J. Microbiol. Biotechnol. (2009), **19**(1), 5–10
doi: 10.4014/jmb.0802.145
First published online 18 July 2008

JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY

Inhella inkyongensis gen. nov., sp. nov., a New Freshwater Bacterium in the Order *Burkholderiales*

Song, Jaeho[1], Hyun-Myung Oh[1], Jung-Sook Lee[2], Seung-Buhm Woo[1], and Jang-Cheon Cho[1]*

[1]*Division of Biology and Ocean Sciences, Inha University, Incheon 402-751, Korea*
[2]*Korean Collection for Type Cultures, Biological Resource Center, Korea Research Institute of Bioscience and Biotechnology, Daejeon 305-333, Korea*

Received: February 20, 2008 / Accepted: June 9, 2008

A freshwater bacterium, designated IMCC1713^T, was isolated from a highly eutrophic artificial pond. Cells of the strain were Gram-negative, chemoheterotrophic, poly-β-hydroxybutyrate granule containing and obligately aerobic short rods that were motile with a single polar flagellum. The 16S rRNA gene sequence similarity analysis showed that the novel strain was most closely related to the species *Roseateles depolymerans* (96.3%), *Mitsuaria chitosanitabida* (96.2%), *Ideonella dechloratans* (96.2%), and *Pelomonas saccharophila* (96.1%) in the *Sphaerotilus-Leptothrix* group within the order *Burkholderiales*. Phylogenetic trees based on 16S rRNA gene sequences indicated that the isolate formed an independent monophyletic clade within the order *Burkholderiales*. The relatively low DNA G+C content (57.4 mol%), together with several phenotypic characteristics, differentiated the novel strain from other members of the *Sphaerotilus-Leptothrix* group. From the taxonomic data, therefore, the strain should be classified as a novel genus and species, for which the name *Inhella inkyongensis* gen. nov., sp. nov. is proposed. The type strain of the proposed species is strain IMCC1713^T (=KCTC 12791^T=NBRC 103252^T=CCUG 54308^T).

Keywords: *Inhella inkyongensis*, *Sphaerotilus-Leptothrix* group, freshwater, novel genus, 16S rRNA gene

The order *Burkholderiales* [5] in the class *Betaproteobacteria* encompasses phenotypically, metabolically, and ecologically diverse members of Gram-negative bacteria. The order currently contains four families, *Burkholderiaceae*, *Oxalobacteraceae*, *Alcaligenaceae*, and *Comamonadaceae*, that have been circumscribed largely based on the 16S rRNA gene sequence-based phylogeny. Although there has been a well-defined taxonomic hierarchy among the members of the order *Burkholderiales*, several genera closely related to the family *Comamonadaceae* have been known to be taxonomically uncertain and placed as *genera insertae sedis* [25]. This phylogenetically uncertain group called the *Sphaerotilus-Leptothrix* group [22] contains metabolically diverse genera, including the bacteriochlorophyll *a*-containing bacteria *Roseateles* [24] and *Rubrivivax* [26], sheathed bacteria such as *Leptothrix* and *Sphaerotilus* [22], and various recalcitrant material-degrading bacteria such as *Mitsuaria* [1], *Paucibacter* [17], *Methylibium* [14], and *Aquincola* [10].

During the course of a study for revealing bacterial diversity in a eutrophic freshwater pond (Inkyong Reservoir) located at Inha University, several novel strains belonging to the *Sphaerotilus-Leptothrix* group were isolated [21]. The present study focuses on the identification and characterization of a novel obligately aerobic and non-phototrophic strain (designated IMCC1713^T) of the *Sphaerotilus-Leptothrix* group. Based on the taxonomic data collected in this study, we propose the inclusion of the strain in a new genus and novel species named *Inhella inkyongensis* gen. nov., sp. nov.

Strain IMCC1713^T was isolated using a standard dilution plating method on R2A agar plates. Strain IMCC1713^T was purified from the agar plate, which was incubated aerobically at 20°C for 5 days. After the optimum growth conditions of the strain had been determined, bacterial cultures were routinely maintained on R2A agar at 25°C and preserved as glycerol suspension (10%) at −70°C.

