Aeromicrobium panaciterrae sp. nov., isolated from soil of a ginseng field in South Korea

Ying-Shun Cui,^{1,2} Wan-Taek Im,¹ Cheng-Ri Yin,² Jung-Sook Lee,³ Keun Chul Lee³ and Sung-Taik Lee¹

Correspondence

Wan-Taek Im
wandra@kaist.ac.kr

Sung-Taik Lee
e_stlee@kaist.ac.kr

¹Department of Biological Sciences, Korea Advanced Institute of Science and Technology, 373-1 Guseong-dong, Yuseong-gu, Daejeon 305-701, Republic of Korea

²Key Laboratory of Natural Resources of the Changbai Mountain and Functional Molecules (Yanbian University), Ministry of Education, Yanji 133002, China

³Korean Collection for Type Cultures (KCTC), Korea Research Institute of Bioscience and Biotechnology, 52 Oeundong, Yusong-gu, Daejeon 305-333, Republic of Korea

A Gram-positive, rod-shaped, non-spore-forming and strictly aerobic bacterium (Gsoil 161^T) was isolated from soil of a ginseng field in Pocheon Province, South Korea. The novel isolate was characterized using a polyphasic approach in order to determine its taxonomic position. On the basis of 16S rRNA gene sequence similarity, strain Gsoil 161^T was shown to belong to the family *Nocardioideae* and was related to *Aeromicrobium marinum* (98.0% similarity to the type strain), *Aeromicrobium alkaliterrae* (97.6%), *Aeromicrobium fastidiosum* (97.0%) and *Aeromicrobium erythreum* (96.7%); the sequence similarity with other species within the family was less than 94.4%. It was characterized chemotaxonomically as having LL-2,6-diaminopimelic acid in the cell-wall peptidoglycan, MK-9(H₄) as the predominant menaquinone and C_{16:0}, 10-methyl C_{18:0} (tuberculostearic acid), C_{16:0} 2-OH, 10-methyl C_{17:0} and 10-methyl-C_{16:0} as the major fatty acids. The G + C content of the genomic DNA was 65.5 mol%. These chemotaxonomic properties and phenotypic characteristics support the affiliation of strain Gsoil 161^T to the genus *Aeromicrobium*. Results of physiological and biochemical tests enabled strain Gsoil 161^T to be differentiated genotypically and phenotypically from currently known *Aeromicrobium* species. Therefore, strain Gsoil 161^T represents a novel species, for which the name *Aeromicrobium panaciterrae* sp. nov. is proposed. The type strain is strain Gsoil 161^T (=KCTC 19131^T=DSM 17939^T=CCUG 52476^T).

The genus *Aeromicrobium* was proposed by Miller *et al.* (1991). According to the original description, the genus comprised non-mycelial, non-sporulating actinomycetes that produced the macrolide antibiotic erythromycin; the type species is *Aeromicrobium erythreum*. In 1994, *Nocardioideae fastidiosa* was transferred to the genus as *Aeromicrobium fastidiosum* (Tamura & Yokota, 1994). Two further species, *Aeromicrobium marinum* (Bruns *et al.*, 2003) and *Aeromicrobium alkaliterrae* (Yoon *et al.*, 2005), were described recently. In this study, taxonomic characterization is reported of an *Aeromicrobium*-like strain, Gsoil 161^T, which was isolated from soil of a ginseng field in South Korea during a study on the culturable aerobic bacterial community.

Strain Gsoil 161^T was originally isolated from soil of a ginseng field in Pocheon Province, South Korea. This soil sample was thoroughly suspended in 50 mM phosphate buffer (pH 7.0) and the suspension was spread on one-tenth-strength modified R2A (l⁻¹: 0.25 g tryptone, 0.25 g peptone, 0.25 g yeast extract, 0.125 g malt extract, 0.125 g beef extract, 0.25 g Casamino acid, 0.25 g soytone, 0.5 g glucose, 0.3 g soluble starch, 0.2 g xylan, 0.3 g sodium pyruvate, 0.3 g K₂HPO₄, 0.05 g MgSO₄, 0.05 g CaCl₂, 15 g agar) agar plates after serial dilution with 50 mM phosphate buffer (pH 7.0). Plates were incubated at 30 °C for 1 month. Single colonies on the plates were purified by transferring them onto new plates and these were incubated again on modified R2A or half-strength modified R2A. Purified colonies were tentatively identified by partial 16S rRNA gene sequences (Im *et al.*, 2005). Strain Gsoil 161^T was one of the isolates that appeared on the modified R2A agar plates under aerobic conditions. As it could grow well on full-strength modified R2A agar and commercial R2A agar

Abbreviation: DAP, diaminopimelic acid.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain Gsoil 161^T is AB245387.

