Description of *Roseateles aquatilis* sp. nov. and *Roseateles terrae* sp. nov., in the class *Betaproteobacteria*, and emended description of the genus *Roseateles*

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Three strains of aerobic Gram-negative bacilli, two isolated from industrial water and freshwater (strains CCUG 48205^T and CCUG 52220) and the third from soil (strain CCUG 52222^T), were analysed phenotypically and genotypically to clarify their taxonomic classification. 16S rRNA gene sequence analysis revealed that the three strains were located on the same phylogenetic branch, closely related to *Roseateles depolymerans*, the only recognized species in the genus. DNA–DNA hybridization studies, analyses of fatty acid contents, and physiological and biochemical tests supported the proposal of two novel species, *Roseateles aquatilis* sp. nov. (type strain, CCUG 48205^T=CECT 7248^T) and *Roseateles terrae* sp. nov. (type strain, CCUG 52222^T=CECT 7247^T).

The genera *Aquabacterium* (Kalmbach *et al.*, 1999), *Ideonella* (Malmqvist *et al.*, 1994), *Rubrivivax* (Willems *et al.*, 1991), *Roseateles* (Suyama *et al.*, 1999), *Azohydromonas* (Xie & Yokota, 2005), *Mitsuaria* (Amakata *et al.*, 2005) and *Pelomonas* (Xie & Yokota, 2005; Gomila *et al.*, 2007) are phylogenetically closely related to the *Sphaerotilus–Leptothrix* group. These genera form the *Rubrivivax* line of descent, which is associated with the family *Comamonadaceae* (Wen *et al.*, 1999). Willems & Gillis (2001) consider these genera to be of *incertae sedis* within the family *Comamonadaceae*. The relative branching order of most species within the *Rubrivivax* group is difficult to determine, mainly because of the limited number of variable positions available for estimating phylogenetic distance values (Spring, 2001).

Abbreviations: ITS, internally transcribed spacer; PHC, poly(hexamethylene carbonate).

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA, ITS1, *nifH* and *puf* gene sequences determined in this study are AM501443–AM501446, AM501462, AM501479 and AM501480, and AM773545–AM773547, respectively.

Carbon source utilization data (Biolog), DNA–DNA hybridization values and a 16S rRNA gene sequence matrix are available as supplementary tables with the online version of this paper.

Roseateles depolymerans 61A^T (=CCUG 52219^T=DSM 11813^T), which is closely related to species of the genera *Mitsuaria* and *Pelomonas*, was isolated in 1998 from river water and described in 1999 as the first bacteriochlorophyll *a*-containing, obligate aerobe belonging to the *Betaproteobacteria*. The strain was isolated originally as a degrader of poly(hexamethylene carbonate) (PHC) and is considered to be an aerobic phototroph (Suyama *et al.*, 1999). Since 2005, three novel strains (CCUG 48205^T, CCUG 52220 and CCUG 52222^T) have been isolated from different sources, including industrial water, freshwater and soil, and were identified provisionally as '*Roseateles depolymerans*-like' organisms. The present study was undertaken to characterize and determine the taxonomic positions of these organisms. The data demonstrated that strains CCUG 48205^T and CCUG 52222^T should be classified as representing two novel species, with the names *Roseateles aquatilis* sp. nov. and *Roseateles terrae* sp. nov., respectively.

The reference strains used in this study included *Pelomonas saccharophila* CCUG 32988^T, *Pelomonas aquatica* CCUG 52575^T, *Pelomonas puraquae* CCUG 52769^T, *Mitsuaria chitosanitabida* IAM 14711^T and *Roseateles depolymerans* CCUG 52219^T, as well as *Roseateles depolymerans* strain CCUG 52220, *Roseateles* sp. strain CCUG 52222^T and

Roseateles depolymerans-like strain CCUG 48205^T. All of the strains used in this study were cultivated in R2A medium (Reasoner & Geldreich, 1985), containing (1⁻¹): 0.5 g yeast extract, 0.5 g acid hydrolysate of casein, 0.5 g glucose, 0.5 g starch, 0.3 g K₂HPO₄, 0.3 g sodium pyruvate, 0.25 g pancreatic digest of casein, 0.25 g peptic digest of animal tissue, 0.0492 g MgSO₄·7H₂O, supplemented with 1.5 % (w/v) agar (Scharlab) for solid medium. The bacteria were incubated for 3–4 days at 30 °C, if not stated otherwise.