PCR amplification of the bacterial 16S rRNA gene was performed with the slightly modified universal bacterial primers 27F-B and 1492R [3]. Sequencing of the 16S rRNA gene was carried out as described previously [3], and the almost complete 16S rRNA gene sequence (1,484 bp) was determined. To determine the approximate phylogenetic affiliation of strain IMCC1713^T, the 16S rRNA gene

*Corresponding author
Phone: 82-32-860-7711; Fax: 82-32-232-0541;
E-mail: chojc@inha.ac.kr

sequence was compared with the sequence databases in RDP-II and GenBank by BLASTN. These comparative sequence analyses showed that the strain belonged to the order *Burkholderiales.* The 16S rRNA gene sequence of strain IMCC1713^T was aligned with related bacterial sequences based on the secondary structure of 16S rRNA using the ARB software package [11]. The 16S rRNA gene sequence similarities were calculated from distance matrices based on the Jukes-Cantor distance formula in the ARB. A total of 1,117 unambiguously aligned nucleotide positions were exported to PAUP* version 4.0 beta 10 for phylogenetic analyses. Phylogenetic trees were inferred by neighbor-joining with the Jukes-Cantor distance model, maximum parsimony, and maximum likelihood with a heuristic search, tree bisection-reconnection (TBR)-branching and a Ti/Tv ratio of 1.648. Robustness of tree topologies from the neighbor-joining and maximum parsimony were evaluated by bootstrap analyses based on 1,000 resamplings of sequences.

Strain IMCC1713^T was most closely related to unidentified betaproteobacterium A1004 (AF236009, GenBank description) and uncultured freshwater clone 134ds10 [19] with 98.8–99.2% 16S rRNA gene sequence similarity. Sequence comparisons with validly published bacteria showed that the strain shared the highest sequence similarity with *Roseateles depolymerans* DSM 11813^T (96.3%), followed by *Mitsuaria chitosanitabida* ATCC BAA-476^T (96.2%), *Ideonella dechloratans* CCUG 30898^T (96.2%), and *Pelomonas saccharophila* DSM 654^T (96.1%). Overall, the strain showed 94.5–96.3% 16S rRNA gene sequence similarity to the validly published species of the *Sphaerotilus-Leptothrix* group and 91.2–92.7% to members of the family *Comamonadaceae.* As shown in the phylogenetic tree (Fig. 1), strain IMCC1713^T formed a unique clade with unidentified bacterium A1004 and uncultured clone 134ds10 that did not associate significantly with any of the known genera in the *Sphaerotilus-Leptothrix* group and the family *Comamonadaceae.* This clade appeared to be monophyletic in the trees generated by three different treeing algorithms, with 100% bootstrap support. These phylogenetic analyses indicated the uniqueness of the 16S rDNA sequence of the strain; therefore, strain IMCC1713^T was considered to represent a new genus and species in the *Sphaerotilus-Leptothrix* group of the order *Burkholderiales*.

For phenotypic and chemotaxonomic characterizations, strain IMCC1713^T was routinely grown and tested on R2A agar at 25°C, unless otherwise specified. The growth temperature range and optimum were tested at 4, 10, 15, 20, 25, 30, 37, and 42°C. The pH range and optimum were examined at pH values from pH 4.0 to 12.0, adjusted with 0.1 M HCl and 0.1 M NaOH. The optimum NaCl concentration for growth was monitored on R2A agar supplemented with 0–15% NaCl (w/v). Cell morphology was observed under a phase contrast microscope (Nikon 80i; Nikon, Japan) and transmission electron microscope (TEM; CM200, Philips, The Netherlands). The presence of intracellular granules and flagellation was also observed using TEM. Colony morphology, size, and color were examined from cultures grown

