100 generations. A consensus tree was developed on the basis of the 10 000 trees developed using the Markov chain Monte Carlo method. The consensus tree was generated using TREEVIEW (Page, 1996).

Strain TRO-001DR8^T could not be assigned to any known genus on the basis of the fatty acid databases in the MIDI Sherlock Microbial Identification System. Two of the fatty acids present in strain TRO-001DR8^T are absent in its closest relative, and two other fatty acids are present in *C. violaceum* ATCC 12472^T but absent in strain TRO-001DR8^T (Table 1). Four other fatty acids were found in both strains. However, their concentrations differed significantly between the two strains (Table 1). Fatty acid content was determined by GC and compared to the fatty acid database in the Microbial Identification System, Sherlock version 4.5 (MIDI).

Cells of strain TRO-001DR8^T are rod-shaped with one polar flagellum and are 1–7 μm in length (mean length ~4 μm). The larger cells are often slightly curved. In stationary phase, cells can form long filaments. Colonies are a tan colour and produce a moderate amount of slime on R2A agar. Unlike *C. violaceum*, strain TRO-001DR8^T does not produce a pigment on peptone agar. No resting stages were observed.

Table 1. Fatty acid content (%) of strain TRO-001DR8^T and *C. violaceum* ATCC 12472^T

All of the major unsaturated fatty acids found in this analysis were *cis* isomers (indicated by the suffix '*c*'). The summed fatty acids below cannot be separated by the GLC of the MIDI system. The position of the double bond in the unsaturated fatty acids is obtained by counting from the methyl (ω) end of the molecule.

Fatty acid	*C. violaceum* ATCC 12472^T	TRO-001DR8^T
$C_{10:0}$ 3-OH	5·2	1·5
$C_{12:0}$	5·6	5·1
$C_{12:0}$ 2-OH	3·0	0·0
$C_{12:0}$ 3-OH	5·0	1·2
$C_{14:0}$	1·7	2·8
$C_{15:0}$	0·0	2·0
$C_{16:1}\omega7c$ + $C_{16:1}\omega7c$ 2-OH iso	35·8	52·5
$C_{16:0}$	23·9	21·7
$C_{17:0}$ cyclo	1·7	0·0
$C_{17:1}\omega6c$	0·0	1·2
$C_{18:1}\omega7c$	15·0	8·0

For light microscopy, strain TRO-001DR8^T was visualized under phase-contrast microscopy and differential interference contrast optics on a Zeiss LSM-5 Pascal laser scanning microscope after 24 and 48 h growth (see Fig. 2a). For electron microscopy, exponential-phase Luria broth-cultured cells were gently deposited on a 300-mesh Formvar-coated grid, washed once with deionized water, briefly stained with 1 % aqueous uranyl acetate and then viewed at 100 kV on a Zeiss Em-10CA transmission electron microscope. The remainder of the culture was pelleted, resuspended in 1 % glutaraldehyde/0·1 M cacodylate (pH 7·2) and then fixed overnight at 4 °C. Cells were secondarily fixed in 1 % osmium tetroxide/0·1 M cacodylate buffer for 1 h and then subjected to 1 % aqueous uranyl acetate treatment for 1 h. After dehydration through an ethanol series and acetone, cells were embedded, thin-sectioned, post-stained with 5 % uranyl acetate and lead citrate and then viewed by transmission electron microscopy as above (Fig. 2b–d).

The DNA–DNA relatedness between strain TRO-001DR8^T and *C. violaceum* ATCC 12472^T was 26 %. The DNA G+C content of strain TRO-001DR8^T is 59·2 mol%. Genome sequencing of *C. violaceum* ATCC 12472^T has revealed that it has a DNA G+C content of 64·83 mol% (Brazilian National Genome Project Consortium, 2003). For DNA–DNA hybridization, cells were disrupted using a French pressure cell and the DNA was purified by hydroxyapatite chromatography (Cashion *et al.*, 1977). DNA–DNA hybridization was done as described by De Ley *et al.* (1970), with the modifications of Huß *et al.* (1983). The G+C content of strain TRO-001DR8^T was determined by using HPLC (Mesbah *et al.*, 1989) after isolation and purification of the DNA (Cashion *et al.*, 1977).

