Herbaspirillum rhizosphaerae sp. nov., isolated from rhizosphere soil of *Allium victorialis* var. *platyphyllum*

Seo-Youn Jung, Mi-Hwa Lee, Tae-Kwang Oh and Jung-Hoon Yoon

Correspondence
Jung-Hoon Yoon
jhyoon@kribb.re.kr

Korea Research Institute of Bioscience and Biotechnology (KRIIBB), PO Box 115, Yusong, Taejeon, South Korea

Two Gram-negative, milky-white-pigmented, motile, slightly curved rod-shaped bacterial isolates, UMS-37^T and UMS-40, were isolated from rhizosphere soil of wild edible greens cultivated on Ulleung island, Korea, and their taxonomic positions were investigated by a polyphasic approach. They grew optimally at 25–30 °C and contained Q-8 as the predominant ubiquinone. The major cellular fatty acids (>10 % of total fatty acids) were C_{16:0}, cyclo C_{17:0} and C_{16:1ω7c} and/or iso-C_{15:0} 2-OH. The DNA G + C contents of the two isolates were 59.8 and 60.0 mol%. Isolates UMS-37^T and UMS-40 exhibited no difference in their 16S rRNA gene sequences and possessed a mean DNA–DNA relatedness level of 94 %; they exhibited 16S rRNA gene sequence similarity levels of 96.8–98.2 % to the type strains of recognized *Herbaspirillum* species. Phylogenetic analyses based on 16S rRNA gene sequences showed that isolates UMS-37^T and UMS-40 formed a distinct phylogenetic lineage within the genus *Herbaspirillum*. DNA–DNA relatedness levels between isolates UMS-37^T and UMS-40 and the type strains of some phylogenetically related *Herbaspirillum* species were in the range 3–56 %. On the basis of differences in phenotypic properties and phylogenetic distinctiveness and genomic data, isolates UMS-37^T and UMS-40 were classified in the genus *Herbaspirillum* within a novel species, for which the name *Herbaspirillum rhizosphaerae* sp. nov. is proposed, with the type strain UMS-37^T (=KCTC 12558^T =CIP 108917^T).

The genus *Herbaspirillum*, which is classified in the family *Oxalobacteraceae* within the *Betaproteobacteria*, was first described with a single species, *Herbaspirillum seropedicae*, that included bacterial strains associated with roots of several cereals (Baldani *et al.*, 1986; Valverde *et al.*, 2003). The genus accommodates bacteria that are Gram-negative, motile by means of one to three or more flagella, generally vibrioid and sometimes helical in shape (Baldani *et al.*, 1986, 1996; Ding & Yokota, 2004; Im *et al.*, 2004). The genus *Herbaspirillum* is also characterized chemotaxonomically by having Q-8 as the predominant ubiquinone and by DNA G + C contents of 57.9–67 mol% (Baldani *et al.*, 1986, 1996; Kirchhof *et al.*, 2001; Valverde *et al.*, 2003; Ding & Yokota, 2004; Im *et al.*, 2004; Rothballer *et al.*, 2006). At present, the genus consists of nine species with validly published names: *H. seropedicae* (Baldani *et al.*, 1986), *Herbaspirillum rubrisubalbicans* (Baldani *et al.*,

1996), *H. frisingense* (Kirchhof *et al.*, 2001), *H. lusitanum* (Valverde *et al.*, 2003), *H. chlorophenolicum* (Im *et al.*, 2004), *H. autotrophicum* (Aragno & Schlegel, 1978; Ding & Yokota, 2004), *H. huttiense* (Leifson, 1962; Ding & Yokota, 2004), *H. putei* (Ding & Yokota, 2004) and *H. hiltneri* (Rothballer *et al.*, 2006). In this study, we report on the detailed taxonomic characterization of two Gram-negative, milky-white-pigmented, slightly curved rod-shaped bacterial isolates, UMS-37^T and UMS-40, which were isolated from the rhizosphere soil of *Allium victorialis* var. *platyphyllum*, wild edible greens cultivated on Ulleung island, Korea.