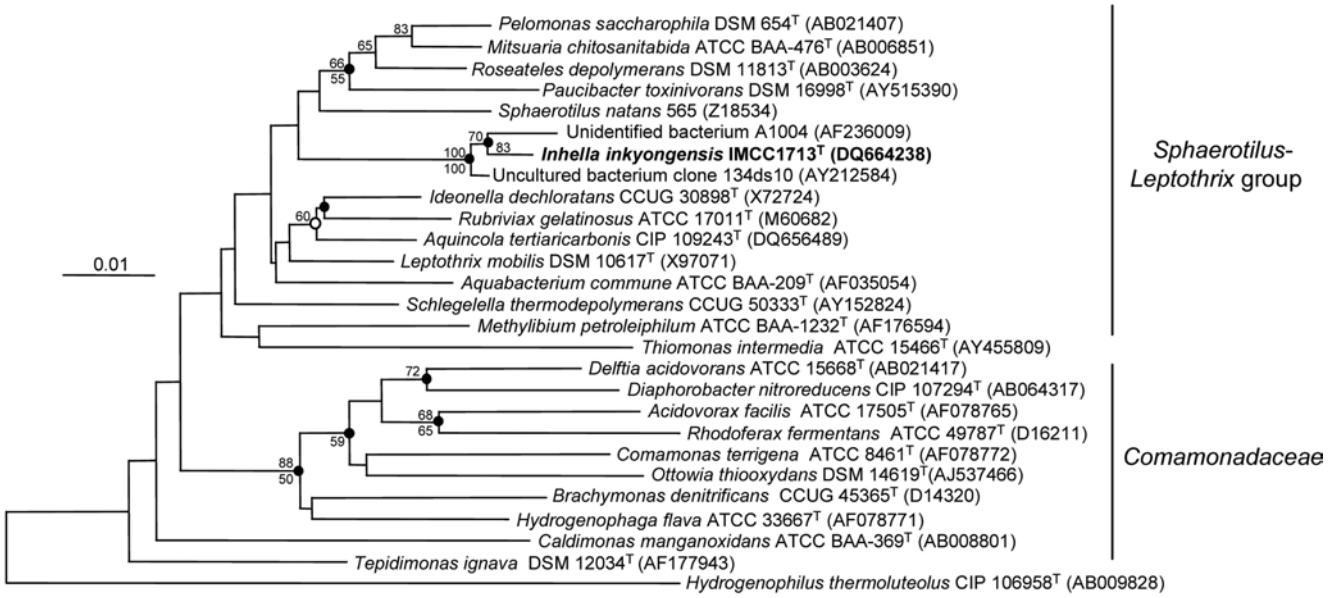

Fig. 1. Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences showing distant relationships between strain IMCC1713^T and representatives of the *Sphaerotilus-Leptothrix* group and the family *Comamonadaceae.* Bootstrap percentages (above 50%) from both neighbor-joining (above nodes) and maximum parsimony (below nodes) are shown. Filled and open circles at each node respectively indicate nodes recovered reproducibly by all treeing methods or by two treeing methods. Bar, 0.01 substitutions per nucleotide position.

aerobically for 5 days. Flagellar motility was tested from wet mounts using a 3-day culture. Gliding motility was tested by phase-contrast microscopy with 17-h incubated cells on microscopic slides coated with R2A agar (0.7% agar) according to the method described previously [2]. The Gram reaction was determined by using both nonstaining method using 3% KOH solution and the Gram-staining kit (Scharlau Chemie, Spain). The presence of poly-β-hydroxybutyrate (PHB) granules was examined using Nile Blue A staining as described by Ostle and Holt [15]. Growth under anaerobic condition was determined by employing AnaeroPACK Anaero (Mitsubishi Gas Chemical Company, Japan). Catalase activity was determined by reaction of 3% (w/v) hydrogen peroxide on fresh colonies, and oxidase activity was tested using Kovacs's solution. Other biochemical tests were carried out on API 20NE (bioMérieux) and API ZYM (bioMérieux) kits according to the manufacturer's instructions. Carbon source oxidation of the strain was tested using Biolog GN2 microplates with sterilized water supplemented with 10 μM NH_4Cl and 1 μM KH_2PO_4. Susceptibility of the strain to antimicrobial agents was tested as described previously [4]. The G+C content of DNA was analyzed by HPLC [13] equipped with a Discovery C18 column (5 μm, 15 cm×4.6 mm, Supelco). For the analysis of cellular fatty acids, the strain was cultured at 25°C for 3 days and analyzed according to the instructions of the Microbial Identification System (MIDI) by the Korean Collection for Type Cultures (KCTC). Respiratory quinones were analyzed using a reversed-phase HPLC [9, 18] by KCTC.

