Alicyclobacillus pohliae sp. nov., a thermophilic, endospore-forming bacterium isolated from geothermal soil of the north-west slope of Mount Melbourne (Antarctica)

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Gram-positive, rod-shaped, endospore-forming, thermophilic bacteria were isolated from a geothermal soil collected on the north-west slope of Mount Melbourne in Antarctica. They grew aerobically at 42–60 °C (optimum 55 °C) and at pH 4.5–7.5 (optimum pH 5.5). Phylogenetic analysis of 16S rRNA gene sequences showed that these isolates were related most closely to the type strain of *Alicyclobacillus pomorum* (91 % similarity). Growth occurred in the presence of ferrous iron at micromolar concentrations and acid was produced from various sugars. Iso-branched fatty acids C_{15:0} (45.56 %) and C_{17:0} (35.81 %) were the most abundant cellular fatty acids. The DNA G + C content was 55.1 mol%. On the basis of phenotypic and phylogenetic characteristics, it is concluded that these strains represent a novel species of the genus *Alicyclobacillus*, for which the name *Alicyclobacillus pohliae* sp. nov. is proposed. The type strain is MP4^T (=CIP 109385^T =NCIMB 14276^T).

Members of the genus *Alicyclobacillus* are heterotrophic, Gram-positive, endospore-forming organisms which inhabit mostly acidic geothermal environments. The genus *Alicyclobacillus* was first described by Wisotzkey *et al.* (1992) and the description has subsequently been emended based on newly obtained data (Goto *et al.*, 2003; Karavaiko *et al.*, 2005). At the time of writing, the genus comprises 17 recognized species: *Alicyclobacillus acidocaldarius*, *A. acidoterrestris* (Wisotzkey *et al.*, 1992), *A. hesperidum* (Albuquerque *et al.*, 2000), *A. acidiphilus* (Matsubara *et al.*, 2002), *A. sendaiensis* (Tsuruoka *et al.*, 2003), *A. vulcanalis* (Simbahan *et al.*, 2004), *A. tolerans*, *A. disulfidooxidans* (Karavaiko *et al.*, 2005), *A. cycloheptanicus* (Deinhard *et al.*, 1987), *A. herbarius* (Goto *et al.*, 2002), *A. pomorum* (Goto *et al.*, 2003), *A. contaminans*, *A. fastidiosus*, *A. kakegawensis*, *A. macrosporangiidus*, *A. sacchari* and *A. shizuokensis* (Goto *et al.*, 2007). Moreover, three Japanese strains and two isolated from the island of São Miguel in the Azores were denoted as representing two genomic species of *Alicyclobacillus* based on 16S rRNA gene sequence analysis and DNA–DNA hybridization assays (Hiraishi *et al.*, 1997; Albuquerque *et al.*, 2000). In addition, a novel subspecies of *A. acidocaldarius*, subsp.

ritmannii, was isolated from a geothermal site in Antarctica (Nicolaus *et al.*, 1998). Although continental Antarctica is largely ice-bound and supports only sparse growth of terrestrial life, it possesses a small number of sites where the ground is warmed by geothermal sources and is supplied with moisture from the condensation of steam emissions (Bargagli *et al.*, 2004). Novel thermophilic species ascribed to the genera *Bacillus*, *Brevibacillus*, *Aneurinibacillus* and *Anoxybacillus* have recently been isolated from Antarctic geothermal grounds (Nicolaus *et al.*, 1996; Logan *et al.*, 2000; Allan *et al.*, 2005; Poli *et al.*, 2006).