Cell size, morphology and flagella insertion were determined using transmission electron microscopy of cells from the exponential growth phase in R2A broth. A Hitachi model H600 electron microscope was used at 75 kV. The samples were negatively stained with phosphotungstic acid (1 % w/v, pH 7.0), as described by Lalucat (1988).

The following phenotypic tests were carried out: API 20NE (identification system for Gram-negative, non-enterobacterial rods) and API 50 CH, according to the instructions of the manufacturer. Biolog tests were also carried out according to the instructions of the manufacturer. Briefly, the procedure was as follows: a homogeneous suspension of cells grown on plates of trypticase soy agar supplemented with sheep blood was made in the GN/GP inoculating fluid and 150 µl of the suspension was dispensed into each well of a GN microplate, which was then incubated for up to 96 h at 30 °C. The absorbance values were recorded periodically over a 4-day period, at 0, 4, 8, 24, 48, 72 and 96 h incubation time, using an automated Microplate reader. Absorbance was measured in dual wavelength mode, at 590 nm (as respiratory reduction will cause the tetrazolium dye to turn purple and absorb light at 590 nm) and at 750 nm as a second reference wavelength.

Conventional phenotypic tests were done according to the methods of Cowan (1974). Growth rates were determined at different temperatures (4, 10, 20, 30, 37 and 45 °C) in R2A medium, and aerobic autotrophic growth with hydrogen was tested in mineral medium, as described by Aragno & Schlegel (1992), at different O₂ partial pressures. For the detection of photosynthetic pigments, cells were grown in R2A medium, PHC medium (or PHC medium supplemented with Tween 80) or in medium lacking organic carbon as described by Suyama *et al.* (1999, 2002). Lipophilic pigments were extracted from fresh wet cells cultured in the four media, under light or in the dark, using acetone [90 % (v/v) with 1 % MgCO₃ at 4 °C for 24 h]. Absorption spectra were recorded spectrophotometrically (Ultrospec III; Pharmacia LKB) between wavelengths of 360 and 900 nm.

Colonies of strains CCUG 52219^T and CCUG 52220 grown on R2A medium for 5 days were 4–5 mm in diameter, transparent, darker in the centre, circular and flat, with an undulate margin, and smooth and translucent. Strain CCUG 52222^T could be differentiated by means of a more mucous appearance of the colonies, which were 4–6 mm in

diameter, less transparent, circular and convex, with an undulate transparent margin. Colonies of strain CCUG 48205^T were 4–6 mm in diameter, clear white, circular and umbonate, with an undulate margin, and translucent. Old colonies showed concentric circles.

Cells were rod-shaped (2 µm in length), Gram-negative and motile, by means of a single polar flagellum. All strains grew at 20 and 30 °C. None of the strains were able to grow at 4 or 45 °C. The range of growth temperatures is indicated in Table 1. Differential phenotypic characteristics and substrates assimilated or utilized as sole carbon and energy sources are listed in Table 1. Carbon sources utilized in Biolog plates are given in Supplementary Table S1, available in IJSEM Online. Differential phenotypic characteristics obtained in the Biolog test are summarized in Table 2. None of the novel isolates were able to produce pigments when incubated for 1 week in the dark or under light in R2A medium or PHC medium, or in PHC medium supplemented with Tween 80, as well as in mineral medium.

Gas chromatography of fatty acid methyl esters was performed at CCUG in a highly standardized way, similar to that of the MIDI Sherlock MIS system. Fatty acids were identified and quantified, and the relative amount of each fatty acid in a strain was expressed as a percentage of the total fatty acids in the profile of that strain. The major cellular fatty acids detected in all *Roseateles* strains analysed (CCUG 48205^T, CCUG 52219^T, CCUG 52220 and CCUG 52222^T) were *cis*-9-hexadecanoic and hexadecanoic acids, as indicated in Table 3. Clear differences between *Roseateles*, *Mitsuaria* and *Pelomonas* could be established in the relative amounts of dodecanoic and *cis*-11-octadecanoic acids. The two novel species proposed in the genus *Roseateles* can be differentiated chemotaxonomically from *Roseateles depolymerans* by the relative amount of dodecanoic acid.