(Difco), strain Gsoil 161^T was routinely cultured on R2A agar at 30 °C and maintained as a glycerol suspension (20 %, w/v) at -70 °C.

Gram-reaction testing was performed by the non-staining method as described by Buck (1982). Cell morphology was observed at $\times 1000$ magnification with a light microscope (Nikon) using cells grown for 24 h at 30 °C on modified R2A agar. Catalase activity was determined by checking bubble production using 3 % (v/v) H₂O₂ and oxidase activity was determined using 1 % (w/v) tetramethyl *p*-phenylenediamine. For single-carbon-source assimilation studies, a defined liquid medium containing basal salts was used, containing (l⁻¹) K₂HPO₄ (1.8 g), KH₂PO₄ (1.08 g), NaNO₃ (0.5 g), NH₄Cl (0.5 g), KCl (0.1 g), MgSO₄ (0.1 g) and CaCl₂ (0.05 g). A vitamin solution (Widdel & Bak, 1992), trace element solution SL-10 (Widdel *et al.*, 1983) and selenite/tungstate solution (Tschech & Pfennig, 1984) were added to this medium and the pH was adjusted to 6.8. This liquid medium was added to 96-well trays and filter-sterilized carbon sources were added to each well (individually at 0.1 %, w/v). Growth was examined visually after incubation at 30 °C for up to 7 days on 96-well plates. A negative control well containing no carbon source was included. A positive control culture was grown in a well containing R2A broth.

Fermentative and oxidative acid production from carbohydrates was tested by growth in O-F basal medium with bromothymol blue (Atlas, 1993) supplemented with 1 % carbohydrate [soft-agar stabs with (fermentative) and without (oxidative) sterile mineral oil overlay]. The O-F medium tubes were incubated at 30 °C for 5 days. Some physiological characteristics such as nitrate reduction, acid production from carbohydrates and activity of some enzymes were determined with API 20E galleries according to the instructions of the manufacturer (bioMérieux). Anaerobic growth was tested in serum bottles containing thioglycolate (1 g l⁻¹) added to R2A broth and replacing the upper air layer with nitrogen gas. The anaerobic nitrate reduction test to determine the final electron acceptor was performed in serum bottles by adding 1 g thioglycolate l⁻¹ and 10 mM KNO₃ to R2A broth, under nitrogen gas. Aerobic nitrate reduction was later confirmed by inoculation into 12 ml R2A broth supplemented with 10 mM KNO₃ in three 25 ml serum bottles. The reduction of nitrate was monitored by an ion chromatograph (model 790 personal IC; Metrohm) equipped with a conductivity detector and an anion exchange column (Metrosep Anion Supp 4; Metrohm). Tests for the degradation of DNA [plates of DNase agar (Scharlau) flooded with 1 M HCl], casein, chitin and starch (Atlas, 1993), lipid (Kouker & Jaeger, 1987), and xylan and cellulose (Ten *et al.*, 2004) were performed and evaluated after 10 days. Growth at different temperatures (4, 15, 20, 25, 30, 37, 42 and 45 °C) and various pH values (pH 5.0–10.0 at intervals of 0.5 pH units) was assessed after 5 days incubation. Salt tolerance was tested on modified R2A agar supplemented with 1–10 % (w/v) NaCl

after 5 days incubation. Growth on nutrient agar (5.0 g peptone, 3.0 g beef extract, 15.0 g agar in 1 l; Difco), trypticase soy agar (TSA) and MacConkey agar was also evaluated at 30 °C.