In the present study, we describe a novel thermophilic bacterium isolated from warm soils collected in the immediate vicinity of a steam vent on the north-west slope of Mount Melbourne, northern Victoria Land, continental Antarctica (74° 20.994' S 164° 39.792' E, 2498 m altitude) (Bargagli *et al.*, 2004). *Brevibacillus levickii* is the only novel species previously recovered from the north-west slope of Mt Melbourne despite repeated soil sampling on separate occasions followed by enrichment and isolation at different times in the laboratory (Allan *et al.*, 2005). In February 2002, four soil samples were collected along a thermal gradient ranging from 25 to 43 °C in the vicinity of a small area (100 cm²) colonized by shoots of the moss *Pohlia nutans* (Bargagli *et al.*, 2004). Analytical determinations of chemical properties of soil water extracts showed pH values ranging

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains MP1, MP2, MP3 and MP4^T are AJ607429, AJ607430, AJ607431 and AJ564766, respectively.

from 5.46 to 5.71 and relatively higher concentrations of sodium, iron and manganese when compared with chemical characteristics of other geothermal sites in Victoria Land. In particular, the iron concentration ranged from 0.94 to $1.22 \mu\text{g g}^{-1}$ (Bargagli *et al.*, 2004). After incubation in DSMZ medium 259 (1^{-1} : 3.5 g $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$, 1.0 g K_2HPO_4 , 0.03 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.5 g NH_4Cl , 4.0 g yeast extract, pH 6.5) at 55°C , all four samples yielded positive cultures. Subcultivation, carried out on tryptone soy agar (TSA; Oxoid) plus $250 \mu\text{M}$ $\text{Fe}_2\text{SO}_4 \cdot 7\text{H}_2\text{O}$ (TSA-Fe) at 55°C , allowed the isolation of four thermophilic strains, designated MP1, MP2, MP3 and MP4^T (Bargagli *et al.*, 2004). Cultures were further propagated on FeP liquid medium [1^{-1} : 5.0 g tryptone (Difco), 0.5 g D-glucose, 0.1 g $(\text{NH}_4)_2\text{SO}_4$, 0.1 g $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$], BAM basal salts medium (Deinhard *et al.*, 1987) and/or DSMZ medium 259, all amended with $250 \mu\text{M}$ $\text{Fe}_2\text{SO}_4 \cdot 7\text{H}_2\text{O}$. In order to establish the requirement for mineral substrates for growth, DSMZ medium 259, containing 0.01 or 0.1 g yeast extract 1^{-1} , was supplemented with $250 \mu\text{M}$ $\text{Fe}_2\text{SO}_4 \cdot 7\text{H}_2\text{O}$, FeCl_3 , $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ or $\text{Na}_2\text{O}_3\text{S}$, or 0.15 % elemental sulfur (S^0). Growth in liquid media was monitored by measuring the optical density at 570 nm (Ultrospec 2100 UV-VIS spectrophotometer; Amersham Biosciences). The temperature range for growth was determined by incubating the four isolates at temperatures ranging from 25 to 70°C . The pH range for growth was determined in FeP medium and modified DSMZ medium 259 with pH adjusted by using 1 M H_2SO_4 . All pH measurements were made at room temperature. Anaerobic growth was tested in triplicate by spreading each strain on FeP agar (1.5 % Bacto agar; Difco). Plates were placed in a GENbag Anaer chamber (bioMérieux) and incubated for 1 week ($\text{O}_2 < 0.1\%$ after 2–5 h). For studies of heat resistance, 1-week-old colonies were resuspended in fresh liquid medium and exposed to temperatures of 80 or 100°C for 10 min. Aliquots were then plated on FeP agar and survival was measured based on the number of colonies formed after 24–48 h incubation at 55°C . After growth on TSA-Fe, colony morphology was observed under a stereomicroscope. Vegetative cells and sporangial morphologies were observed visually by bright-field light microscopy and phase-contrast microscopy, respectively. Gram determination was carried out either by staining (Gram stain kit; Medical Chemical) or a chemical method (Powers, 1995). Catalase and oxidase activities were determined according to Smibert & Krieg (1981). Sensitivity of the four isolates to ampicillin, chloramphenicol, tetracycline, gentamicin, penicillin G, bacitracin, erythromycin, kanamycin and streptomycin was tested on FeP agar. Acidification was examined with the API 50 CH test system (bioMérieux) essentially as described by Albuquerque *et al.* (2000) at the optimum growth temperature. Acidification of the medium was monitored every 2 days for 10 days. All tests were repeated in triplicate. The ability to oxidize different carbon sources was examined by using Biolog GN and GP microplates. Cell suspensions, after growth on TSA-Fe, were prepared by following the manufacturer's instructions and then used to inoculate the wells of Biolog

microtitre plates. Microplates were incubated in a humidified chamber at 55°C , and readings of the rate of colour development were taken after 24, 48 and 72 h (Viti & Giovannetti, 2005). Results of the phenotypic characterization tests are given in the species description and in Table 1.

