Herminiimonas contaminans sp. nov., isolated as a contaminant of biopharmaceuticals

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A Gram-staining-negative, rod-shaped, non-spore-forming bacterium isolated as a contaminant from a biopharmaceutical process (strain CCUG 53591^T) was studied for its taxonomic allocation. On the basis of 16S rRNA gene sequence similarity data, this strain was clearly allocated to the genus *Herminiimonas*. *Herminiimonas saxobsidens* was shown to be the most closely related species on the basis of 16S rRNA gene sequence similarity (99.9%), followed by *Herminiimonas glaciei* (99.6%) and *Herminiimonas arsenicoxydans* (98.8%). Strain ND5, previously reported as *H. glaciei*, but showing 100% 16S rRNA gene sequence similarity to strain CCUG 53591^T, was included in the comparative study. Similarities to all other species of the genus *Herminiimonas* were below 98.0%. Chemotaxonomic data (major ubiquinone, Q-8; major polar lipids, phosphatidylethanolamine, phosphatidylglycerol and diphosphatidylglycerol; and major fatty acids, C_{17:0} cyclo, C_{19:0} cyclo ω8c and C_{16:0}, with C_{10:0} 3-OH as hydroxylated fatty acid) supported the affiliation of the isolate to the genus *Herminiimonas*. DNA–DNA hybridization results (mean values) for strain CCUG 53591^T with *H. saxobsidens* CCUG 59860^T (34%), *H. glaciei* DSM 21140^T (57%), *H. arsenicoxydans* DSM 17148^T (17%) and *Herminiimonas fonticola* S-94^T (11%) clearly supported the separate taxonomic position of this strain. Strain ND5 showed DNA–DNA similarities of 78, 56 and 52% to strain CCUG 53591^T, *H. glaciei* DSM 21140^T and *H. saxobsidens* CCUG 59860^T, respectively. Phenotypic differentiation of the isolate from the most closely related species was possible by various features. Hence, strain CCUG 53591^T represents a novel species, for which the name *Herminiimonas contaminans* sp. nov. is proposed, with the type strain CCUG 53591^T (=CCM 7991^T). Strain ND5 is a second strain of this species.

The genus *Herminiimonas* was proposed by Fernandes *et al.* (2005) for a group belonging to the *Betaproteobacteria* isolated from different water habitats. At the time of writing, the genus contained five species with validly published names: *Herminiimonas aquatilis*, which was isolated from drinking water (Kämpfer *et al.*, 2006); *Herminiimonas fonticola*, initially isolated from bottled mineral water (Fernandes *et al.*, 2005); *Herminiimonas arsenicoxydans*, isolated from arsenic-contaminated industrial wastewater sludge (Muller *et al.*, 2006); *Herminiimonas saxobsidens*, isolated recently from lichen-colonized rock (Lang *et al.*, 2007); and *Herminiimonas glaciei* isolated from 3042 m deep Greenland ice (Loveland-Curtze *et al.*, 2009).

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of *Herminiimonas contaminans* CCUG 53591^T is HE610501.

Members of the genus are characterized as aerobic, Gram-negative, motile and non-spore-forming.

In 2006, strain CCUG 53591^T was isolated in Sweden as a contaminant of a biopharmaceutical production process. This strain was presumptively morphologically identified as an ultramicrobacterium because of its small cell size. Cultivation for further analyses was done on tryptone soy agar (TSA; Oxoid) at 28 °C for 48 h.