Based on phenotypic characterization, strain IMCC1713^T was Gram-negative, chemoheterotrophic, obligately aerobic, PHB granule-containing, short to straight rods that were motile with a single flagellum (Fig. 2). The details of phenotypic and chemotaxonomic characteristics are given in the species description and Table 1. Strain IMCC1713^T shared general characteristics of the *Sphaerotilus-Leptothrix* group, such as flagellation and Q-8 as the major quinone content, with the other genera of the group. The strain, however, could be differentiated from the other genera of the *Sphaerotilus-Leptothrix* group by several phenotypic characteristics and the DNA G+C content (Table 1). The DNA G+C content of strain IMCC1713^T was 57 mol%, which was 8–17 mol% higher than that of other members of the *Sphaerotilus-Leptothrix* group. The strain clearly differed from the most closely related genera, *Roseateles*, *Mitsuaria*, *Ideonella*, and *Pelomonas*, the 16S rRNA gene sequences of which were higher than 96%, by several phenotypic properties including the presence of PHB granules, growth temperature, bacteriochlorophyll *a*, and carbon source oxidation pattern. It is clear from the formation of an independent phyletic line in the phylogenetic analyses (Fig. 1), the low G+C content of DNA, and the differential phenotypic characteristics (Table 1) that strain IMCC1713^T cannot be affiliated with any of the known genera. Conclusively, based on the taxonomic results collected in this study, strain IMCC1713^T should be classified as a novel species within a new genus, for which the name *Inhella inkyongensis* gen. nov., sp. nov. is proposed.

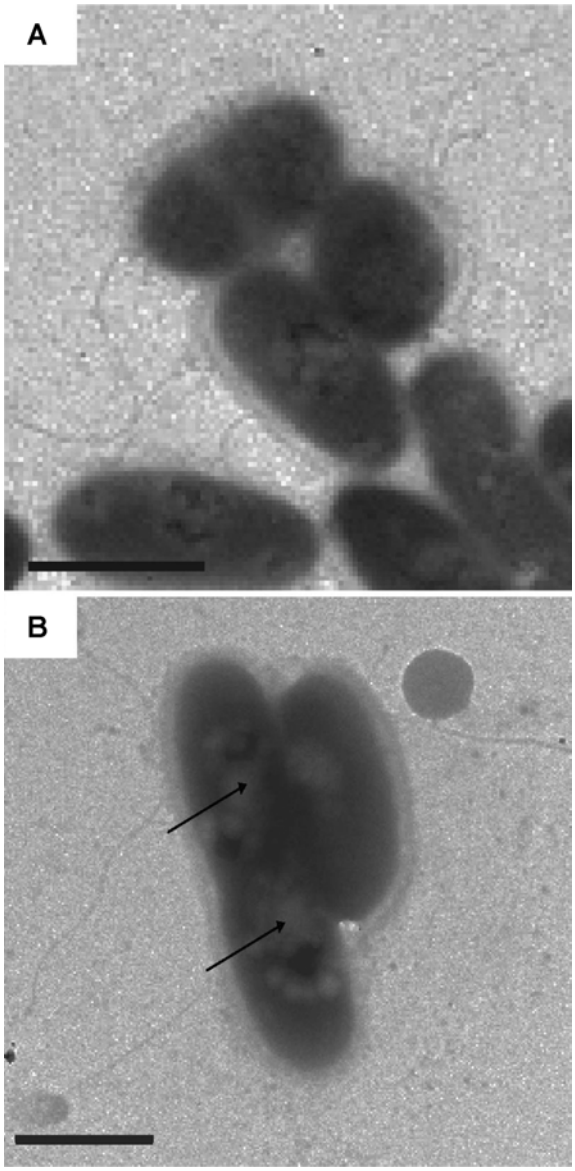

Fig. 2. Transmission electron micrographs of negatively stained cells of strain IMCC1713^T.
A. Cells showing a polar flagellum. **B**. Cells containing PHB granule. Arrows in panel B indicate PHB granules. Bars, 1 μm.