The partial 16S rRNA gene sequences of strains MP1, MP2 and MP3 as well the almost-complete sequence of strain MP4^T were reported previously by Bargagli *et al.* (2004). For the present study, more complete sequences of the 16S rRNA gene of strains MP1, MP2 and MP3 were determined. By comparing 16S rRNA gene sequences between positions 36 and 1490 (*Escherichia coli* numbering system), strains MP1, MP3 and MP4^T showed 100 % identity to each other, while strain MP2 differed from the other three at one nucleotide position. The four 16S rRNA gene sequences were then used to retrieve closely matching type sequences at the Ribosomal Database Project (Cole *et al.*, 2003). Phylogenetic analysis was performed by using the software package TREECON for Windows (Van de Peer & De Wachter, 1997) after multiple alignment of the data by CLUSTAL_X (Thompson *et al.*, 1997). Distances were converted to evolutionary distances according to the Jukes and Cantor method, and clustering with the neighbour-joining method was determined by using bootstrap values based on 1000 replications (Fig. 1). The 16S rRNA gene sequence of strain MP4^T was a continuous stretch of 1505 bp. After a neighbour-joining analysis, 16S rRNA gene sequence similarity calculations indicated that the closest relatives of strain MP4^T were the type strains of *A. pomorum* (91.0 %), *A. sacchari* (90.9 %), *A. hesperidum* and *A. macrosporangiidus* (90.8 %), *A. acidoterrestris* (90.7 %) and *A. cycloheptanicus* (90.6 %). Lower sequence similarities (<90.5 %) were found with the type strains of all other recognized species of the genus.

GC analysis of methylated fatty acids was performed following the analytical procedures described by Kämpfer & Kroppenstedt (1996). Analysis of the fatty acids at the conditions applied revealed that strain MP4^T was dominated by iso-branched fatty acids iso- $\text{C}_{15:0}$ (45.56 %) and iso- $\text{C}_{17:0}$ (35.81 %). In addition, smaller amounts of anteiso-branched fatty acids were found (anteiso- $\text{C}_{15:0}$, 6.4 %; anteiso- $\text{C}_{17:0}$, 3.36 %). The DNA base composition for MP4^T , determined according to the method of Mesbah *et al.* (1989), was 55.1 mol% G + C, which is within the range reported for recognized *Alicyclobacillus* species (48.7–62.7 mol%) (Goto *et al.*, 2003; Karavaiko *et al.*, 2005).

On the basis of the data presented, we conclude that strains MP1, MP2, MP3 and MP4^T represent a single novel species of the genus *Alicyclobacillus*, for which the name *Alicyclobacillus pohliae* sp. nov. is proposed.

Description of *Alicyclobacillus pohliae* sp. nov.

Alicyclobacillus pohliae (poh'li.æ. N.L. gen. fem. n. *pohliae* of *Pohlia*, a genus of moss colonizing the area where the type strain was isolated).