Isolates UMS-37^T and UMS-40 were isolated from the same rhizosphere soil sample of *Allium victorialis* var. *platyphyllum* using the standard dilution plating technique at 30 °C on nutrient agar (NA; Difco). To investigate their morphological, physiological and biochemical characteristics, the isolates were routinely cultivated at 30 °C on NA. Growth at various temperatures from 4 to 40 °C was measured on NA and tolerance to various NaCl concentrations was measured in nutrient broth (NB; Difco). Optimal pH and pH range for growth were determined in NB that was adjusted to various pH values (pH 4.5–10.5 at intervals of 0.5 pH units). Growth under anaerobic

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of isolates UMS-37^T and UMS-40 are respectively DQ188986 and DQ188985.

Detailed DNA–DNA hybridization results are available as supplementary material with the online version of this paper.

conditions was determined after incubation in an anaerobic chamber on NA and on NA supplemented with nitrate, both of which had been prepared anaerobically using nitrogen. The cell morphology and presence of flagella were examined by light microscopy (Nikon E600) and transmission electron microscopy by using cells grown on NA. The Gram reaction was determined by using the bioMérieux Gram stain kit according to the manufacturer's instructions. Catalase and oxidase activities and hydrolysis of casein, starch, hypoxanthine, xanthine, tyrosine and Tweens 20, 40, 60 and 80 were determined as described by Cowan & Steel (1965). Hydrolysis of aesculin, gelatin and urea and nitrate reduction were studied as described by Lanyi (1987). Acid production from carbohydrates was determined as described by Leifson (1963). Utilization of various substrates for growth was determined as described by Yurkov *et al.* (1994). The API ZYM system (bioMérieux) was used to determine enzyme activity. Antibiotic sensitivity was tested by spreading bacterial suspension on NA and applying discs impregnated with the following antibiotics (content per disc): ampicillin (10 µg), carbenicillin (100 µg), cephalothin (30 µg), chloramphenicol (100 µg), gentamicin (30 µg), lincomycin (15 µg), kanamycin (30 µg), neomycin (30 µg), novobiocin (5 µg), oleandomycin (15 µg), penicillin G (20 U), polymyxin B (100 U), streptomycin (50 µg) and tetracycline (30 µg).

Isolates UMS-37^T and UMS-40 were cultivated for 3 days in NB at 30 °C to obtain the cell mass required for isoprenoid quinone analysis and DNA extraction. Isoprenoid quinones were analysed as described previously (Komagata & Suzuki, 1987), using reversed-phase HPLC. For fatty acid methyl ester (FAME) analysis, cell mass of isolates UMS-37^T and UMS-40 was harvested from NA plates after cultivation for 3 days at 30 °C. The FAMES were extracted and prepared according to the standard protocol of the MIDI/Hewlett Packard Microbial Identification System (Sasser, 1990). Chromosomal DNA was extracted and purified by a procedure described previously (Yoon *et al.*, 1996). The DNA G+C content was determined by the method of Tamaoka & Komagata (1984) with the modification that DNA was hydrolysed and the resultant nucleotides were analysed by reversed-phase HPLC. DNA–DNA reassociation was determined by the microplate hybridization method (Ezaki *et al.*, 1989) using photobiotin-labelled DNA probes. Type strains of eight *Herbaspirillum* species were used as reference strains for DNA–DNA hybridization: *H. autotrophicum* DSM 732^T, *H. huttiense* DSM 10281^T and *H. frisingense* DSM 13128^T were obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ), Braunschweig, Germany; *H. chlorophenolicum* KCTC 12096^T was obtained from the Korean Collection for Type Cultures (KCTC), Taejeon, Korea, and *H. hiltneri* LMG 23131^T, *H. lusitanum* LMG 21710^T, *H. rubrisubalbicans* LMG 2286^T and *H. seropedicae* LMG 6513^T were obtained from the BCCM/LMG Bacteria Collection, Ghent University, Ghent, Belgium. 16S rRNA gene amplification

was performed according to the method described previously using two universal primers (Yoon *et al.*, 1998). Sequencing of the amplified 16S rRNA gene and phylogenetic analysis were performed as described by Yoon *et al.* (2003). PCR primers and conditions used to detect the *nifD* and *nifH* genes were described by Stoltzfus *et al.* (1997), Poly *et al.* (2001) and Im *et al.* (2004).