Gram-staining of cells from this strain was performed as described by Gerhardt *et al.* (1994). Gram-behaviour was also tested with the KOH test carried out as described by Moaledj (1986). Catalase activity was tested by bubble formation after covering a colony with 3% H₂O₂. Urease activity was tested on urea agar base (Merck) according to the instructions of the manufacturer. In addition, cell

morphology and motility were observed under a Zeiss light microscope at $\times 1000$ magnification with cells grown for 3 days at 28 °C on TSA. The 16S rRNA gene sequence was analysed as described previously (Kämpfer *et al.*, 2003). Phylogenetic analysis was performed in ARB release 5.2 (Ludwig *et al.*, 2004) using the 'All-Species Living Tree Project' (LTP; Yarza *et al.*, 2008) database release LTPs106 (August 2011). Sequences not included in the LTP database were aligned with SINA (v1.2.9) according to the SILVA seed alignment (<http://www.arb-silva.de>; Pruesse *et al.*, 2007) and implemented in the ARB database. The alignment was controlled manually based on secondary structure information. Pairwise sequence similarities were calculated in ARB without the use of an evolutionary substitution model. Phylogenetic trees were constructed with the maximum-likelihood method using RAxML v7.04 (Stamatakis, 2006) with GTR-GAMMA and rapid bootstrap analysis and the neighbour-joining method with the Jukes-Cantor correction (Jukes & Cantor, 1969). Phylogenetic trees were calculated with 100 resamplings (bootstrap analysis; Felsenstein, 1985) and based on 16S rRNA gene sequences between positions 56 and 1455 (*Escherichia coli* numbering, according to Brosius *et al.*, 1978). The sequenced 16S rRNA gene fragment of strain CCUG 53591^T was a continuous stretch of 1442 nt between positions 33 and 1484 (*E. coli* numbering). Strain CCUG 53591^T shared 97.8–99.9% sequence similarity to members of the genus *Hermiimonas*, with the type strains of *H. saxobsidens* (99.9%) and *H. glaciei* (99.6%) as closest relatives, followed by *H. arsenicoxydans* (98.8%), *H. aquatilis* (97.9%) and *H. fonticola* (97.8%). Furthermore, strain

CCUG 53591^T shared identical 16S rRNA gene sequence with strain ND5, which was recently characterized as a member of *H. glaciei* genospecies based on ΔT_m values (Sahin *et al.*, 2010). Phylogenetic trees showed that, independent of the method used, strain CCUG 53591^T formed a distinct cluster with the type strains of *H. saxobsidens* and *H. glaciei*. A phylogenetic tree based on the maximum-likelihood algorithm is shown in Fig. 1.

For production of biomass that was subjected to analyses of polyamines, quinone system, polar lipids and the diagnostic peptidoglycan diamino acid, strain CCUG 53591^T and *H. glaciei* DSM 21140^T were grown on peptone yeast extract (PYE) broth (0.3% peptone from casein, 0.3% yeast extract, pH 7.2) at 28 °C. Polyamines were extracted from biomass that was harvested at the late exponential growth phase as recommended by Busse & Auling (1988), whereas quinones, polar lipids and cell wall amino acids were extracted from cells that were harvested at the stationary growth phase. Polyamines were extracted as described by Busse & Auling (1988) applying the slight modification in the gradient as reported by Busse *et al.* (1997). Quinones and polar lipids were extracted by the integrated method reported previously (Tindall, 1990a, b; Altenburger *et al.*, 1996). HPLC analyses of polyamines and quinones were carried out using the equipment described by Stolz *et al.* (2007). Diagnostic peptidoglycan diamino acid was extracted and analysed as reported by Schleifer (1985). The polyamine pattern of strain CCUG 53591^T was composed of putrescine [100.0 μmol (g dry mass)⁻¹], 2-hydroxyputrescine [49.7 μmol (g dry mass)⁻¹], spermine

Fig. 1. Maximum-likelihood tree showing the phylogenetic position of strain CCUG 53591^T. The tree was generated in ARB using RAxML (GTR-GAMMA, Rapid Bootstrap analysis, 100 bootstraps) and based on 16S rRNA gene sequences between positions 56 and 1455 [*E. coli* numbering, Brosius *et al.* (1978)]. GenBank accession numbers are given in parentheses. Numbers at branch nodes refer to bootstrap values >70% (100 replicates). *Oxalobacter formigenes* OxB^T was used as outgroup. Bar, 0.10 substitutions per site.