Table 1. Phenotypic characteristics of strain MP4^T and recognized species of the genus *Alicyclobacillus*

Strains: 1, MP4^T (data from the present study); 2, *A. tolerans* DSM 16297^T; 3, *A. disulfidooxidans* DSM 12064^T (data in columns 2 and 3 from Karavaiko *et al.*, 2005); 4, *A. acidocaldarius* subsp. *acidocaldarius* ATCC 27009^T; 5, *A. acidoterrestris* ATCC 49025^T; 6, *A. cycloheptanicus* DSM 4006^T (data in columns 4–6 from Goto *et al.*, 2007); 7, *A. hesperidum* DSM 12489^T (Albuquerque *et al.*, 2000); 8, *A. acidiphilus* TA67^T (Matsubara *et al.*, 2002); 9, *A. herbarius* CP1^T (Goto *et al.*, 2002); 10, *A. pomorum* 3A^T (Goto *et al.*, 2003); 11, *A. sendaiensis* NTAP-1^T (Tsuruoka *et al.*, 2003); 12, *A. vulcanalis* DSM 16176^T (Simbahan *et al.*, 2004); 13, *A. sacchari* RB718^T; 14, *A. fastidiosus* S-TAB^T; 15, *A. kakagawensis* 5-A83^T; 16, *A. shizuokensis* 4-A336^T; 17, *A. macrosporangiidus* 5-A239-2O-A^T; 18, *A. contaminans* 3-A191^T (data in columns 13–18 from Goto *et al.*, 2007). +, Positive; –, negative; w, weakly positive; v, variable between strains; NR, not reported. All strains produce acid from glucose.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
pH for growth																		
Range	4.5–7.5	1.5–5.0	0.5–6.0	2.0–6.0	2.2–5.8	3.0–5.5	2.0–6.0	2.5–5.5	3.0–6.5	3.0–6.0	2.5–6.5	2.0–6.0	2.0–6.0	2.0–5.5	3.0–6.5	3.0–6.5	3.0–6.5	3.0–6.0
Optimum	5.0	2.0–2.7	1.5–2.5	4.0	4.0	4.5	4.0	3.0	4.5–5.0	4.0–4.5	3.5	4.0	4.0–4.5	4.0–4.5	4.0–4.5	4.0–4.5	4.0–4.5	4.0–4.5
Growth temperature (°C)																		
Range	42–60	<20–55	4–40	45–70	<35–55	40–53	35–60	20–55	35–65	30–60	40–65	35–65	30–55	20–55	40–60	35–60	35–60	35–60
Optimum	55	38–42	35	60	42–55	48	50	50	55–60	45–50	55	55	45–50	40–45	50–55	45–50	50–55	50–55
DNA G + C content (mol%)	55.1	48.1–49.3	53.0	60.3	52.2	55.6	54.0	54.1	56.2	53.1	62.3	62.0	56.6	53.9	61.3–61.7	60.5	62.5	60.1–60.6
Oxidase	–	NR	NR	–	–	+	–	–	–	–	+	–	–	–	–	–	–	–
Catalase	–	NR	NR	+	+	+	w	+	+	+	+	–	–	+	w	+	w	–
Mineral substrates	Fe ²⁺	Fe ²⁺ , S ⁰ , sulfide minerals	S ⁰ , FeS, S ₂ O ₃ ^{2–} , Fe ³⁺	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Acid production from:																		
Glycerol	–	w	+	+	+	–	+	–	+	+	+	+	+	–	–	–	+	+
Erythritol	–	–	–	–	+	–	–	–	–	–	–	–	–	–	v	–	–	–
D-Arabinose	+	w	+	+	+	+	+	+	+	–	–	–	–	+	+	–	+	–
L-Arabinose	+	w	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+
Ribose	+	+	+	NR	NR	NR	+	+	+	+	+	+	+	+	+	+	+	+
D-Xylose	+	+	+	+	–	+	+	+	+	–	+	+	+	+	+	+	+	+
L-Xylose	+	+	+	+	–	+	+	–	–	–	–	–	–	–	+	+	+	–
Methyl β-xyloside	–	NR	NR	–	–	–	+	–	NR	NR	–	–	+	+	–	–	–	–
D-Galactose	+	+	–	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+
D-Fructose	+	+	–	NR	NR	NR	+	+	+	+	+	+	+	+	+	+	+	+
Mannose	+	+	–	NR	NR	NR	+	+	+	+	+	+	+	+	+	+	+	+
L-Sorbose	+	+	+	–	–	+	+	+	–	+	–	–	–	–	+	–	+	v
Rhamnose	+	–	–	+	+	+	–	–	+	–	–	–	+	+	+	–	+	v
Inositol	+	–	–	–	+	–	–	–	–	–	–	+	–	+	v	–	–	–
Mannitol	–	+	–	+	+	+	+	–	+	+	+	+	+	+	+	+	–	+
Sorbitol	–	–	+	–	+	+	–	+	–	–	–	–	–	–	+	–	+	–
Methyl α-D-mannoside	–	NR	NR	–	–	–	+	–	+	–	–	–	–	–	+	–	+	–
Methyl α-D-glucoside	w	+	+	+	–	–	+	+	+	+	+	w	+	–	+	–	–	–
Amygdalin	–	+	+	–	–	–	+	–	+	+	–	–	–	–	+	–	–	–
Arbutin	w	–	–	+	–	–	+	+	+	–	+	w	+	–	+	+	–	+
Aesculin	+	+	+	+	–	+	+	+	+	+	–	–	–	–	+	+	+	+
Salicin	w	–	–	+	–	–	+	+	+	+	+	–	+	–	+	+	+	v
Cellobiose	+	–	+	+	+	–	+	+	+	–	+	+	+	–	+	+	–	+
Maltose	+	w	+	+	+	–	+	+	+	+	+	+	+	–	+	+	+	+
Lactose	+	w	–	+	+	–	+	+	+	–	+	–	+	–	+	–	+	v
Melibiose	+	–	+	+	w	–	–	–	+	–	–	+	+	+	–	+	–	–
Sucrose	+	+	+	+	+	–	+	+	+	+	+	+	+	–	+	+	+	+
Trehalose	w	+	–	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+
Inulin	–	NR	NR	–	–	–	+	–	NR	NR	–	–	+	–	–	–	–	–
Melezitose	w	+	+	–	–	–	+	+	+	–	–	–	+	–	v	–	–	–
Raffinose	w	w	+	+	–	–	–	+	+	–	+	+	+	+	–	–	–	–
Glycogen	+	+	+	+	–	–	+	–	–	–	+	+	+	–	–	–	–	–