As *C. violaceum* was the closest cultured relative of strain TRO-001DR8^T, *C. violaceum* ATCC 12472^T was used as the reference strain (Table 2). Selected strains from closely related genera in the *Neisseriaceae* are also included in Supplementary Table S1 in IJSEM Online. Strain TRO-001DR8^T cannot utilize the following substrates utilized by *C. violaceum*: D-mannose, D-trehalose, L-alaninamide, L-phenylalanine, D-serine, L-threonine, inosine, uridine,

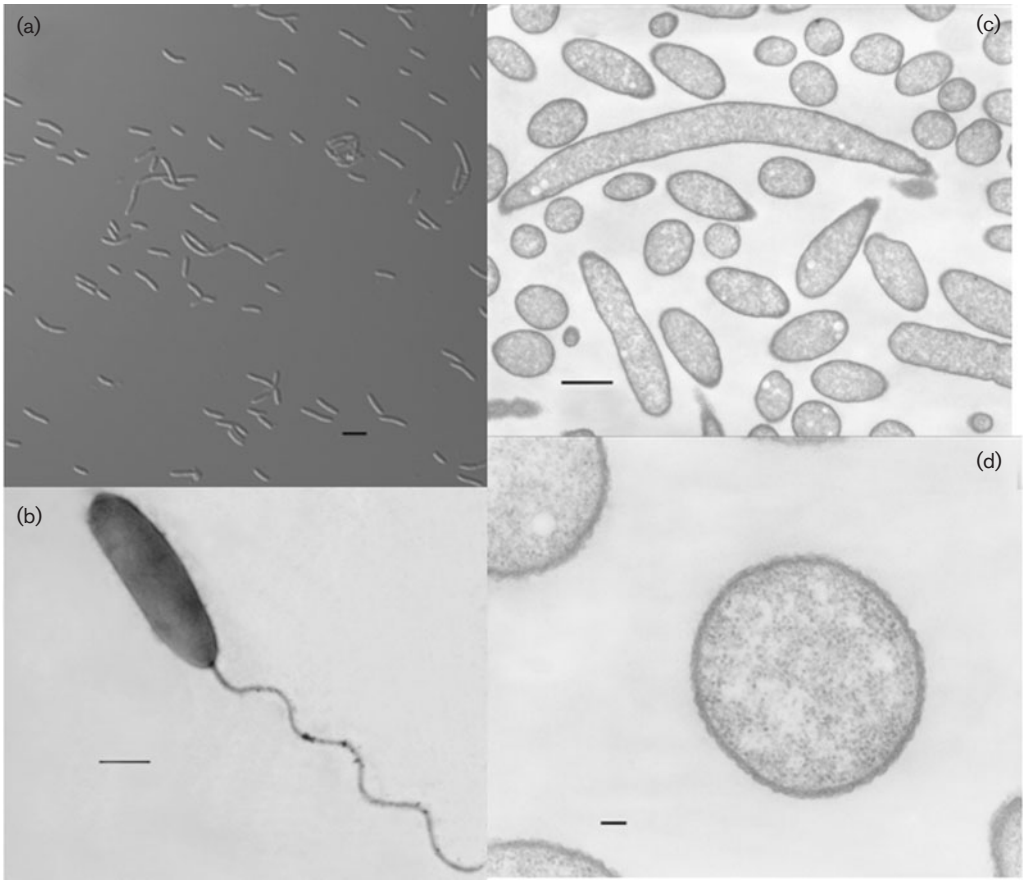

Fig. 2. (a) Differential interference contrast image of strain TRO-001DR8^T (note the variability in length from approximately 3 to 9 μm). Bar, 5 μm. (b) Electron micrograph of a cell negatively stained with 1 % aqueous uranyl acetate, showing the single polar flagellum. Bar, 1 μm. (c) Electron micrograph of thin-sectioned cells; most cells contain lipid-like inclusions. Bar, 1 μm. (d) Higher-magnification electron micrograph of cell cross-section, showing typical Gram-negative cell-wall structure. Bar, 0·1 μm.