Genomic DNA was isolated according to the method of Marmur (1961). DNA–DNA hybridizations were performed, in duplicate, using a non-radioactive method as described by Ziemke *et al.* (1998). Reference DNAs of one strain of each group (*P. saccharophila* CCUG 32988^T, *P. puraquae* CCUG 52769^T, *P. aquatica* CCUG 52575^T, *M. chitosanitabida* IAM 14711^T, *Roseateles depolymerans* CCUG 52219^T, *Roseateles* sp. strain CCUG 52222^T and *Roseateles depolymerans*-like strain CCUG 48205^T) were double-labelled with DIG-11-dUTP and biotin-16-dUTP using a Nick Translation kit (Roche). DNA–DNA hybridizations were performed using chromosomal DNA of each type strain as probes against the other strains (see Supplementary Table S2 in IJSEM Online). Strain CCUG 52220 showed 100 % DNA–DNA relatedness to the type strain of *Roseateles depolymerans*. The levels of DNA–DNA relatedness of strains CCUG 48205^T and CCUG 52222^T to the other strains were less than 57 %, and was 37.1 % between the two strains. This indicates that three different genomic groups can be delineated in the genus *Roseateles*.

Table 1. Differential phenotypic characteristics between species of the genera *Mitsuaria*, *Pelomonas* and *Roseateles*

Strains: 1, *Roseateles depolymerans* CCUG 52219^T; 2, *Roseateles depolymerans* CCUG 52220; 3, strain CCUG 52222^T (*Roseateles terrae* sp. nov.); 4, strain CCUG 48205^T (*Roseateles aquatilis* sp. nov.); 5, *M. chitosanitabida* IAM 14711^T; 6, *P. saccharophila* CCUG 32988^T; 7, *P. puraquae* CCUG 52769^T; 8, *P. aquatica* CCUG 52575^T. All strains are oxidase-positive. +, Positive; –, negative; w, weak; v, variable; ND, not determined.

Characteristic	1	2	3	4	5	6	7	8
Flagella	Several, polar*	One, polar	One, polar	One, polar	One, polar	One, polar	One, polar	One, polar
Growth at:								
10 °C	+	+	w	–	+	–	+	–
37 °C	+	+	+	–	+	–	+	+
Autotrophic growth with H ₂	–	–	–	–	–	+	+	–
Pigment	+	–	–	–	–	–	–	–
Growth on PHC	+	+	w	+	w	–	–	–
Amplification of:								
<i>cbbL</i>	–	–	–	–	–	+	+	–
<i>hox</i>	–	ND	ND	ND	–	ND	+	–
<i>nifH</i>	+	+	+	–	–	+	+	–
<i>puf</i>	+	+	+	–	–	–	–	–
Catalase	+	+	+	–	–	–	–	–
Nitrate reduction	–	–	–	–	+	+	v	+
Urease	+	w	–	–	–	–	–	–
β-Glucosidase	+	+	+	–	+	+	+	+
Gelatin hydrolysis	+	+	+	+	+	–	+	–
β-Galactosidase	+	+	+	–	+	+	+	–
Assimilation of:								
D-Glucose	+	+	+	+	+	+	+	–
L-Arabinose	+	+	+	–	–	+	+	–
D-Mannose	+	+	+	–	–	v	–	–
D-Mannitol	+	+	+	–	–	–	–	–
N-Acetyl-D-glucosamine	–	–	–	–	–	+	–	–
Maltose	+	+	+	+	+	+	+	–
Gluconate	+	+	+	+	+	+	–	–
Capric acid	w	–	+	–	+	–	–	–
Adipic acid	w	w	–	–	–	–	–	–
Malic acid	w	+	w	+	+	v	+	–
Citrate	w	+	–	–	+	–	w	–
Phenylacetic acid	–	–	–	–	w	–	–	–

*Data from Suyama *et al.* (1999).