Fig. 2. Two-dimensional TLC of polar lipid extracts from strain CCUG 53591^T, stained with molybdophosphoric acid. DPG, Diphosphatidylglycerol; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PS, phosphatidylserine; PL1, unidentified phospholipid; L1, L2, unidentified polar lipids not containing a sugar, amino or phosphate moiety.

[1.6 $\mu\text{mol (g dry mass)}^{-1}$], spermidine [0.4 $\mu\text{mol (g dry mass)}^{-1}$], *sym*-homospermidine [0.3 $\mu\text{mol (g dry mass)}^{-1}$], cadaverine and *sym*-norspermidine [each 0.1 $\mu\text{mol (g dry mass)}^{-1}$]. *H. glaciei* DSM 21140^T contained putrescine [72.4 $\mu\text{mol (g dry mass)}^{-1}$], 2-hydroxyputrescine [43.5 $\mu\text{mol (g dry mass)}^{-1}$], spermine [0.7 $\mu\text{mol (g dry mass)}^{-1}$],

spermidine [0.3 $\mu\text{mol (g dry mass)}^{-1}$], cadaverine [0.1 $\mu\text{mol (g dry mass)}^{-1}$] and traces [$<0.1 \mu\text{mol (g dry mass)}^{-1}$] of *sym*-norspermidine and *sym*-homospermidine. Though the contents of the major polyamines putrescine and 2-hydroxyputrescine were approximately three to fourfold higher than those in *H. aquatilis* CCUG 44693^T, the only other species of the genus so far analysed for its polyamine content (Kämpfer *et al.*, 2006), no significant qualitative differences were detected. The quinone system of strain CCUG 53591^T consisted of ubiquinones Q-8 (62.8%), Q-7 (36.8%) and Q-9 (0.5%). The quinone system of *H. glaciei* DSM 21140^T was analysed as well because this trait was not included in the original description of this species (Loveland-Curtze *et al.*, 2009) and it revealed a quinone system comprising Q-8 (94.1%), Q-7 (5.4%) and Q-9 (0.5%). These quinone systems with Q-8 as the major compound were in agreement with the affiliation of the two strains to the class *Betaproteobacteria* and the description of the genus *Herminiimonas* (Fernandes *et al.*, 2005) but high amounts of Q-7 clearly distinguished strain CCUG 53591^T from its close relative *H. glaciei* DSM 21140^T and from *H. aquatilis*, the only other species of the genus for which detailed analyses of the quinone system has been carried out. The polar lipid profile of strain CCUG 53591^T exhibited the major polar lipids that were listed in the original genus description, namely phosphatidylethanolamine, phosphatidylglycerol and diphosphatidylglycerol. Furthermore, major amounts of phosphatidylserine, an unidentified phospholipid PL1 and two minor lipids (L1 and L2) were detected (Fig. 2). The polar lipid profile of *H. glaciei* was substantially identical showing only some quantitative differences and the absence of L1 (results not shown). As in other *Proteobacteria*, *meso*-diaminopimelic acid is the diagnostic diamino acid of the peptidoglycan.

Table 1. Comparison of the fatty acid contents (percentage of total) of strain CCUG 53591^T and recognized species of the genus *Herminiimonas*

Strains: 1, CCUG 53591^T; 2, ND5; 3, *H. glaciei* DSM 21140^T; 4, *H. saxobidens* CCUG 59860^T; 5, *H. arsenicoxydans* ULPAs1^T; 6, *H. aquatilis* CCUG 36956^T (Kämpfer *et al.*, 2006); 7, *H. fonticola* S-94^T (Fernandes *et al.*, 2005). Fatty acids comprising less than 3% of total in all species studied are not shown. —, Not detected. Data for strains 1–5 are from this study. Strains were grown on TSA for 48 h at 28 °C prior to extraction. Data in parentheses are from Loveland-Curtze *et al.* (2009).