For phylogenetic analysis of strain Gsoil 161^T, DNA was extracted using a genomic DNA extraction kit (Solgent). The 16S rRNA gene was amplified from chromosomal DNA using the universal bacterial primer set 9F and 1512R (Weisburg *et al.*, 1991) and purified PCR products were sequenced by Solgent (Daejeon, South Korea) (Kim *et al.*, 2005). Full sequences of the 16S rRNA gene were compiled using SeqMan software (DNASTAR). The 16S rRNA gene sequences of related taxa were obtained from GenBank. Multiple alignments were performed using CLUSTAL X (Thompson *et al.*, 1997). Gaps were edited in the program BIOEDIT (Hall, 1999). Evolutionary distances were calculated using the Kimura two-parameter model (Kimura, 1983). Phylogenetic trees were constructed by using the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1971) methods using the program MEGA3 (Kumar *et al.*, 2004) with bootstrap values based on 1000 replications (Felsenstein, 1985).

For the measurement of G + C content, genomic DNA of the novel strain was extracted and purified as described by Moore & Dowhan (1995). The DNA was enzymically degraded into nucleosides and G + C content was determined as described by Mesbah *et al.* (1989) using reverse-phase HPLC. Isoprenoid quinones were extracted with chloroform/methanol (2 : 1, v/v), evaporated under vacuum and re-extracted in *n*-hexane/water (1 : 1, v/v). The crude *n*-hexane/quinone solution was purified using Sep-Pak Vac cartridges silica (Waters) and subsequently analysed by HPLC as described previously (Hiraishi *et al.*, 1996). Cellular fatty acid profiles were determined for strains grown on modified R2A broth for 6 days. Cellular fatty acids were saponified, methylated and extracted according to the protocol of the Sherlock Microbial Identification System (MIDI). The fatty acids analysed by GC (Hewlett Packard 6890) were identified by the Microbial Identification software package (Sasser, 1990). The presence of diamino-pimelic acid (DAP) isomers in the cell wall peptidoglycan was determined by using TLC after hydrolysis with 6 M HCl at 100 °C for 18 h (Komagata & Suzuki, 1987).

Strain Gsoil 161^T was Gram-positive, strictly aerobic, non-spore-forming, non-motile and rod-shaped. Colonies grown on R2A agar plates (Difco) for 5 days were smooth, circular, light-yellowish white in colour, convex and 1–3 mm in diameter. After 5 days incubation, the novel strain did not grow well on TSA; it showed very thin layer growth on the surface of TSA plates. However, it did grow well on nutrient agar and modified R2A agar. Strain Gsoil 161^T was able to grow at 15–30 °C, but not at 4 or 35 °C or above. Physiological characteristics of strain Gsoil 161^T are summarized in the species description and a comparison of selective characteristics with related type strains is given in Table 1.

Table 1. Physiological characteristics of strain Gsoil 161^T and related type strains of the genus *Aeromicrobium*

Strains: 1, Gsoil 161^T (data from this study); 2, *A. alkaliterrae* KCTC 19073^T (data from Yoon *et al.*, 2005); 3, *A. erythreum* DSM 8599^T (Miller *et al.*, 1991; Bruns *et al.*, 2003; Yoon *et al.*, 2005); 4, *A. fastidiosum* KCTC 9576^T (Tamura & Yokota, 1994; Bruns *et al.*, 2003; Yoon *et al.*, 2005); 5, *A. marinum* DSM 15272^T (Bruns *et al.*, 2003; Yoon *et al.*, 2005). +, Positive; –, negative; ND, not defined. All strains are non-spore-forming, assimilate D-galactose, trehalose and glycerol, do not assimilate lactose or rhamnose and have MK-9(H₄) as the predominant menaquinone.

Characteristic	1	2	3	4	5
Cell morphology	Rods	Rods, cocci	Irregular rods, cocci	Short rods, cocci	Rods
Motility	–	–	–	+	–
Temperature range (°C)	15–30	4–35	21–40	5–30	4–35
pH range	5.0–8.5	6.0–11.0	5.0–9.0	5.0–8.0	5.5–9.5
Tolerance of NaCl (% w/v)	0–3	0–8	0–4	0–4	0.08–10.7
Oxidase	–	–	+	+	–
Urease	–	–	–	–	ND
Catalase	–	+	+	+	+
Arginine dihydrolase	+	–	–	–	+
Nitrate reduction	–	–	–	ND	–
Hydrolysis of:					
Starch	–	–	+	+	–
Casein	–	+	+	+	–
Cellulose	–	–	+	–	–
Gelatin	+	+	+	+	ND
Carbon source assimilation:*					
Acetate	+	–	+	+	+
Alanine	+	–	+	+	–
Citrate	–	–	+	+	–
D-Cellobiose	+	+	–	+	+
D-Fructose	–	–	+	+	–
D-Glucose	+	+	+	+	–
D-Mannose	+	–	–	+	–
D-Xylose	–	–	+	+	–
L-Arabinose	–	+	+	+	–
Maltose	+	+	–	–	–
Mannitol	–	–	–	–	+
Salicin	+	+	–	–	–
Succinate	+	+	+	–	+
Sucrose	+	+	+	+	–
DNA G + C content (mol%)	65.5	71.5	71–73	71–72	70.6
Peptidoglycan type	LL-DAP	LL-DAP	Ala, Glu, Gly, LL-DAP	Ala, Glu, Gly, LL-DAP	LL-DAP