Table 2. Differential carbon source utilization (Biolog) among the *Roseateles* strains studied

Strains: 1, *Roseateles depolymerans* CCUG 52219^T; 2, *Roseateles depolymerans* CCUG 52220; 3, strain CCUG 52222^T (*Roseateles terrae* sp. nov.); 4, strain CCUG 48205^T (*Roseateles aquatilis* sp. nov.). +, Positive; –, negative.

Carbon source	1	2	3	4
L-Arabinose, D-fructose, D-galactose, D-mannose, D-arabitol, D-mannitol, glycogen, L-pyroglyutamic acid, succinic acid monomethyl ester, urocanic acid	+	+	+	–
N-Acetyl-D-galactosamine, D-cellobiose, α-D-glucose 1-phosphate, α-cyclodextrin, inosine, γ-aminobutyric acid, L-aspartic acid, citric acid, γ-hydroxybutyric acid, α-ketobutyric acid, DL-lactic acid, sebacic acid, succinic acid, L-alanine, L-leucine, L-ornithine, L-proline, L-serine, L-threonine	+	+	–	+
Malonic acid	+	+	–	–
D-Glucosaminic acid	–	–	+	–
D-Alanine, D-glucose 6-phosphate	–	–	–	+

Table 3. Cellular fatty acid composition (%) of the bacteria studied and reference strains

Strains: 1, *P. saccharophila* CCUG 32988^T; 2, *P. puraquae* CCUG 52769^T; 3, *P. aquatica* CCUG 52575^T; 4, *M. chitosanitabida* IAM 14711^T; 5, *Roseateles depolymerans* CCUG 52219^T; 6, *Roseateles depolymerans* CCUG 52220; 7, strain CCUG 52222^T (*Roseateles terrae* sp. nov.); 8, strain CCUG 48205^T (*Roseateles aquatilis* sp. nov.). —, Below the limit of detection.

Fatty acid	1	2	3	4	5	6	7	8
10:0 3-OH	9.2	7.1	9.4	3.8	5.8	5.0	7.2	4.1
12:0	7.4	4.9	7.4	—	0.9	0.8	2.5	—
12:0 2-OH	3.8	2.7	1.8	2.1	2.2	1.9	1.7	2.0
14:0	1.3	1.0	1.8	0.8	1.2	1.1	1.7	0.5
15:0	1.0	0.6	1.4	0.2	1.3	1.5	—	0.3
16:0 iso	—	—	—	—	—	0.3	—	—
16:1 ω 7c	41.9	47.7	43.1	44.1	42.8	41.9	45.5	44.9
16:0	32.0	30.1	32.0	31.0	24.0	28.2	25.9	27.7
18:2 ω 6,9c/17:0	—	—	—	—	0.3	0.3	—	—
18:1 ω 9c	—	—	—	—	0.3	0.3	—	—
18:1 ω 7c/12t/9t	3.3	3.7	3.2	12.9	13.0	10.5	11.0	15.5
18:0	—	0.5	—	0.5	0.4	0.3	—	0.6
Unidentified	—	—	—	—	0.1	0.2	—	0.6

The 16S rRNA, internally transcribed spacer 1 (ITS1; between the 16S and the 23S genes), *nifH* (nitrogenase) and Rubisco genes were amplified from genomic DNA by PCR, and sequenced as described previously (Gomila *et al.*, 2007). Gene sequences were aligned with the homologous sequences of the closest relatives retrieved using the BLAST analysis tool and the NCBI nucleotide sequence database. The alignments were done using a hierarchical method for multiple alignments implemented in the program CLUSTAL_X (Thompson *et al.*, 1997). Automatically aligned sequences were checked manually. Sequence similarities and evolutionary distances were calculated with programs implemented in PHYLIP (Phylogeny Inference Package, version 3.5c) (Felsenstein, 1989). The 16S rRNA gene sequence of strain CCUG 52220 was 99.7% similar to that of *Roseateles depolymerans* CCUG 52219^T (Fig. 1). Strain CCUG 52222^T clustered in the same phylogenetic branch of the 16S rRNA gene sequence tree, with a sequence similarity of 99.6% with that of the type strain, and was similar to the sequences of the species in the genus *Pelomonas* (between 97.2 and 97.9%) and to the type strain of *M. chitosanitabida* (98.1%). Strain CCUG 48205^T also grouped in the *Roseateles* branch, with a similarity of 98.6% to the 16S rRNA gene sequence of the type strain of *Roseateles depolymerans* (see Supplementary Table S3 in IJSEM Online). The type strain of *Roseateles depolymerans*, as well as strains CCUG 52220 and CCUG 52222^T, showed two ITS1 amplicons of different sizes and were not sequenced. Rubisco genes *cbbL* of green or red form I enzymes and *cbbM* of the form II enzyme did not amplify in any of the strains. The *nifH* genes in strains CCUG 52219^T, CCUG 52220 and CCUG 52222^T were