Morphological, cultural, physiological and biochemical characteristics of isolates UMS-37^T and UMS-40 are shown in Table 1 or are given in the species description. The isolates did not grow on semi-solid, nitrogen-free JNFB medium (Döbereiner, 1995), and *nifD* and *nifH* genes were not detected by PCR-based methods, indicating that the two isolates do not possess the ability to fix atmospheric nitrogen. The 16S rRNA gene sequences of isolates UMS-37^T and UMS-40 determined in this study comprised 1488 nucleotides and were identical. Comparative 16S rRNA gene sequence analysis and estimation of phylogenetic relationships showed that isolates UMS-37^T and UMS-40 are phylogenetically most closely related to the genus *Herbaspirillum* (Fig. 1). They exhibited 16S rRNA gene sequence similarity of 96.8–98.2% to the type strains of *Herbaspirillum* species. Sequence similarities to other species included in the phylogenetic analysis were less than 95.7%. Isolates UMS-37^T and UMS-40 exhibited a mean DNA–DNA relatedness value of 94% when their DNAs were used individually as labelled DNA probes for cross-hybridization. This value indicated that isolates UMS-37^T and UMS-40 are members of the same genomic species (Wayne *et al.*, 1987). Levels of DNA–DNA relatedness between isolates UMS-37^T and UMS-40 and the type strains of eight *Herbaspirillum* species that showed 16S rRNA gene sequence similarity values of >97.0% to the two isolates were in the range 3–56% (DNA–DNA relatedness levels are detailed in Supplementary Table S1 available in IJSEM Online).

The chemotaxonomic properties of isolates UMS-37^T and UMS-40 were in agreement with their phylogenetic affiliation to the genus *Herbaspirillum*. Isolates UMS-37^T and UMS-40 contained Q-8 as the predominant ubiquinone, as reported for other *Herbaspirillum* species (Im *et al.*, 2004; Ding & Yokota, 2004). The cellular fatty acid profiles of isolates UMS-37^T and UMS-40 are shown in Table 2, together with those of some type strains of *Herbaspirillum* species. The fatty acid profiles were characterized by large amounts of straight-chain, unsaturated, hydroxy and cyclopropane fatty acids; the major components (>10% of total fatty acids) were C_{16:0}, cyclo C_{17:0} and C_{16:1ω7c} and/or iso-C_{15:0} 2-OH. The fatty acid profiles of two isolates were similar to those of *Herbaspirillum* species, although there were differences in the proportions of some fatty acids, which might be caused by different cultivation or analytical conditions (Table 2). The DNA G+C contents of isolates UMS-37^T and UMS-40 were 60.0 and 59.8 mol%, respectively. The differential phenotypic properties, together with the phylogenetic distinctiveness and DNA–DNA relatedness data, provide decisive evidence that

Table 1. Differential phenotypic characteristics of *Herbaspirillum* species

Strains: 1, *H. rhizosphaerae* sp. nov. strains UMS-37^T and UMS-40; 2, *H. seropedicae* LMG 6513^T (unless indicated, data from Baldani *et al.*, 1986, 1996; Valverde *et al.*, 2003; Im *et al.*, 2004; Rothballer *et al.*, 2006); 3, *H. rubrisubalbicans* LMG 2286^T (Baldani *et al.*, 1996; Valverde *et al.*, 2003); 4, *H. frisingense* DSM 13128^T (Kirchhof *et al.*, 2001; Valverde *et al.*, 2003); 5, *H. lusitanum* LMG 21710^T (Valverde *et al.*, 2003; Rothballer *et al.*, 2006); 6, *H. chlorophenolicum* KCTC 12096^T (Im *et al.*, 2004; Rothballer *et al.*, 2006); 7, *H. autotrophicum* DSM 732^T (Aragno & Schlegel, 1978; Ding & Yokota, 2004; Rothballer *et al.*, 2006); 8, *H. huttiense* DSM 10281^T (Leifson, 1962; Ding & Yokota, 2004; Rothballer *et al.*, 2006); 9, *H. putei* 7-2^T (Ding & Yokota, 2004; Rothballer *et al.*, 2006); 10, *H. hiltneri* (three strains) (Rothballer *et al.*, 2006). +, Positive; –, negative; w, weakly positive; ND, no data available. All strains are positive for oxidase (not determined for *H. rubrisubalbicans* LMG 2286^T) and leucine arylamidase. All strains are negative for lipase (C14), trypsin, α -chymotrypsin, α -galactosidase, β -glucuronidase, α -glucosidase, β -glucosidase, *N*-acetyl- β -glucosaminidase, α -mannosidase and α -fucosidase.