*Carbon source assimilation tests for all strains were conducted under the conditions used in this study.

The 16S rRNA gene sequence of strain Gsoil 161^T determined in this study was a continuous stretch of 1496 bp (positions 17–1538 with respect to the *Escherichia coli* numbering system). Sequence similarity calculations after neighbour-joining analysis indicated that the closest relatives of strain Gsoil 161^T were *A. marinum* (98.0 % 16S rRNA gene sequence similarity to the type strain), *A. alkaliterrae* (97.6 %), *A. fastidiosum* (97.0 %) and *A. erythreum* (96.7 %). Lower sequence similarities (< 94.4 %) were found to all other recognized species of the family *Nocardioidaceae*. This relationship between strain Gsoil 161^T and other members of the genus *Aeromicrobium* was also evident in the phylogenetic tree (Fig. 1, using sequences

of over 1300 bp). Strain Gsoil 161^T and the four *Aeromicrobium* species mentioned above formed a monophyletic clade with a bootstrap value of 100 %, which was supported by the two tree-making methods used in this study.

DNA–DNA hybridization between Gsoil 161^T and type strains of members of the genus *Aeromicrobium* was assessed according to Ezaki *et al.* (1989) using photobiotin-labelled DNA probes and microdilution wells. DNA–DNA relatedness values of strain Gsoil 161^T with the four most closely related *Aeromicrobium* species were 21 % with *A. marinum* DSM 15272^T, 3 % with *A. alkaliterrae* KCTC 19073^T, 18 % with *A. fastidiosum* KCTC 9576^T and 10 % with *A. erythreum*

Fig. 1. Neighbour-joining phylogenetic tree constructed from a comparative analysis of 16S rRNA gene sequences showing the relationship between strain Gsoil 161^T and related species. Filled circles at nodes indicate generic branches that were also recovered by using the maximum-parsimony algorithm. Bootstrap values (expressed as percentages of 1000 replications) > 70% are shown at branch points. Bar, 0.01 substitutions per nucleotide position.

DSM 8599^T, values that are low enough to propose strain Gsoil 161^T as a representative of a novel species of genus *Aeromicrobium* (Wayne *et al.*, 1987).

The G+C content of genomic DNA of strain Gsoil 161^T was 65.5 mol%. The respiratory quinone system supports affiliation of strain Gsoil 161^T to the genus *Aeromicrobium*, where the majority of species have MK-9(H₄) as the predominant quinone. The cell wall peptidoglycan of strain Gsoil 161^T contained LL-DAP. The fatty acid profile of strain Gsoil 161^T was composed mainly of 10-methyl C_{18:0} (tuberculostearic acid; 14.5%), C_{16:0} (13.0%), C_{16:0} 2-OH (12.8%), 10-methyl C_{17:0} (11.9%), 10-methyl C_{16:0} (11.7%), C_{16:1ω7c/iso-C_{15:0} 2-OH (6.6%), C_{18:1ω9c} (5.3%), C_{15:0} (4.9%), C_{17:0} (4.8%), C_{17:1ω8c} (4.7%), C_{17:0} 2-OH (2.7%), C_{15:0} 2-OH (1.8%), iso-C_{16:0} (1.5%) and C_{17:1ω6c} (1.4%). The fatty acid profile of strain Gsoil 161^T differed significantly from those of the most closely related *Aeromicrobium* species. However, some of the major components of the fatty acid profiles of these taxa were the same, i.e. C_{16:0} and C_{16:0} 2-OH.}

In summary, the characteristics of strain Gsoil 161^T are consistent with descriptions of the genus *Aeromicrobium* with regard to morphological, biochemical and chemotaxonomic properties. However, on the basis of phylogenetic distance from known *Aeromicrobium* species indicated by 16S rRNA gene sequence similarities and the combination of unique phenotypic characteristics (Table 1), Gsoil 161^T should be placed in the genus *Aeromicrobium* as a representative of a novel species, for which the name *Aeromicrobium panaciterrae* sp. nov. is proposed.