PCR-amplified and sequenced, and showed the same nucleotide sequence.

Amplification of the partial *puf* (photosynthetic) gene operon was done for the four *Roseateles* strains using primers *pufLF* (5'-CTKTTTCGACTTCTGGGTSGG-3') and *pufMR* (5'-CCCATSGTCCAGCGCCAGAA-3'). The following PCR conditions were employed: an initial cycle at 94 °C for 4 min, 35 cycles at 94 °C for 1 min, 56 °C for 1 min and 72 °C for 1.5 min, and a final cycle at 72 °C for 10 min. A fragment of about 1.5 kb in size was amplified for strains CCUG 52219^T, CCUG 52220 and CCUG 52222^T. The PCR product was sequenced and compared with databases, confirming that photosynthetic genes are present in these strains. DNA of strain CCUG 48205^T did not amplify. A Southern blot analysis was also performed with the four *Roseateles* strains by digestion of the genomic DNAs with the restriction enzymes *ApaI* and *EcoRI* (GE Health Care), followed by hybridization with the amplified *puf* gene fragment of strain CCUG 52219^T as a probe. Strain CCUG 48205^T did not hybridize with the probe, but the other three strains gave a positive signal (data not shown).

In addition to the phylogenetic, genomic and chemotaxonomic differences, strain CCUG 48205^T could be differentiated from other *Roseateles* strains by its narrow range of temperatures for growth, by the assimilation of different carbon sources, catalase, β -glucosidase and β -galactosidase reactions and by the absence of *nif* and *puf* genes. Strain CCUG 52222^T could be differentiated from *Roseateles depolymerans* by its profile of carbon sources assimilated and by the urease reaction.

Based on these results, we conclude that strain CCUG 52220 belongs to the species *Roseateles depolymerans* and that strains CCUG 48205^T and CCUG 52222^T represent two novel species, with the names *Roseateles aquatilis* sp. nov. and *Roseateles terrae* sp. nov., respectively.

Emended description of the genus *Roseateles* Suyama *et al.* 1999

Roseateles (Ro.se.a.te'les. L. adj. *roseus* rose-coloured, pink; Gr. adj. *ateles* defective, incomplete; N.L. masc. n. *roseateles* the rose-coloured incomplete photosynthetic bacterium).

Cells are motile, Gram-negative straight rods. Cells of some strains are pink in colour under conditions suitable for photosynthetic pigment production. *Cis*-9-hexadecanoic and hexadecanoic acids are the major cellular fatty acids. Low levels of dodecanoic acid and high levels of *cis*-11-octadecanoic acid differentiate the genus from other genera in the same phylogenetic branch. Other characteristics of the genus are given by Suyama *et al.* (1999).

Description of *Roseateles aquatilis* sp. nov.

Roseateles aquatilis (a.qua.ti'lis. L. masc. adj. *aquatilis* aquatic, growing in water).

Fig. 1. Phylogenetic tree, based on 16S rRNA gene sequence comparisons over 1380 aligned bases, showing the relationship between the genera *Mitsuaria*, *Pelomonas* and *Roseateles* in the *Betaproteobacteria*. The tree was constructed using the neighbour-joining method, following distance analysis calculated by using the Jukes and Cantor method (Jukes & Cantor, 1969). The topology of the tree was visualized by means of the program TreeView (Page, 1996). Bootstrap values greater than 500 based on 1000 replications are indicated at nodes. Sequences indicated in bold were determined in this study. GenBank accession numbers are given in parentheses. Bar, 0.01 nucleotide substitutions per site.