Table 2. Some genotypic, phenotypic and nutritional characteristics that distinguish strain TRO-001DR8^T from the closest genus type strain *C. violaceum* ATCC 12472^T

Characteristic	TRO-001DR8^T	*C. violaceum* ATCC 12472^T
Colony colour	Tan	Violet
DNA G+C content (mol%)	59·2	64
Highest NaCl tolerance (%)	<1·5	<4*
Isolation source	Humic lake	Soil/water*
Growth temperature (°C)	28–37	28–40
Fermentation of ribose	−	+
Indole production	+	−
Gelatinase	−	+
Assimilation of:		
α-D-Glucose 1-phosphate	−	+
2,3-Butanediol	−	+
2-Aminoethanol	−	+
DL-α-Glycerol phosphate	−	+
D-Glucose 6-phosphate	−	+
D-Mannose	−	+
D-Serine	−	+
D-Trehalose	−	+
Inosine	−	+
L-Alaninamide	−	+
L-Phenylalanine	−	+
L-Threonine	−	+
Thymidine	−	+
Uridine	−	+
γ-Aminobutyric acid	+	−
p-Hydroxyphenylacetic acid	+	−
α-Ketoglutaric acid	+	−
cis-Aconitic acid	+	−
Citric acid	+	−
L-Leucine	+	−
Resistance to antibiotics (μg ml^{-1})		
Kanamycin (50)	−	+
Tetracycline (10)	−	+
Trimethoprim (25)	−	+

*From Gillis & Logan (2005).

thymidine, 2-aminoethanol, 2,3-butanediol, DL-α-glycerol phosphate, α-D-glucose 1-phosphate and D-glucose 6-phosphate. Strain TRO-001DR8^T does utilize the following substrates not utilized by *C. violaceum: cis*-aconitic acid, citric acid, *p*-hydroxyphenylacetic acid, α-ketoglutaric acid, L-leucine and γ-aminobutyric acid. Unlike *Chromobacterium*, strain TRO-001DR8^T ferments (but does not oxidize) glucose and does not hydrolyse gelatin. Strain TRO-001DR8^T is also more sensitive to salt as it cannot grow on 1·5% NaCl whereas *C. violaceum* can tolerate concentrations up to 6% NaCl. The carbon-assimilation tests were done using API 20NE (bioMérieux) according to the manufacturer's instructions. Additional carbon-assimilation tests were done using the Biolog GN2 MicroPlate according to the manufacturer's instructions. Cultures were inoculated on Biolog Universal Growth agar with 5% sheep blood or chocolate agar and then incubated at 30 °C for 4–6 and 16–24 h prior to testing with the Biolog system. On the basis of the results of the Biolog GN carbon-utilization tests and other API 20NE metabolic tests, strain TRO-001DR8^T could not be assigned to any known genus.

OF medium (Hugh & Leifson, 1953) was used for the oxidation/fermentation test. Phenol red broth with a Durham tube inserted was used to identify acid/gas production. Tests for catalase and cytochrome *c* oxidase activity were completed using cells scraped from Luria agar and subsequently treated with 3% (w/v) hydrogen peroxide or tetramethyl-*p*-phenylenediamine (Difco), respectively. Tests for nitrate reduction, indole production, arginine dihydolase, urease, aesculin ferric citrate and protease activity were done with an API 20NE kit. Cells grown in Luria broth (for 24 h at 28 °C) were wet-mounted and viewed under a light microscope to test for motility. To test for amylase activity, colonies were grown on starch agar for 14 days at 37 °C (Difco) and then flooded with iodine. Growth over a range of temperatures (4–45 °C) was tested with strains inoculated on Luria agar. Optimal growth was observed at 35 °C and pH 6·0.