Fatty acid	1	2	3	4	5	6	7
C _{10:0} 3-OH	6.9	7.9	9.0 (8.6)	9.8 (8.9)	4.3 (8.4)	5.2	6.0
C _{14:0}	—	—	— (0.3)	— (0.3)	3.7 (4.1)	—	4.8
C _{15:1} ω 6c	—	—	—	—	—	3.6	—
C _{16:1} ω 7c*	3.8	5.8	6.1 (11.6)	8.1 (19.3)	43.7 (31.0)	47.7	45.6
C _{16:1} ω 5c	—	—	—	— (—)	1.9 (—)	1.5	0.8
C _{16:0}	23.1	24.3	22.6 (30.9)	17.3 (33.2)	27.0 (26.6)	11.9	26.1
C _{17:1} ω 6c	—	—	—	— (—)	—	16.0	—
C _{17:0} cyclo	46.7	45.8	40.0 (28.4)	38.0 (19.7)	13.7 (13.6)	—	7.9
C _{18:1} ω 7c	1.0	2.1	2.9 (5.7)	2.8 (8.9)	3.8 (4.5)	9.1	7.3
C _{18:1} ω 7c 11 methyl	—	—	— (0.5)	— (0.6)	— (4.2)	—	—
C _{19:0} cyclo ω 8c	15.0	18.9	19.1 (8.4)	25.5 (5.0)	2.4 (1.6)	—	0.4

*Labelled as summed feature 3 in the MIDI system (C_{16:1} ω 7c/iso-C_{15:0} 2-OH).

Table 2. Differential characteristics of strain CCUG 53591^T and recognized species of the genus *Hermiiniimonas*

Strains: 1, CCUG 53591^T; 2, ND5; 3, *H. glaciei* UMB49^T; 4, *H. saxobsidens* NS11^T (data from Lang *et al.*, 2007); 5, *H. arsenicoxydans* ULPAs1^T (Muller *et al.*, 2006); 6, *H. aquatilis* CCUG 36956^T (Kämpfer *et al.*, 2006; Lang *et al.*, 2007); 7, *H. fonticola* S-94^T (Fernandes *et al.*, 2005). Data for physiological tests for strains 1–5 are from this study, unless indicated otherwise. +, Positive; – negative; ND, not determined/no data.

Characteristic	1	2	3	4	5	6	7
Cell morphology	Thin rods	Thin rods	Thin rods	Ovoid rods	Curved rods	Short rods	Short rods
Cell size (µm)	0.5–1.0 × 0.5–0.6	0.5–0.9 × 0.3–0.4	0.5–0.9 × 0.3–0.4	0.8 × 0.4	1–2 × 0.5–0.7	0.8–1.2 × 1.0–1.2	1–4 × 0.7
Colony colour	Translucent white, translucent tan	Translucent white, translucent tan	Translucent white, translucent tan	Translucent, cream coloured	Pale yellow/straw coloured	Yellowish	Translucent yellow
Colony size	<1 mm (R2A; 3–4 days), 1–1.5 mm (TSA; 3–4 days)	<1 mm (R2A; 3–4 days), 1–1.5 mm (TSA; 3–4 days)	<1 mm (R2A; 3–4 days), 1–1.5 mm (TSA; 3–4 days)	1.5 mm (NA*; 3 days)	ND	2–3 mm	<1 mm (3 days)
Flagella	Motile (type of flagellation ND)	Motile 1 or 2 polar and/or 1–3 lateral	1 or 2 polar and/or 1–3 lateral	Polar flagella	1 polar	Motile	1 polar
Temperature range for growth (°C)	4–30	ND	1–35	4–37	4–30	ND	10–35
Nitrate reduction	–	–	–	+	+	+	–
Assimilation of:†							
Citrate	–	–	+	–	–	–	+
Malate	+	+	+	+	–	–	+
Succinate	ND	ND	+	+	ND	–	+
Lactate	+	+	+	+	+	+	+
Alanine	–	–	+	+	–	–	+
DNA G + C content (mol%)	ND	55.4‡	59	ND	54	ND	52

*NA, nutrient agar.

†Results were congruent with those reported by Loveland-Curtze *et al.* (2009) for *H. glaciei*, by Lang *et al.* (2007) for *H. saxobsidens* and by Muller *et al.* (2006) for *H. arsenicoxydans*.