Colonies are 4–6 mm in diameter, clear white, circular and umbonate, with an undulate margin and translucent. Four-day-old colonies show concentric circles. Non-pigmented. Oxidase-positive and catalase-negative. Growth occurs in R2A medium at 20–30 °C, but not at 10 or 45 °C. Cells are rod-shaped (2 µm in length and 0.5 µm in width) with single polar flagella. Nitrate reduction, indole formation, glucose fermentation, arginine dihydrolase, urease, β -galactosidase (PNPG) and aesculin tests are negative. Gelatin hydrolysis is positive. D-Glucose, maltose, gluconate and malic acid are assimilated. L-Arabinose, D-mannose, D-mannitol, N-acetyl-D-glucosamine, capric acid, adipic acid, citrate and phenylacetic acid are not assimilated. Photosynthetic genes (*puf*) are not detected.

The type strain, CCUG 48205^T (=CECT 7248^T), was isolated from industrial water in Sweden.

Description of *Roseateles terrae* sp. nov.

Roseateles terrae (ter'rae. L. gen. n. *terrae* of the soil).

Colonies are circular, 4–6 mm in diameter and convex, with an undulate transparent margin. Non-pigmented. Oxidase- and catalase-positive. Growth occurs in R2A medium at 10–37 °C, but not at 4 or 45 °C. Cells are rod-shaped (2 µm in length and 0.5 µm in width) with single polar flagella. Nitrate reduction, indole formation, glucose fermentation, arginine dihydrolase and urease tests are negative. β -Galactosidase (PNPG), aesculin and gelatin

hydrolysis are positive. D-Glucose, L-arabinose, D-mannose, D-mannitol, maltose, gluconate and capric acid are assimilated. Assimilation of malic acid is extremely weak. N-Acetyl-D-glucosamine, adipic acid, citrate and phenylacetic acid are not assimilated. Photosynthetic genes (*puf*) are detected, but are not expressed under the experimental conditions used.

The type strain, CCUG 52222^T (=CECT 7247^T), was isolated from soil in Japan.