The GenBank accession number for the 16S rRNA gene sequences of strain IMCC1713^T is DQ664238.

Description of *Inhella* gen. nov.

Inhella (In.hella. N.L. fem. n. *Inhella* named after Inha University, where the isolation source of the type species is located.).

Table 1. Differential characteristics of *Inhella inkyongensis* IMCC1713^T and other related genera in the *Sphaerotilus-Leptothrix* group of the order *Burkholderiales*.

Characteristics	1	2	3	4	5	6	7	8	9	10	11	12
Flagellation[a]	OP	OP	OP	OP, SP	OP	V	OP	SP	OP	OP	T	OP
Growth at 37°C	–	–	ND	V	–	V	+	+	+	V	+	+
Anaerobic growth	–	+	+	–	+	V	–	+	ND	–	+	–
Catalase	–	W	+	V	–	–	+	W	+	ND	ND	W
Oxidase	+	+	+	–	+	+	+	+	+	+	ND	+
Bacteriochlorophyll *a*	–	–	+	+	–	–	–	–	–	–	–	–
Sheath formation	–	–	–	–	–	–	–	–	–	+	+	–
PHB granules	+	ND	+	+	+	+	–	–	+[g]	+	+	+
Nitrate reduction	W	+	–[b]	–	+	V	+	+	V	ND	ND	–
Indole production	–	–	–[c]	+[d]	ND	–	–	ND	ND	ND	ND	–
Gelatinase	W	+	+	+	–	–	ND	ND	+	ND	W	–
Carbon oxidation:												
Fructose	–	–	+	V	–	+[f]	–	+	+[g]	–	+	ND
Galactose	–	–	ND	V	–	–[f]	–	ND	+[h]	–	+	ND
Sucrose	–	–	ND	–	–	–	–	ND	+[i]	–	+	ND
Acetic acid	–	–	+	–	+	+	+	+	+[i]	V	+	+
Lactic acid	–	–	V	V	V	+[f]	ND	+	+	V	+	+
Succinic acid	+	–	+	V	+	–[f]	ND	+	V	–	+	ND
Glycerol	W	W	–	V	V	–[f]	+	ND	V	–	+	ND
Quinone	Q-8 Q-6	ND	Q-8 MK-8	Q-8[e]	ND	Q-8	Q-8	Q-8	Q-8[g]	Q-8	Q-8	Q-8
DNA G+C content (mol%)	57	67	70-74	66[e]	65-66	66-69	69	68	69[g]	68-71	69	69-71

Taxa: 1, IMCC1713^T (present study); 2, *Paucibacter* [17]; 3, *Rubrivivax* [16, 26]; 4, *Roseateles* [7, 24]; 5, *Aquabacterium* [8]; 6, *Methylibium* [14, 20, 28]; 7, *Mitsuaria* [1]; 8, *Ideonella* [12]; 9, *Pelomonas* [6, 27]; 10, *Leptothrix* [23, 25]; 11, *Sphaerotilus* [22, 25]; 12, *Aquincola* [10]. Symbols: +, positive; W, weakly positive; –, negative; V, variable; ND, no data available.

[a] OP, One polar; SP, several polar; T, tuft subpolar.

[b] Data for *Rubrivivax gelatinosus*.

[c] Data for *Rubrivivax benzoatilyticus*.

[d] Data for *Roseateles terrae* and *Roseateles aquatilis*.

[e] Data for *Roseateles depolymerans*.

[f] Data for *Methylibium aquaticum* and *Methylibium fulvum*.

[g] Data for *Pelomonas saccharophila*.

[h] Data for *Pelomonas saccharophila* and *Pelomonas aquatica*.