Table 1. cont.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Xylitol	—	NR	NR	—	+	—	—	+	—	—	—	—	—	—	+	—	+	—
Gentiobiose	+	NR	NR	—	—	—	w	+	+	—	—	—	+	—	+	+	+	v
Turanose	+	w	—	—	—	—	+	+	+	+	+	+	+	—	+	—	—	—
D-Lyxose	+	NR	NR	—	—	+	+	—	—	—	—	—	—	+	+	—	+	—
D-Tagatose	+	w	+	—	—	+	+	—	—	+	—	—	—	+	v	—	—	v
D-Fucose	+	—	+	—	—	—	+	—	+	—	—	—	—	+	—	—	—	—
L-Fucose	+	NR	NR	—	—	—	+	—	NR	NR	—	—	—	+	—	—	—	—
D-Arabitol	—	NR	NR	—	—	—	+	—	NR	—	—	—	—	—	+	—	+	—
5-Ketogluconate	+	+	+	—	—	+	+	—	+	+	—	w	—	—	—	—	—	+

Fig. 1. Phylogenetic tree (constructed according to the neighbour-joining method) showing the position of the four novel strains in relation to recognized species of the genus *Alicyclobacillus* and a phylogenetically related *Bacillus* species, based on 16S rRNA gene sequences. Numbers represent percentages from 1000 replicate bootstrap samplings (values <80% not shown). *Arthrobacter globiformis* JCM 1332^T served as an outgroup. Bar, genetic divergence of 0.1.