‡Data from Sahin *et al.* (2010).

Fatty acid analysis was performed according to the method described by Kämpfer & Kroppenstedt (1996). The cells of all strains under comparison were extracted after 48 h growth on TSA in the same growth phase (stationary phase). Because colony development of the type strains of the most closely related species was very similar it can be assumed that strains were in similar growth phases. The fatty acid profiles of all strains are shown in Table 1 (identified with the Sherlock version 2.11, TSBA40 Rev. 4.1). No pronounced qualitative differences in the fatty acid profiles were found for the strains but quantitative differences could be observed. All type strains of members of the genus *Herminiimonas*, as well as strain CCUG 53591^T, had fatty acid profiles composed of C_{17:0} cyclo, summed feature 3 (C_{16:1}ω7c and/or iso-C_{15:0} 2-OH) and C_{16:0} as the major components, as well as C_{18:1}ω7c, C_{10:0} 3-OH and C_{19:0} cyclo ω8c in moderate amounts.

Results of the physiological characterization are given in the species description and in Table 2 (differential characters) obtained using methods described previously (Kämpfer *et al.*, 1991). Urease was weakly positive after 6 days of incubation at 28 °C. Only a few carbon sources, mainly organic acids, could be used as sole sources of carbon. DNA–DNA hybridization results (mean values of duplicates are shown in parentheses) of strain CCUG 53591^T with the type strains of *H. saxobidens* CCUG 59860^T (34%), *H. glaciei* DSM 21140^T (57%), *H. arsenicoxydans* DSM 17148^T (17%) and *H. fonticola* S-94^T (11%) clearly supported the separate taxonomic position of this strain. Strain ND5 showed DNA–DNA similarities of 78, 56 and 52% to strain CCUG 53591^T, *H. glaciei* DSM 21140^T and *H. saxobidens* CCUG 59860^T, respectively. It is an interesting result of this study that strains showing a very high 16S rRNA gene sequence similarity (99.6–99.8%) show significant phenotypic and also genotypic differences sufficient for them to be classified as separate species, thus confirming the results of previous studies on bacteria of this genus (Kämpfer *et al.*, 2006; Muller *et al.*, 2006; Lang *et al.*, 2007; Loveland-Curtze *et al.*, 2009; Sahin *et al.*, 2010). On the basis of these results as well as the phenotypic traits, we describe a novel species, for which the name *Herminiimonas contaminans* sp. nov. is proposed.

Description of *Herminiimonas contaminans* sp. nov.

Herminiimonas contaminans (con.ta'mi.nans. L. part. adj. *contaminans* contaminating, polluting, referring to the isolation of the type strain as a contaminant of a biopharmaceutical process).

Cells appear as non-spore-forming rods (approximately 1 µm in length and 0.5 µm in width). Gram-stain-negative, catalase- and oxidase-positive, showing an oxidative metabolism. Motile. Urease weakly positive. Good growth occurs on R2A agar, TSA, PYE agar and nutrient agar at 25–30 °C. Growth was visible on TSA at temperatures ranging from 15 °C to 37 °C. No growth was observed at