[i] Data of *Pelomonas saccharophila* and *Pelomonas puraquae*.

Cells are Gram-negative, flagellated, and short to long rod-shaped. Chemoheterotrophic and obligately aerobic. PHB granules are present as storage material. Oxidase-postive and catalase-negative. The predominant fatty acids are $C_{16:1}$ ω7c and/or iso-$C_{15:0}$ 2-OH, $C_{16:0}$, and $C_{10:0}$ 3-OH. The major quinone is Q-8. Q-6 is also present. Phylogenetically, the genus belongs to the *Sphaerotilus-Leptothrix* group. The type species of the genus is *Inhella inkyongensis*.

Description of *Inhella inkyongensis* sp. nov.

Inhella inkyongensis (in.kyong.en'sis. N.L. fem. adj. *inkyongensis* from Inkyong Reservoir, where the type strain was isolated.)

The description is the same as that for the genus, with the following additional properties. Cells are 0.9–2.6 μm long and 0.4–0.9 μm wide. Colonies on R2A (5 days of incubation) are circular, convex, smooth, 0.5–1.0 mm in diameter. Growth occurs at 15–30°C (optimum; 25°C), pH 6.0–9.0 (optimum; 7.0), and with 0–0.7% NaCl (optimally without NaCl). Indole production, arginine dihydrolase, acid production from glucose, urease, and PNPG (β-galactosidase) are negative. Esculin hydrolysis, gelatinase, and nitrate reduction are weakly positive. Enzyme activities for acid phosphatase and naphthol-AS-BI-phosphohydrolase are positive. However, alkaline phosphatase, esterase lipase (C8), esterase (C4), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, and acid phosphatase activities are negative. In Biolog GN2 plates, pyruvic acid methyl ester, β-hydroxybutyric acid, α-ketoglutaric acid, and succinic acid are oxidized by the type strain. Glycogen, Tween 80, Tween 40, cis-aconitic acid, citric acid, L-alanyl-glycine, L-glutamic acid, L-ornithine, L-proline, L-threonine, glycerol, and urocanic acid are weakly oxidized. Susceptible to vancomycin, chloramphenicol, erythromycin, gentamicin, kanamycin,

streptomycin, and tetracycline, but resistant to ampicillin, rifampicin, and penicillin G. The cellular fatty acids are composed of $C_{16:1}$ ω7c and/or iso-$C_{15:0}$ 2-OH (36.0%), $C_{16:0}$ (16.4%), $C_{10:0}$ 3-OH (6.1%), $C_{14:0}$ (4.9%), $C_{15:0}$ (4.7%), $C_{15:1}$ ω6c (4.6%), $C_{18:1}$ ω7c (4.6%), $C_{17:0}$ (3.5%), unknown 18.846 peak and/or $C_{19:1}$ ω6c (2.6%), $C_{12:0}$ (2.4%), and $C_{12:0}$ 3-OH (1.9%). Traces of iso-$C_{16:0}$, $C_{17:1}$ ω8c, and $C_{18:1}$ ω9c are also present. The respiratory quinones detected are Q-8 (87.9%) and Q-6 (12.1%). The DNA G+C content is 57.4±0.3 mol% (determined by HPLC). The type strain, IMCC1713^T (=KCTC 12791^T, NBRC 103252^T and CCUG 54308^T), was isolated from an artificial freshwater pond (Inkyong Reservoir) located inside Inha University, Korea.

Acknowledgment

This study was supported by Inha University Research Grant.