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References

- Amakata, D., Matsuo, Y., Shimono, K., Kweon Park, J., Soo Yun, C., Matsuda, H., Yokota, A. & Kawamukai, M. (2005). *Mitsuaria chitosanitabida* gen. nov., sp. nov., an aerobic, chitosanase-producing member of the 'Betaproteobacteria'. *Int J Syst Evol Microbiol* **55**, 1927–1932.
- Aragno, M. & Schlegel, H. G. (1992). The mesophilic hydrogen-oxidizing (Knallgas) bacteria. In *The Prokaryotes*, vol. 1, pp. 344–384, 2nd edn. Edited by A. Balows, H. G. Trüper, M. Dworkin, W. Harder & K. H. Schleifer. New York: Springer.
- Cowan, S. T. (1974). *Cowan and Steel's Manual for the Identification of Medical Bacteria*, 2nd edn. London: Cambridge University Press.
- Felsenstein, J. (1989). PHYLIP – phylogeny inference package (version 3.2). *Cladistics* **5**, 164–166.
- Gomila, M., Bowien, B., Falsen, E., Moore, E. R. B. & Lalucat, J. (2007). Description of *Pelomonas aquatica* sp. nov. and *Pelomonas puraquae* sp. nov., isolated from industrial and haemodialysis water. *Int J Syst Evol Microbiol* **57**, 2629–2635.
- Jukes, T. H. & Cantor, C. R. (1969). Evolution of protein molecules. In *Mammalian Protein Metabolism*, vol. 3, pp. 21–132. Edited by H. N. Munro. New York: Academic Press.
- Kalmbach, S., Manz, W., Wecke, J. & Szewzyk, U. (1999). *Aquabacterium* gen. nov., with description of *Aquabacterium citratiphilum* sp. nov., *Aquabacterium parvum* sp. nov. and *Aquabacterium commune* sp. nov., three *in situ* dominant bacterial species from the Berlin drinking water system. *Int J Syst Bacteriol* **49**, 769–777.
- Lalucat, J. (1988). Analysis of refractile (R) bodies. In *Methods in Microbiology – Electron Microscopy in Microbiology*, vol. 20, pp. 79–90. Edited by F. Mayer. London: Academic Press.
- Malmqvist, A., Welander, T., Moore, E., Ternström, A., Molin, G. & Stenström, I.-M. (1994). *Ideonella dechloratans* gen. nov., sp. nov., a new bacterium capable of growing anaerobically with chlorate as an electron acceptor. *Syst Appl Microbiol* **17**, 58–64.
- Marmur, J. (1961). A procedure for the isolation of deoxyribonucleic acid from microorganisms. *J Mol Biol* **3**, 208–218.
- Page, R. D. M. (1996). TreeView: an application to display phylogenetic trees on personal computers. *Comput Appl Biosci* **12**, 357–358.
- Reasoner, D. J. & Geldreich, E. E. (1985). A new medium for the enumeration and subculture of bacteria from potable water. *Appl Environ Microbiol* **49**, 1–7.
- Spring, S. (2006). The genera *Leptothrix* and *Sphaerotilus*. In *The Prokaryotes: a Handbook on the Biology of Bacteria*, 3rd edn, vol. 5, pp. 758–777. Edited by M. Dworkin, S. Falkow, E. Rosenberg, K. H. Schleifer & E. Stackebrandt. New York: Springer.
- Suyama, T., Shigematsu, T., Takaichi, S., Nodasaka, Y., Fujikawa, S., Hosoya, H., Tokiwa, Y., Kanagawa, T. & Hanada, S. (1999). *Roseateles depolymerans* gen. nov., sp. nov., a new bacteriochlorophyll *a*-containing obligate aerobe belonging to the β -subclass of the *Proteobacteria*. *Int J Syst Bacteriol* **49**, 449–457.
- Suyama, T., Shigematsu, T., Suzuki, T., Tokiwa, Y., Kanagawa, T., Nagashima, K. V. P. & Hanada, S. (2002). Photosynthetic apparatus in *Roseateles depolymerans* 61A is transcriptionally induced by carbon limitation. *Appl Environ Microbiol* **68**, 1665–1673.
- Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997). The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* **25**, 4876–4882.
- Wen, A., Fegan, M., Hayward, C., Chakraborty, S. & Sly, L. I. (1999). Phylogenetic relationships among members of the *Comamonadaceae*, and description of *Delftia acidovorans* (den Dooren de Jong 1926 and Tamaoka *et al.* 1987) gen. nov., comb. nov. *Int J Syst Bacteriol* **49**, 567–576.
- Willems, A. & Gillis, M. (2005). Family IV. *Comamonadaceae* Willems, De Ley, Gillis and Kersters 1991a, 447^{VP}. In *Bergey's Manual of Systematic Bacteriology*, 2nd edn. *The Proteobacteria: The Alpha-, Beta-, Delta-, and Epsilonproteobacteria*, vol. 2, part C, pp. 686–763. Edited by D. J. Brenner, N. R. Krieg, J. T. Staley & G. M. Garrity. New York: Springer.
- Willems, A., Gillis, M. & De Ley, J. (1991). Transfer of *Rhodocyclus gelatinosus* to *Rubrivivax gelatinosus* gen. nov., comb. nov., and phylogenetic relationships with *Leptothrix*, *Sphaerotilus natans*, *Pseudomonas saccharophila*, and *Alcaligenes latus*. *Int J Syst Bacteriol* **41**, 65–73.
- Xie, C.-H. & Yokota, A. (2005). Reclassification of *Alcaligenes latus* strains IAM 12599^T and IAM 12664 and *Pseudomonas saccharophila* as *Azohydromonas lata* gen. nov., comb. nov., *Azohydromonas australiaca* sp. nov. and *Pelomonas saccharophila* gen. nov., comb. nov., respectively. *Int J Syst Evol Microbiol* **55**, 2419–2425.
- Ziemke, F., Höfle, M. G., Lalucat, J. & Rosselló-Mora, R. (1998). Reclassification of *Shewanella putrefaciens* Owen's genomic group II as *Shewanella baltica* sp. nov. *Int J Syst Bacteriol* **48**, 179–186.