Aerobic, Gram-positive, spore-forming rods measuring $1.5\text{--}2.5 \times 0.4\text{--}0.6\text{ }\mu\text{m}$. Round spores lie terminally in swollen sporangia. Oxidase- and catalase-negative. Does not grow in the presence of 5% NaCl. Colonies are cream-coloured, entire and convex and grow to 1.5–2 mm in diameter after 48 h at 55 °C in TSA-Fe. Iso-branched fatty acids iso-C_{15:0} (45.56%) and iso-C_{17:0} (35.81%) are the major cellular fatty acids. Facultatively anaerobic. Optimal growth occurs aerobically at 55 °C (range 42–60 °C) and at pH 5.5 (range pH 4.5–7.5). Growth on yeast extract (0.01%) occurs in the presence of ferrous iron (Fe²⁺) at micromolar concentrations. Sensitive to (ml⁻¹) ampicillin (25 µg), chloramphenicol (10 µg), tetracycline (50 µg), gentamicin (30 µg), penicillin G (25 µg), bacitracin (10 µg), erythromycin (5 µg), kanamycin (30 µg) and streptomycin (25 µg). Acid is produced from D- and L-arabinose, ribose, D- and L-xylose, D-galactose, glucose, D-fructose, mannose, L-sorbose, rhamnose, inositol, N-acetylglucosamine, aesculin, D-cellobiose, maltose, lactose, melibiose, sucrose, glycogen, gentiobiose, D-turanose,

D-lyxose, D-tagatose, D- and L-fucose and 2- and 5-ketogluconate. Oxidizes D-xylose, D-ribose, α-ketovaleric acid, L-proline and pyruvic acid methyl ester. The DNA G + C content is 55.1 mol%.

The type strain, MP4^T (=CIP 109385^T =NCIMB 14276^T), was isolated from geothermal soil on the north-west slope of Mount Melbourne on the Antarctic continent. Strains MP1, MP2 and MP3, isolated from the same vicinity, are also included in the species.

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References

Albuquerque, L., Rainey, F. A., Chung, A. P., Sunna, A., Nobre, M. F., Grote, R., Antranikian, G. & De Costa, M. S. (2000). *Alicyclobacillus*