References

1. Amakata, D., Y. Matsuo, K. Shimono, J. K. Park, C. S. Yun, H. Matsuda, A. Yokota, and M. Kawamukai. 2005. *Mitsuaria chitosanitabida* gen. nov., sp. nov., an aerobic, chitosanase-producing member of the '*Betaproteobacteria*'. *Int. J. Syst. Evol. Microbiol.* **55:** 1927–1932.
2. Bowman, J. P. 2000. Description of *Cellulophaga algicola* sp. nov., isolated from the surfaces of Antarctic algae, and reclassification of *Cytophaga uliginosa* (ZoBell and Upham 1944) Reichenbach 1989 as *Cellulophaga uliginosa* comb. nov. *Int. J. Syst. Evol. Microbiol.* **50:** 1861–1868.
3. Cho, J.-C. and S. J. Giovannoni. 2004. Cultivation and growth characteristics of a diverse group of oligotrophic marine *Gammaproteobacteria*. *Appl. Environ. Microbiol.* **70:** 432–440.
4. Choo, Y.-J., K. Lee, J. Song, and J.-C. Cho. 2007. *Puniceicoccus vermicola* gen. nov., sp. nov., a novel marine bacterium, and description of *Puniceicoccaceae* fam. nov., *Puniceicoccales* ord. nov., *Opitutaceae* fam. nov., *Opitutales* ord. nov. and *Opitutae classis* nov. in the phylum '*Verrucomicrobia*'. *Int. J. Syst. Evol. Microbiol.* **57:** 532–537.
5. Garrity, G. M., J. A. Bell, and T. Lilburn. 2005. Order I. *Burkholderiales* ord. nov., pp. 575–763. *In* D. J. Brenner, N. R. Krieg, J. T. Staley, and G. M. Garrity (eds.), *Bergey's Manual of Systematic Bacteriology*, Vol. 2 (The *Proteobacteria*), Part C (The *Alpha-, Beta-, Delta-,* and *Epsilonproteobacteria*), 2nd Ed. Springer, New York.
6. Gomila, M., B. Bowien, E. Falsen, E. R. Moore, and J. Lalucat. 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.
7. Gomila, M., B. Bowien, E. Falsen, E. R. Moore, and J. Lalucat. 2008. Description of *Roseateles aquatilis* sp. nov. and *Roseateles terrae* sp. nov., in the class *Betaproteobacteria*, and emended description of the genus *Roseateles*. *Int. J. Syst. Evol. Microbiol.* **58:** 6–11.
8. Kalmbach, S., W. Manz, J. Wecke, and U. Szewzyk. 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.
9. Komagata, K. and K. Suzuki. 1987. Lipids and cell-wall analysis in bacterial systematics. *Methods Microbiol.* **19:** 161–203.
10. Lechner, U., D. Brodkorb, R. Geyer, G. Hause, C. Hartig, G. Auling, *et al.* 2007. *Aquincola tertiaricarbonis* gen. nov., sp. nov., a tertiary butyl moiety-degrading bacterium. *Int. J. Syst. Evol. Microbiol.* **57:** 1295–1303.
11. Ludwig, W., O. Strunk, R. Westram, L. Richter, H. Meier, Yadhukumar, *et al.* 2004. ARB: A software environment for sequence data. *Nucleic Acids Res.* **32:** 1363–1371.
12. Malmqvist, Å., T. Welander, E. Moore, A. Ternström, G. Molin, and I. Stenström. 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.
13. Mesbah, M., U. Premachandran, and W. B. Whitman. 1989. Precise measurement of the G+C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int. J. Syst. Bacteriol.* **39:** 159–167.
14. Nakatsu, C. H., K. Hristova, S. Hanada, X. Y. Meng, J. R. Hanson, K. M. Scow, and Y. Kamagata. 2006. *Methylibium petroleiphilum* gen. nov., sp. nov., a novel methyl tert-butyl ether-degrading methylotroph of the *Betaproteobacteria*. *Int. J. Syst. Evol. Microbiol.* **56:** 983–989.
15. Ostle, A. G. and J. G. Holt. 1982. Nile blue A as a fluorescent stain for poly-beta-hydroxybutyrate. *Appl. Environ. Microbiol.* **44:** 238–241.
16. Ramana Ch, V., C. Sasikala, K. Arunasri, P. Anil Kumar, T. N. Srinivas, S. Shivaji, P. Gupta, J. Suling, and J. F. Imhoff. 2006. *Rubrivivax benzoatilyticus* sp. nov., an aromatic, hydrocarbon-degrading purple betaproteobacterium. *Int. J. Syst. Evol. Microbiol.* **56:** 2157–2164.
17. Rapala, J., K. A. Berg, C. Lyra, R. M. Niemi, W. Manz, S. Suomalainen, L. Paulin, and K. Lahti. 2005. *Paucibacter toxinivorans* gen. nov., sp. nov., a bacterium that degrades cyclic cyanobacterial hepatotoxins microcystins and nodularin. *Int. J. Syst. Evol. Microbiol.* **55:** 1563–1568.
18. Shin, Y.-K., J.-S. Lee, C.-O. Chun, H.-J. Kim, and Y.-H. Park. 1996. Isoprenoid quinone profiles of the *Leclercia adecarboxylata* KCTC 1036^T. *J. Microbiol. Biotechnol.* **6:** 68–69.
19. Simpson, J. M., J. W. Santo Domingo, and D. J. Reasoner. 2004. Assessment of equine fecal contamination: The search for alternative bacterial source-tracking targets. *FEMS Microbiol. Ecol.* **47:** 65–75.
20. Song, J. and J.-C. Cho. 2007. *Methylibium aquaticum* sp. nov., a betaproteobacterium isolated from a eutrophic freshwater pond. *Int. J. Syst. Evol. Microbiol.* **57:** 2125–2128.
21. Song, J., S.-J. Yang, and J.-C. Cho. 2007. "Bring to lab" of 19 novel species among 60 isolates retrieved from a freshwater pond. *J. Microbiol. Biotechnol.* **17:** 168–175.
22. Spring, S. 2002. The genera *Leptothrix* and *Sphaerotilus*., pp. release 3.9, 1 April 2002. *In* M. Dworkin, K. H. Schleifer, and E. Stackebrandt (eds.), *The Prokaryotes: An Evolving Electronic Resource for the Microbiological Community*, 3rd Ed. Springer-Verlag, New York.