Description of *Aeromicrobium panaciterrae* sp. nov.

Aeromicrobium panaciterrae (pa.na.ci.ter'rae. N.L. n. *Panax*,

-acis scientific name of ginseng; L. n. *terra* soil; N.L. gen. n. *panaciterrae* of soil of a ginseng field).

Cells are Gram-positive, strictly aerobic, non-spore-forming, non-motile and rod-shaped, 0.2–0.4 µm in diameter and 1.0–1.5 µm in length after culture for 3 days on modified R2A broth. Colonies grown on R2A agar (Difco) for 5 days are smooth, circular, and light yellowish in colour, convex and 1–3 mm in diameter. Grows well at 15–30 °C and pH 5.0–8.5, but does not grow at 4 or 35 °C. Growth occurs in the absence of NaCl and in the presence of 3.0% (w/v) NaCl, but not in 4.0% (w/v) NaCl. Grows on nutrient agar. Does not grow on MacConkey agar. Catalase-positive and oxidase-negative. Does not reduce nitrate to nitrite. H₂S is not produced. Negative for production of urease, lysine decarboxylase, ornithine decarboxylase, β-galactosidase and tryptophan deaminase. Positive for gelatinase and arginine dihydrolase. Does not produce any acid or gas from glucose, but glucose is utilized as a sole carbon source. Does not degrade xylan, chitin, cellulose, casein, DNA or starch. The following compounds are utilized as sole carbon sources: acetate, alanine, D-cellobiose, D-galactose, D-glucose, D-mannose, trehalose, glycerol, maltose, salicin, succinate, sucrose, melibiose, L-lactate, L-malate, glycine and D-glycogen. The following compounds are not utilized as sole carbon sources: citrate, D-fructose, D-xylose, lactose, L-arabinose, mannitol, rhamnose, L-serine, L-sorbose, sodium formate, D-ribose, L-lysine, D-fucose, xylitol and D-lyxose. MK-9(H₄) is the predominant menaquinone, and C_{16:0}, 10-methyl C_{18:0}, C_{16:0} 2-OH, 10-methyl C_{17:0} and 10-methyl C_{16:0} are the major components of the cellular fatty acids. The cell wall peptidoglycan contains LL-DAP.

The type strain is Gsoil 161^T (=KCTC 19131^T=DSM 17939^T=CCUG 52476^T), isolated from soil of a ginseng field in Pocheon Province, South Korea. The DNA G+C content of Gsoil 161^T is 65.5 mol% (as determined by HPLC).

Acknowledgements

This work was supported by the 21C Frontier Microbial Genomics and Application Center Program, Ministry of Science & Technology (Grant MG05-0101-4-0), Republic of Korea, and sponsored by the Scientific Research Foundation for the Returned Overseas Chinese Scholars, State Education Ministry. We thank Jean Euzéby for his help with the etymology of the species epithet.