Strain TRO-001DR8^T will grow on Luria agar supplemented with ampicillin, rifampicin and streptomycin but not with chloramphenicol, kanamycin, spectinomycin, tetracycline and trimethoprim. In contrast, *C. violaceum* ATCC 12472^T expresses resistance to a wider array of antibiotics, being insensitive to kanamycin, tetracycline and trimethoprim. Antibiotic sensitivity was tested using Luria agar supplemented with the following: spectinomycin, streptomycin or tetracycline, each at 10 μg ml^{-1}; ampicillin, chloramphenicol or trimethoprim, each at 25 μg ml^{-1}; kanamycin at 50 μg ml^{-1}.

Description of *Aquitalea* gen. nov.

Aquitalea (A.qui.ta′le.a. L. fem. n. *aqua -ae*, water; L. fem. n. *talea -ae* a slender staff, rod, stick; N.L. fem. n. *Aquitalea* a rod of water).

Cells are Gram-negative, non-spore-forming, short rods. Rods are straight or slightly curved and 1–7 μm in length. Strain is aerobic, facultatively anaerobic, chemoheterotrophic and sensitive to NaCl. Cells are motile with one polar flagellum and are catalase- and oxidase-positive. DNA G+C content is 59·2 mol% (HPLC). Predominant fatty acids are $C_{16:1}\omega7c$ (53%) and $C_{16:0}$ (22%), $C_{18:1}\omega7c$ (8%) and $C_{12:0}$ (5%). Phylogenetically belongs to the family *Neisseriaceae*.

The type species is *Aquitalea magnusonii*.

Description of *Aquitalea magnusonii* gen. nov., sp. nov.

Aquitalea magnusonii (mag′nu.son′i.i. N.L. gen. n. *magnusonii* of Magnuson, in honour of Professor Emeritus John J.

Magnuson, an ecologist at the University of Wisconsin-Madison who has contributed greatly to the study of the biodiversity, biogeography and climate-change analysis of lake ecosystems.

In addition to possessing the characteristics of the genus, described above, this species can grow on glycogen, pyruvic acid methyl ester, succinic acid monomethyl ester, acetic acid, *cis*-aconitic acid, citric acid, β-hydroxybutyric acid, *p*-hydroxyphenylacetic acid, α-ketoglutaric acid, DL-lactic acid, propionic acid, succinic acid, bromosuccinic acid, L-asparagine, L-aspartic acid, L-glutamic acid, L-histidine, L-proline, L-serine and γ-aminobutyric acid. Reduces nitrate and produces indole from L-tryptophan. Produces catalase, arginine dihydrolase and cytochrome oxidase. Resistant to ampicillin (25 $\mu g\ ml^{-1}$), rifampicin (25 $\mu g\ ml^{-1}$) and streptomycin (10 $\mu g\ ml^{-1}$), but sensitive to chloramphenicol (25 $\mu g\ ml^{-1}$), kanamycin (50 $\mu g\ ml^{-1}$), spectinomycin (10 $\mu g\ ml^{-1}$), tetracycline (10 $\mu g\ ml^{-1}$) and trimethoprim (25 $\mu g\ ml^{-1}$). Growth occurs between pH 5 and 8. Growth occurs between 28 and 40 °C but not at 4 or 45 °C. Strain TRO-001DR8^T ferments, but does not oxidize, glucose.

The type strain, TRO-001DR8^T (=ATCC BAA-1216^T=BCCM/LMG 23054^T), was isolated from a humic lake in northern Wisconsin, USA.

Acknowledgements

This work was supported by funds from the National Science Foundation (MCB 9977903, MCB 0401987 and DEB 0217533) and the Florida Agricultural Experiment Station. We thank Donna Williams of the Microbiology and Cell Science Department at the University of Florida for the microscopy done in this study. We thank Stuart Jones of the University of Wisconsin-Madison for collecting lake samples for us.

References

Borneman, J., Skroch, P. W., O'Sullivan, K. M., Palus, J. A., Rumjanek, N. G., Jansen, J. L., Nienhuis, J. & Triplett, E. W. (1996). Molecular microbial diversity of an agricultural soil in Wisconsin. *Appl Environ Microbiol* **62**, 1935–1943.

Brazilian National Genome Project Consortium (2003). The complete genome sequence of *Chromobacterium violaceum* reveals remarkable and exploitable bacterial adaptability. *Proc Natl Acad Sci U S A* **100**, 11660–11665.