4 °C or 37 °C. The colonies are beige-coloured, translucent and shiny with entire edges within 24 h of incubation, with a diameter of approximately 1 mm. The polar lipid profile was composed of the major components phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol and phosphatidylserine, and moderate amounts of an unidentified phospholipid (PL1) and two minor lipids (L1 and L2). The quinone system contains ubiquinone Q-8 and Q-7. Putrescine and 2-hydroxyputrescine are predominant in the polyamine pattern. *meso*-Diaminopimelic acid is the diagnostic diamino acid of the peptidoglycan. The fatty acid profile is composed mainly of C_{17:0} cyclo, C_{19:0} cyclo ω8c and C_{16:0}, with C_{10:0} 3-OH as hydroxylated fatty acid. Carbon source utilization and hydrolysis of chromogenic substrates (including differentiating characters for all species of the genus *Herminiimonas*) are indicated in Table 2. Growth with acetate, propionate, fumarate, DL-lactate, malate or 2-oxoglutarate is positive. Growth with D-glucose, maltose, D-mannose, D-fructose and trehalose, *N*-acetylglucosamine, *N*-acetylglucosamine, L-arabinose, L-arbutin, cellobiose, D-galactose, gluconate, glycerol, D-mannitol, maltitol, α-melibiose, L-rhamnose, D-ribose, sucrose, salicin, D-xylose, adonitol, *i*-inositol, D-sorbitol, putrescine, *cis*-aconitate, *trans*-aconitate, 4-aminobutyrate, adipate, azelate, glutarate, DL-3-hydroxybutyrate, itaconate, pyruvate, substrate, citrate, mesaconate, L-alanine, β-alanine, L-ornithine, L-phenylalanine, L-serine, L-aspartate, L-histidine, L-leucine, L-proline, L-tryptophan, 3-hydroxybenzoate, 4-hydroxybenzoate and phenylacetate is negative. The chromogenic substrate L-alanine-*p*-nitroanilide is hydrolysed. The following substrates are not hydrolysed: *p*-nitrophenyl-α-D-glucopyranoside, *p*-nitrophenyl-β-D-glucopyranoside, *p*-nitrophenyl-β-D-galactopyranoside, *p*-nitrophenyl-β-D-xylopyranoside, *p*-nitrophenyl-β-D-glucuronide, bis-*p*-nitrophenyl-phosphate, bis-*p*-nitrophenyl-phenyl-phosphonate, bis-*p*-nitrophenyl-phosphoryl-choline, 2-deoxythymidine-2'-*p*-nitrophenyl-phosphate, γ-L-glutamate-*p*-nitroanilide and L-proline-*p*-nitroanilide.

The type strain is CCUG 53591^T (=CCM 7991^T), isolated in Sweden in 2006 from a biopharmaceutical process. Strain ND5 previously identified as *H. glaciei* (Sahin *et al.*, 2010) is a second strain of this species.

Acknowledgements

We are grateful to the depositor and to the entire Culture Collection University Göteborg (CCUG) staff for their devoted work. We thank Dr T. Iizuka for providing strain ND5 for comparative analyses. We thank Dr Jean Euzéby (Société de Bactériologie Systématique et Vétérinaire, Prefecture de la Haute-Garonne, France) for his advice with the nomenclature.

References

- Altenburger, P., Kämpfer, P., Makristathis, A., Lubitz, W. & Busse, H.-J. (1996). Classification of bacteria isolated from a medieval wall painting. *J Biotechnol* **47**, 39–52.