References

- Atlas, R. M. (1993).** *Handbook of Microbiological Media*. Edited by L. C. Parks. Boca Raton, FL: CRC Press.
- Bruns, A., Philipp, H., Cypionka, H. & Brinkhoff, T. (2003).** *Aeromicrobium marinum* sp. nov., an abundant pelagic bacterium isolated from the German Wadden Sea. *Int J Syst Evol Microbiol* **53**, 1917–1923.
- Buck, J. D. (1982).** Nonstaining (KOH) method for determination of Gram reactions of marine bacteria. *Appl Environ Microbiol* **44**, 992–993.
- 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 strains. *Int J Syst Bacteriol* **39**, 224–229.
- Felsenstein, J. (1985).** Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.
- Fitch, W. M. (1971).** Toward defining the course of evolution: minimum change for a specific tree topology. *Syst Zool* **20**, 406–416.
- Hall, T. A. (1999).** BIOEDIT: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp Ser* **41**, 95–98.
- Hiraishi, A., Ueda, Y., Ishihara, J. & Mori, T. (1996).** Comparative lipoquinone analysis of influent sewage and activated sludge by high-performance liquid chromatography and photodiode array detection. *J Gen Appl Microbiol* **42**, 457–469.
- Im, W.-T., Jung, H.-M., Cui, Y.-S., Liu, Q.-M., Zhang, S.-L. & Lee, S.-T. (2005).** Cultivation of the three hundreds of bacterial species from soil of a ginseng field and mining the novel lineage bacteria. In *Proceedings of the International Meeting of the Federation of Korean Microbiological Societies*, abstract A035, p. 169. Seoul: Federation of Korean Microbiological Societies.
- Kim, M. K., Im, W.-T., Ohta, H., Lee, M. & Lee, S.-T. (2005).** *Sphingopyxis granuli* sp. nov., a β -glucosidase-producing bacterium in the family *Sphingomonadaceae* in α -4 subclass of the *Proteobacteria*. *J Microbiol* **43**, 152–157.
- Kimura, M. (1983).** *The Neutral Theory of Molecular Evolution*. Cambridge: Cambridge University Press.
- Komagata, K. & Suzuki, K. (1987).** Lipid and cell-wall analysis in bacterial systematics. *Methods Microbiol* **19**, 161–207.
- Kouker, G. & Jaeger, K.-E. (1987).** Specific and sensitive plate assay for bacterial lipases. *Appl Environ Microbiol* **53**, 211–213.
- Kumar, S., Tamura, K. & Nei, M. (2004).** MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. *Brief Bioinform* **5**, 150–163.
- 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.
- Miller, E. S., Woese, C. R. & Brenner, S. (1991).** Description of the erythromycin-producing bacterium *Arthrobacter* sp. strain NRRL B-3381 as *Aeromicrobium erythreum* gen. nov., sp. nov. *Int J Syst Bacteriol* **41**, 363–368.
- Moore, D. D. & Dowhan, D. (1995).** Preparation and analysis of DNA. In *Current Protocols in Molecular Biology*, pp. 2–11. Edited by F. M. Ausubel, R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith & K. Struhl. New York: Wiley.
- Saitou, N. & Nei, M. (1987).** The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Sasser, M. (1990).** *Identification of bacteria by gas chromatography of cellular fatty acids*, MIDI Technical Note 101. Newark, DE: MIDI Inc.
- Tamura, T. & Yokota, A. (1994).** Transfer of *Nocardioideae fastidiosa* Collins and Stackebrandt 1989 to the genus *Aeromicrobium* as *Aeromicrobium fastidiosum* comb. nov. *Int J Syst Bacteriol* **44**, 608–611.
- Ten, L. N., Im, W.-T., Kim, M.-K., Kang, M.-S. & Lee, S.-T. (2004).** Development of a plate technique for screening of polysaccharide-degrading microorganisms by using a mixture of insoluble chromogenic substrates. *J Microbiol Methods* **56**, 375–382.
- 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.
- Tschech, A. & Pfennig, N. (1984).** Growth yield increase linked to caffeine reduction in *Acetobacterium woodii*. *Arch Microbiol* **137**, 163–167.
- 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.
- Weisburg, W. G., Barns, S. M., Pelletier, D. A. & Lane, D. J. (1991).** 16S ribosomal DNA amplification for phylogenetic study. *J Bacteriol* **173**, 697–703.
- Widdel, F. & Bak, F. (1992).** Gram-negative mesophilic sulfate reducing bacteria. In *The Prokaryotes*, 2nd edn, pp. 3352–3378. Edited by A. Balows, H. G. Trüper, M. Dworkin, W. Harder & K. H. Schleifer. New York: Springer.
- Widdel, F., Kohring, G. & Mayer, F. (1983).** Studies on dissimilatory sulfate-reducing bacteria that decompose fatty acids. III. Characterization of the filamentous gliding *Desulfonema limicola* gen. nov., sp. nov., and *Desulfonema magnum* sp. nov. *Arch Microbiol* **134**, 286–294.
- Yoon, J.-H., Lee, C.-H. & Oh, T.-K. (2005).** *Aeromicrobium alkaliterrae* sp. nov., isolated from an alkaline soil, and emended description of the genus *Aeromicrobium*. *Int J Syst Evol Microbiol* **55**, 2171–2175.