Cashion, P., Holder-Franklin, M. A., McCully, J. & Franklin, M. (1977). A rapid method for the base ratio determination of bacterial DNA. *Anal Biochem* **81**, 461–466.

Chelius, M. K. & Triplett, E. W. (2000). *Dyadobacter fermentans* gen. nov., sp. nov., a novel gram-negative bacterium isolated from surface-sterilized *Zea mays* stems. *Int J Syst Evol Microbiol* **50**, 351–358.

De Ley, J., Cattoir, H. & Reynaerts, A. (1970). The quantitative measurement of DNA hybridization from renaturation rates. *Eur J Biochem* **12**, 133–142.

Dessaux, Y., Elmerich, C. & Faure, D. (2004). Violacein: a molecule of biological interest originating from the soil-borne bacterium *Chromobacterium violaceum*. *Rev Med Interne* **25**, 659–662 (in French).

Gillis, M. & Logan, N. A. (2005). Genus IV. *Chromobacterium* Bergonzini 1881, 153^{AL}. In *Bergey's Manual of Systematic Bacteriology*, vol. 2, pp. 824–827. Edited by D. J. Brenner, N. R. Kreig & J. T. Staley. New York: Springer.

Grimes, D. J., Woese, C. R., MacDonell, M. T. & Colwell, R. R. (1997). Systematic study of the genus *Vogesella* gen. nov. and its type species, *Vogesella indigofera* comb. nov. *Int J Syst Bacteriol* **47**, 19–27.

Hongoh, Y., Ohkuma, M. & Kudo, T. (2003). Molecular analysis of bacterial microbiota in the gut of the termite *Reticulitermes speratus* (Isoptera; Rhinotermitidae). *FEMS Microbiol Ecol* **44**, 231–242.

Hugh, R. & Leifson, E. (1953). The taxonomic significance of fermentative versus oxidative metabolism of carbohydrates by various gram-negative bacteria. *J Bacteriol* **66**, 24–26.

Huß, V. A. R., Festl, H. & Schleifer, K. H. (1983). Studies on the spectrophotometric determination of DNA hybridization from renaturation rates. *Syst Appl Microbiol* **4**, 184–192.

Khan, S. T., Horiba, Y., Yamamoto, M. & Hiraishi, A. (2002). Members of the family *Comamonadaceae* as primary poly(3-hydroxybutyrate-co-3-hydroxyvalerate)-degrading denitrifiers in activated sludge as revealed by a polyphasic approach. *Appl Environ Microbiol* **68**, 3206–3214.

Mesbah, M., Premachandran, U. & Whitman, W. B. (1989). Precise measurement of the G+C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int J Syst Bacteriol* **39**, 159–167.

Page, R. D. M. (1996). TREEVIEW: an application to display phylogenetic trees on personal computers. *Comput Appl Biosci* **12**, 357–358.

Patureau, D., Godon, J. J., Dabert, P., Bouchez, T., Bernet, N., Delgenes, J. P. & Moletta, R. (1998). *Microvirgula aerodenitrificans* gen. nov., sp. nov., a new gram-negative bacterium exhibiting co-respiration of oxygen and nitrogen oxides up to oxygen-saturated conditions. *Int J Syst Bacteriol* **48**, 775–782.

Pot, B. & Gillis, M. (2005). Genus III. *Aquaspirillium* Hylemon, Wells, Kreig and Jannasch 1973b. 36l^{AL}. In *Bergey's Manual of Systematic Bacteriology*, vol. 2, pp. 801–823. Edited by D. J. Brenner, N. R. Kreig & J. T. Staley. New York: Springer.

Ronquist, F. & Huelsenbeck, J. P. (2003). MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**, 1572–1574.

Yuen, K. Y., Woo, P. C., Teng, J. L., Leung, K. W., Wong, M. K. & Lau, S. K. (2001). *Laribacter hongkongensis* gen. nov., sp. nov., a novel gram-negative bacterium isolated from a cirrhotic patient with bacteremia and empyema. *J Clin Microbiol* **39**, 4227–4232.