Characteristic	1	2	3	4	5	6	7	8	9	10
Cell shape	Slight curved rod	Rod	Slight curved rod	Slight curved rod	Curved rod	Slight curved rod	Curved to spiral	Rod	Concave	Slight curved rod
Cell size (μm)	0.3–0.4 $\times$ 1.8–2.2	0.6–0.7 $\times$ 1.5–5.0	0.6–0.7 $\times$ 1.5–5.0	0.5–0.7 $\times$ 1.4–1.8	0.5 $\times$ 1.6	0.7 $\times$ 2.3	0.6–0.8 $\times$ 2.0–5.0	0.4 $\times$ 1.8	0.4–0.5 $\times$ 1.4–1.8	0.5–0.6 $\times$ 1.6–2.0
Pigment	Milky white	Brownish	–	White	White	Brownish	Cream	–	Yellow–brown	ND
Motility	+	+	+	+	+	+	+	+	–	+
Optimal growth temperature ($^{\circ}\text{C}$)	25–30	34	25–30	30–37	28	30	28	25–30	25–37	26–34
Optimal growth pH	6.5–7.5	5.3–8.0	6.7–6.8	6.0–7.0	5.0–8.0	6.0–7.0	6.0–8.0*	Neutrophilic	6.0–7.0	6–8
Nitrate reduction	–	+	+	+	–	–	–	–	–	ND
Catalase activity	+	+	+	+	+	+	+	–	+	+
Detection of:										
<i>nifD</i>	–	+	+	+	+	–	–	–	–	–
<i>nifH</i>	–	+	+	+	+	–	–	–	+	–
Hydrolysis of:										
Tween 40	–	–*	–*	+	–*	–*	–*	–*	–*	–*
Tween 80	–	–*	–	+	–*	–*	–*	–*	–*	–*
Growth on:										
D-Mannose	+	+	+	+	+	–	–	–	w	–
L-Rhamnose	–	+	–	–	+	–	–	–	–	–
D-Glucose	+	+	+	+	+	+	–	–	–	+
D-Galactose	+	+	+	+	+	+	–	–	–	+
Sorbitol	w	+	+	+	–	–	–	+	+	+
D-Fructose	+	+	+	+	+	–	–	–	–	–
API ZYM test results†										
Alkaline phosphatase	–	+	+	+	+	–	–	+	+	w
Esterase (C4)	+	+	+	+	+	–	–	+	w	w
Esterase lipase (C8)	+	+	+	+	+	–	–	+	w	–
Valine arylamidase	+	+	+	+	+	–	–	–	–	–
Cystine arylamidase	–	+	+	+	+	–	–	–	–	–
Acid phosphatase	–	+	+	+	+	–	w	+	+	–
Naphthol-AS-BI-phosphohydrolase	+	+	+	+	+	–	w	w	+	–
β -Galactosidase	–	+	+	+	–	–	–	–	–	–
DNA G + C content (mol%)	59.8–60.0	64.5	62.5	64	57.9	61.3	61.7	63.3	62.9	60.9–61.5

*Data from this study.

†Data for the type strains of *H. chlorophenolicum*, *H. autotrophicum*, *H. huttiense*, *H. putei* and *H. hiltneri* from this study.

isolates UMS-37^T and UMS-40 differ from recognized *Herbaspirillum* species (Table 1). On the basis of the data presented, isolates UMS-37^T and UMS-40 should be

classified as members of a novel species of the genus *Herbaspirillum*, for which the name *Herbaspirillum rhizosphaerae* sp. nov. is proposed.

Fig. 1. Neighbour-joining tree showing the phylogenetic positions of isolates UMS-37^T and UMS-40 and other related taxa, based on 16S rRNA gene sequences. Only bootstrap values (expressed as percentages of 1000 replications) greater than 50 % are shown at branching points. *Comamonas testosteroni* ATCC 11996^T was used as an outgroup. Bar, 0.01 substitutions per nucleotide position.