- Brosius, J., Palmer, M. L., Kennedy, P. J. & Noller, H. F. (1978). Complete nucleotide sequence of a 16S ribosomal RNA gene from *Escherichia coli*. *Proc Natl Acad Sci U S A* 75, 4801–4805.
- Busse, H.-J. & Auling, G. (1988). Polyamine pattern as a chemotaxonomic marker within the Proteobacteria. *Syst Appl Microbiol* 11, 1–8.
- Busse, H.-J., Bunka, S., Hensel, A. & Lubitz, W. (1997). Discrimination of members of the family Pasteurellaceae based on polyamine patterns. *Int J Syst Bacteriol* 47, 698–708.
- Felsenstein, J. (1985). Confidence limits of phylogenies: an approach using the bootstrap. *Evolution* 39, 783–791.
- Fernandes, C., Rainey, F. A., Nobre, M. F., Pinhal, I., Folhas, F. & da Costa, M. S. (2005). *Hermiimonas fonticola* gen. nov., sp. nov., a Betaproteobacterium isolated from a source of bottled mineral water. *Syst Appl Microbiol* 28, 596–603.
- Gerhardt, P., Murray, R. G. E., Wood, W. A. & Krieg, N. R. (editors) (1994). *Methods for General and Molecular Bacteriology*. Washington, DC: American Society for Microbiology.
- Jukes, T. H. & Cantor, C. R. (1969). Evolution of the protein molecules. In *Mammalian Protein Metabolism*, pp. 21–132. Edited by H. N. Munro. New York: Academic Press.
- Kämpfer, P. & Kroppenstedt, R. M. (1996). Numerical analysis of fatty acid patterns of coryneform bacteria and related taxa. *Can J Microbiol* 42, 989–1005.
- Kämpfer, P., Steiof, M. & Dott, W. (1991). Microbiological characterisation of a fuel-oil contaminated site including numerical identification of heterotrophic water and soil bacteria. *Microb Ecol* 21, 227–251.
- Kämpfer, P., Dreyer, U., Neef, A., Dott, W. & Busse, H.-J. (2003). *Chryseobacterium defluvii* sp. nov., isolated from wastewater. *Int J Syst Evol Microbiol* 53, 93–97.
- Kämpfer, P., Busse, H.-J. & Falsen, E. (2006). *Hermiimonas aquatilis* sp. nov., a new species from drinking water. *Syst Appl Microbiol* 29, 287–291.
- Lang, E., Swiderski, J., Stackebrandt, E., Schumann, P., Spröer, C. & Sahin, N. (2007). *Hermiimonas saxobsidens* sp. nov., isolated from a lichen-colonized rock. *Int J Syst Evol Microbiol* 57, 2618–2622.
- Loveland-Curtze, J., Miteva, V. I. & Brenchley, J. E. (2009). *Hermiimonas glaciei* sp. nov., a novel ultramicrobacterium from 3042 m deep Greenland glacial ice. *Int J Syst Evol Microbiol* 59, 1272–1277.
- Ludwig, W., Strunk, O., Westram, R., Richter, L., Meier, H., Yadhukumar, A., Buchner, A., Lai, T., Steppi, S. & other authors (2004). ARB: a software environment for sequence data. *Nucleic Acids Res* 32, 1363–1371.
- Moaledj, K. (1986). Comparison of Gram-staining and alternate methods, KOH test and aminopeptidase activity in aquatic bacteria: their application to numerical taxonomy. *J Microbiol Methods* 5, 303–310.
- Muller, D., Simeonova, D. D., Riegel, P., Mangenot, S., Koechler, S., Lièremont, D., Bertin, P. N. & Lett, M.-C. (2006). *Hermiimonas arsenicoxydans* sp. nov., a metalloresistant bacterium. *Int J Syst Evol Microbiol* 56, 1765–1769.
- Pruesse, E., Quast, C., Knittel, K., Fuchs, B. M., Ludwig, W., Peplies, J. & Glöckner, F. O. (2007). SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. *Nucleic Acids Res* 35, 7188–7196.
- Sahin, N., Gonzalez, J. M., Iizuka, T. & Hill, J. E. (2010). Characterization of two aerobic ultramicrobacteria isolated from urban soil and a description of *Oxalicibacterium solurbis* sp. nov. *FEMS Microbiol Lett* 307, 25–29.
- Schleifer, K. H. (1985). Analysis of the chemical composition and primary structure of murein. *Methods Microbiol* 18, 123–156.
- Stamatakis, A. (2006). RAXML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* 22, 2688–2690.
- Stolz, A., Busse, H.-J. & Kämpfer, P. (2007). *Pseudomonas knackmussii* sp. nov. *Int J Syst Evol Microbiol* 57, 572–576.
- Tindall, B. J. (1990a). Lipid composition of *Halobacterium lacusprofundi*. *FEMS Microbiol Lett* 66, 199–202.
- Tindall, B. J. (1990b). A comparative study of the lipid composition of *Halobacterium saccharovorum* from various sources. *Syst Appl Microbiol* 13, 128–130.
- Yarza, P., Richter, M., Peplies, J., Euzéby, J., Amann, R., Schleifer, K. H., Ludwig, W., Glöckner, F. O. & Rosselló-Móra, R. (2008). The All-Species Living Tree project: a 16S rRNA-based phylogenetic tree of all sequenced type strains. *Syst Appl Microbiol* 31, 241–250.