23. Spring, S., P. Kämpfer, W. Ludwig, and K. H. Schleifer. 1996. Polyphasic characterization of the genus *Leptothrix*: New descriptions of *Leptothrix mobilis* sp. nov. and *Leptothrix discophora* sp. nov. nom. rev. and emended description of *Leptothrix cholodnii* emend. *Syst. Appl. Microbiol.* **19:** 634–643.
24. Suyama, T., T. Shigematsu, S. Takaichi, Y. Nodasaka, S. Fujikawa, H. Hosoya, Y. Tokiwa, T. Kanagawa, and S. Hanada. 1999. *Roseateles depolymerans* gen. nov., sp. nov., a new bacteriochlorophyll *a*-containing obligate aerobe belonging to the beta-subclass of the *Proteobacteria*. *Int. J. Syst. Evol. Microbiol.* **49:** 449–457.
25. Willems, A. and M. Gillis. 2005. Family IV. *Comamonadaceae* Willems, De Ley, Gillis and Kersters 1991a, 447^{VP}, pp. 686–763. *In* D. J. Brenner, N. R. Krieg, J. T. Staley, and G. M. Garrity (eds.), *Bergey's Manual of Systematic Bacteriology*, Vol. 2 (The *Proteobacteria*), Part C (The *Alpha-*, *Beta-*, *Delta-*, and *Epsilonproteobacteria*), 2nd Ed. Springer, New York.
26. Willems, A., M. Gillis, and J. De Ley. 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.
27. Xie, C. H. and A. Yokota. 2005. Reclassification of *Alcaligenes latus* strains IAM 12599^{T} and IAM 12664 and *Pseudomonas saccharophila* as *Azohydromonas lata* gen. nov., comb. nov., *Azohydromonas australica* sp. nov. and *Pelomonas saccharophila* gen. nov., comb. nov., respectively. *Int. J. Syst. Evol. Microbiol.* **55:** 2419–2425.
28. Yoon, M. H., L. N. Ten, W. T. Im, and S. T. Lee. 2007. *Methylibium fulvum* sp. nov., a member of the *Betaproteobacteria* isolated from ginseng field soil, and emended description of the genus *Methylibium*. *Int. J. Syst. Evol. Microbiol.* **57:** 2062–2066.