Description of *Herbaspirillum rhizosphaerae* sp. nov.

Herbaspirillum rhizosphaerae (rhi.zo.sphae'rae. N.L. gen. n. *rhizosphaerae* of the rhizosphere).

Cells are Gram-negative, slightly curved rods, 0.3–0.4 × 1.8–2.2 µm. Motile by means of bipolar flagella. No growth occurs under anaerobic conditions on NA or on NA with nitrate. Growth occurs at 4 and 34 °C, with optimum growth at 25–30 °C. Optimal pH for growth is 6.5–7.5; growth occurs weakly at pH 5.0 but not at pH 4.5. No growth occurs in the presence of more than 3 % (w/v) NaCl. Hypoxanthine, tyrosine and urea are hydrolysed, but aesculin, casein, gelatin, starch, xanthine and Tweens 20 and 60 are not. Acetate, citrate, glycerol, pyruvate, ribose and succinate are utilized as sole carbon and energy sources. D-Xylose is utilized, but adonitol, benzoate, D-cellobiose, formate, L-glutamate, maltose, salicin, sucrose and trehalose are not utilized. Utilization of L-malate (positive for type strain) and L-arabinose (negative for type strain) is variable. Acid is produced from D-glucose, D-galactose, D-mannitol, D-mannose, D-ribose, D-xylose and L-arabinose. Acid is not produced from D-cellobiose, lactose, *myo*-inositol, maltose, melibiose, D-melezitose, D-sorbitol, sucrose, L-rhamnose or D-raffinose. Acid production from D-fructose (positive for type strain) and trehalose (negative for type strain) is variable. Susceptible to chloramphenicol, gentamicin, kanamycin, neomycin, oleandomycin, streptomycin and tetracycline but not to ampicillin, carbenicillin, cephalothin, lincomycin, novobiocin, penicillin G or polymyxin B. The major cellular fatty acids (>10 % of total fatty acids) are C_{16:0}, cyclo C_{17:0} and C_{16:1}ω7c and/or iso-C_{15:0} 2-OH. The predom-

Table 2. Cellular fatty acid compositions (%) of strains UMS-37^T and UMS-40 and the type strains of some *Herbaspirillum* species

Strains: 1, strain UMS-37^T; 2, strain UMS-40; 3, *H. seropedicae* IAM 14977^T (data from Im *et al.*, 2004); 4, *H. rubrisubalbicans* IAM 14976^T (Im *et al.*, 2004); 5, *H. frisingense* IAM 14974^T (Im *et al.*, 2004); 6, *H. chlorophenolicum* CPW301^T (Im *et al.*, 2004); 7, *H. huttiense* IAM 14941^T (Ding & Yokota, 2004); 8, *H. putei* 7-2^T (Ding & Yokota, 2004); 9, *H. hiltneri* N3^T (mean values of two measurements from Rothballer *et al.*, 2006). Fatty acids that represented <1.0 % in all isolates and strains were omitted. –, Not detected/not reported.

Fatty acid	1	2	3	4	5	6	7	8	9
Saturated fatty acids									
C _{12:0}	5.2	6.7	1.0	3.2	0.7	3.7	4.0	3.5	–
C _{14:0}	0.4	0.6	5.2	0.8	4.0	0.2	–	0.5	0.5 ± 0.3
C _{16:0}	36.0	31.6	22.8	26.3	27.4	33.7	18.0	24.6	26.4 ± 2.5
C _{18:0}	1.2	1.0	0.6	1.8	1.7	2.0	4.0	1.7	2.7 ± 0.3
Unsaturated fatty acids									
C _{18:1} ω6c	–	–	1.9	2.5	–	–	–	–	–
C _{18:1} ω7c	6.0	5.2	13.6	22.1	23.8	13.4	30.0	22.5	18.2 ± 4.9
Hydroxy fatty acids									
C _{10:0} 3-OH	1.8	2.1	1.8	1.4	1.4	1.3	1.8	1.6	–
C _{12:0} 2-OH	3.7	5.0	1.8	0.4	1.1	2.7	–	0.3	–
C _{12:0} 3-OH	4.6	5.5	4.2	3.1	3.3	3.3	3.9	3.3	–
C _{14:0} 2-OH	–	–	1.6	2.0	1.1	0.1	2.8	2.4	<0.1
Cyclopropane fatty acids									
cyclo C _{17:0}	26.8	28.7	4.8	3.3	1.8	21.8	6.7	0.9	4.0 ± 0.9
cyclo C _{19:0} ω8c	1.5	1.6	–	0.2	0.1	1.4	–	–	0.1 ± 0.1
Summed feature 3*	12.8	11.1	39.6	31.7	32.5	14.9	28.8	38.0	44.5 ± 1.8†

*Summed features represent groups of two or three fatty acids that could not be separated by GLC with the MIDI system. Summed features 3 contained C_{16:1}ω7c and/or iso-C_{15:0} 2-OH.

†Data for C_{16:1}ω7c.

inant ubiquinone is Q-8. The DNA G+C content of the type strain is 60.0 mol%.

The type strain, UMS-37^T (=KCTC 12558^T =CIP 108917^T), was isolated from rhizosphere soil of *Allium victorialis* var. *platyphyllum*, wild edible greens cultivated on Ulleung island, Korea.

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

- Aragno, M. & Schlegel, H. G. (1978). *Aquaspirillum autotrophicum*, a new species of hydrogen-oxidizing, facultatively autotrophic bacteria. *Int J Syst Bacteriol* **28**, 112–116.
- Baldani, J. I., Baldani, V. L. D., Seldin, L. & Döbereiner, J. (1986). Characterization of *Herbaspirillum seropedicae* gen. nov., sp. nov., a root-associated nitrogen-fixing bacterium. *Int J Syst Bacteriol* **36**, 86–93.
- Baldani, J. I., Pot, B., Kirchhof, G., Falsen, E., Baldani, V. L. D., Olivares, F. L., Hoste, B., Kersters, K., Hartmann, A. & other authors (1996). Emended description of *Herbaspirillum*; inclusion of [*Pseudomonas*] *rubrisubalbicans*, a mild plant pathogen, as *Herbaspirillum rubrisubalbicans* comb. nov.; and classification of a group of clinical isolates (EF group 1) as *Herbaspirillum* species 3. *Int J Syst Bacteriol* **46**, 802–810.
- Cowan, S. T. & Steel, K. J. (1965). *Manual for the Identification of Medical Bacteria*. London: Cambridge University Press.
- Ding, L. & Yokota, A. (2004). Proposals of *Curvibacter gracilis* gen. nov., sp. nov. and *Herbaspirillum putei* sp. nov. for bacterial isolates isolated from well water and reclassification of [*Pseudomonas*] *huttiensis*, [*Pseudomonas*] *lanceolata*, [*Aquaspirillum*] *delicatum* and [*Aquaspirillum*] *autotrophicum* as *Herbaspirillum huttiense* comb. nov., *Curvibacter lanceolatus* comb. nov., *Curvibacter delicatus* comb. nov. and *Herbaspirillum autotrophicum* comb. nov. *Int J Syst Evol Microbiol* **54**, 2223–2230.
- Döbereiner, J. (1995). Isolation and identification of aerobic nitrogen-fixing bacteria from soil and plants. In *Methods in Applied Soil Microbiology and Biochemistry*, pp. 134–141. Edited by K. Alef & P. Nannipieri. London: Academic Press.
- Ezaki, T., Hashimoto, Y. & Yabuuchi, E. (1989). Fluorometric deoxyribonucleic acid-deoxyribonucleic acid hybridization in micro-dilution wells as an alternative to membrane filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial isolates. *Int J Syst Bacteriol* **39**, 224–229.
- Im, W.-T., Bae, H.-S., Yokota, A. & Lee, S. T. (2004). *Herbaspirillum chlorophenolicum* sp. nov., a 4-chlorophenol-degrading bacterium. *Int J Syst Evol Microbiol* **54**, 851–855.
- Kirchhof, G., Eckert, B., Stoffels, M., Baldani, J. I., Reis, V. M. & Hartmann, A. (2001). *Herbaspirillum frisingense* sp. nov., a new nitrogen-fixing bacterial species that occurs in C4-fibre plants. *Int J Syst Evol Microbiol* **51**, 157–168.
- Komagata, K. & Suzuki, K. (1987). Lipids and cell-wall analysis in bacterial systematics. *Methods Microbiol* **19**, 161–207.
- Lanyi, B. (1987). Classical and rapid identification methods for medically important bacteria. *Methods Microbiol* **19**, 1–67.
- Leifson, E. (1962). The bacterial flora of distilled and stored water. III. New species of the genera *Corynebacterium*, *Flavobacterium*, *Spirillum* and *Pseudomonas*. *Int Bull Bacteriol Nomencl Taxon* **12**, 161–170.
- Leifson, E. (1963). Determination of carbohydrate metabolism of marine bacteria. *J Bacteriol* **85**, 1183–1184.
- Poly, F., Monrozier, L. J. & Bally, R. (2001). Improvement in the RFLP procedure for studying the diversity of *nifH* genes in communities of nitrogen fixers in soil. *Res Microbiol* **152**, 95–103.
- Rothballer, M., Schmid, M., Klein, I., Gattinger, A., Grundmann, S. & Hartmann, A. (2006). *Herbaspirillum hiltneri* sp. nov., isolated from surface-sterilized wheat roots. *Int J Syst Evol Microbiol* **56**, 1341–1348.
- Sasser, M. (1990). *Identification of bacteria by gas chromatography of cellular fatty acids*, MIDI Technical Note 101. Newark, DE: MIDI Inc.
- Stoltzfus, J. R., So, R., Malarvithi, P. P., Ladha, J. K. & de Bruijn, F. J. (1997). Isolation of endophytic bacteria from rice and assessment of their potential for supplying rice with biologically fixed nitrogen. *Plant Soil* **194**, 25–36.
- Tamaoka, J. & Komagata, K. (1984). Determination of DNA base composition by reversed-phase high-performance liquid chromatography. *FEMS Microbiol Lett* **25**, 125–128.
- Valverde, A., Velázquez, E., Gutiérrez, C., Cervantes, E., Ventosa, A. & Igual, J.-M. (2003). *Herbaspirillum lusitanum* sp. nov., a novel nitrogen-fixing bacterium associated with root nodules of *Phaseolus vulgaris*. *Int J Syst Evol Microbiol* **53**, 1979–1983.
- Wayne, L. G., Brenner, D. J., Colwell, R. R., Grimont, P. A. D., Kandler, O., Krichevsky, M. I., Moore, L. H., Moore, W. E. C., Murray, R. G. E. & other authors (1987). International Committee on Systematic Bacteriology. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int J Syst Bacteriol* **37**, 463–464.
- Yoon, J.-H., Kim, H., Kim, S.-B., Kim, H.-J., Kim, W. Y., Lee, S. T., Goodfellow, M. & Park, Y.-H. (1996). Identification of *Saccharomonospora* isolates by the use of genomic DNA fragments and rRNA gene probes. *Int J Syst Bacteriol* **46**, 502–505.
- Yoon, J.-H., Lee, S. T. & Park, Y.-H. (1998). Inter- and intraspecific phylogenetic analysis of the genus *Nocardioides* and related taxa based on 16S rRNA sequences. *Int J Syst Bacteriol* **48**, 187–194.
- Yoon, J.-H., Kang, K. H. & Park, Y.-H. (2003). *Psychrobacter jeotgali* sp. nov., isolated from jeotgal, a traditional Korean fermented seafood. *Int J Syst Evol Microbiol* **53**, 449–454.
- Yurkov, V., Stackebrandt, E., Holmes, A., Fuerst, J. A., Hugenholtz, P., Golecki, J., Gad'on, N., Gorlenko, V. M., Kompantseva, E. I. & Drews, G. (1994). Phylogenetic positions of novel aerobic, bacteriochlorophyll *a*-containing bacteria and description of *Roseococcus thiosulfatophilus* gen. nov., sp. nov., *Erythromicrobium ramosum* gen. nov., sp. nov., and *Erythrobacter litoralis* sp. nov. *Int J Syst Bacteriol* **44**, 427–434.