- hesperidum* sp. nov. and a related genomic species from solfataric soils of São Miguel in the Azores. *Int J Syst Evol Microbiol* **50**, 451–457.
- Allan, R. N., Lebbe, L., Heyrman, J., De Vos, P., Buchanan, C. J. & Logan, N. A. (2005). *Brevibacillus levickii* sp. nov. and *Aneurinibacillus terranovensis* sp. nov., two novel thermoacidophiles isolated from geothermal soils of northern Victoria Land, Antarctica. *Int J Syst Evol Microbiol* **55**, 1039–1050.
- Bargagli, R., Skotnicki, M. L., Marri, L., Pepi, M., Mackenzie, A. & Agnorelli, C. (2004). New record of moss and thermophilic bacteria species and physico-chemical properties of geothermal soils on the northwest slope of Mt. Melbourne (Antarctica). *Polar Biol* **27**, 423–431.
- Cole, J. R., Chai, B., Marsh, T. L., Farris, R. J., Wang, Q., Kulam, S. A., Chandra, S., McGarrell, D. M., Schmidt, T. M. & other authors (2003). The Ribosomal Database Project (RDP-II): previewing a new autoaligner that allows regular updates and the new prokaryotic taxonomy. *Nucleic Acids Res* **31**, 442–443.
- Deinhard, G., Saar, J., Krischke, W. & Poralla, K. (1987). *Bacillus cycloheptanicus* sp. nov., a new thermophile containing ω -cycloheptane fatty acids. *Syst Appl Microbiol* **10**, 68–73.
- Goto, K., Matsubara, H., Mochida, K., Matsumura, T., Hara, Y., Niwa, M. & Yamasato, K. (2002). *Alicyclobacillus herbarius* sp. nov., a novel bacterium containing omega-cycloheptane fatty acids, isolated from herbal tea. *Int J Syst Evol Microbiol* **52**, 109–113.
- Goto, K., Mochida, K., Asahara, M., Suzuki, M., Kasai, H. & Yokota, A. (2003). *Alicyclobacillus pomorum* sp. nov., a novel thermo-acidophilic, endospore-forming bacterium that does not possess ω -alicyclic fatty acids, and emended description of the genus *Alicyclobacillus*. *Int J Syst Evol Microbiol* **53**, 1537–1544.
- Goto, K., Mochida, K., Kato, Y., Asahara, M., Fujita, R., An, S. Y., Kasai, H. & Yokota, A. (2007). Proposal of six species of moderately thermophilic, acidophilic, endospore-forming bacteria: *Alicyclobacillus contaminans* sp. nov., *Alicyclobacillus fastidiosus* sp. nov., *Alicyclobacillus kakegawensis* sp. nov., *Alicyclobacillus macrosporangiidus* sp. nov., *Alicyclobacillus sacchari* sp. nov. and *Alicyclobacillus shizuokensis* sp. nov. *Int J Syst Evol Microbiol* **57**, 1276–1285.
- Hiraishi, A., Inagaki, K., Tanimoto, Y., Iwasaki, M., Kishimoto, N. & Tanaka, H. (1997). Phylogenetic characterization of a new thermo-acidophilic bacterium isolated from hot springs in Japan. *J Gen Appl Microbiol* **43**, 295–304.
- Kämpfer, P. & Kroppenstedt, R. M. (1996). Numerical analysis of fatty acid patterns of coryneform bacteria and related taxa. *Can J Microbiol* **42**, 989–1005.
- Karavaiko, G. I., Bogdanova, T. I., Tourova, T. P., Kondrat'eva, T. F., Tsaplina, I. A., Egorova, M. A., Krasil'nikova, E. N. & Zakharchuk, L. M. (2005). Reclassification of '*Sulfobacillus thermosulfidooxidans* subsp. *thermotolerans*' strain K1 as *Alicyclobacillus tolerans* sp. nov. and *Sulfobacillus disulfidooxidans* Dufresne et al. 1996 as *Alicyclobacillus disulfidooxidans* comb. nov., and emended description of the genus *Alicyclobacillus*. *Int J Syst Evol Microbiol* **55**, 941–947.
- Logan, N. A., Lebbe, L., Hoste, B., Goris, J., Forsyth, G., Heyndrick, M., Murray, B. L., Syme, N., Wynn-Williams, D. D. & De Vos, P. (2000). Aerobic endospore-forming bacteria from geothermal environments in northern Victoria Land, Antarctica, and Candlemas Island, South Sandwich Archipelago, with the proposal of *Bacillus fumarioli* sp. nov. *Int J Syst Evol Microbiol* **50**, 1741–1753.
- Matsubara, H., Goto, K., Matsumura, T., Mochida, K., Iwaki, M., Niwa, M. & Yamasato, K. (2002). *Alicyclobacillus acidiphilus* sp. nov., a novel thermo-acidophilic, ω -alicyclic fatty acid-containing bacterium isolated from acidic beverages. *Int J Syst Evol Microbiol* **52**, 1681–1685.
- 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.
- Nicolaus, B., Lama, L., Esposito, E., Manca, M. C., Di Prisco, G. & Gambacorta, A. (1996). '*Bacillus thermoantarcticus*' sp. nov., from Mount Melbourne, Antarctica: a novel thermophilic species. *Polar Biol* **16**, 101–104.
- Nicolaus, B., Improt, R., Manca, M. C., Lama, L., Esposito, E. & Gambacorta, A. (1998). Alicyclobacilli from an unexplored geothermal soil in Antarctica: Mount Rittmann. *Polar Biol* **19**, 133–141.
- Poli, A., Esposito, E., Lama, L., Orlando, P., Nicolaus, G., de Appolonia, F., Gambacorta, A. & Nicolaus, B. (2006). *Anoxybacillus amylolyticus* sp. nov., a thermophilic amylase producing bacterium isolated from Mount Rittmann (Antarctica). *Syst Appl Microbiol* **29**, 300–307.
- Powers, E. M. (1995). Efficacy of the Ryu nonstaining KOH technique for rapidly determining gram reactions of food-borne and waterborne bacteria and yeasts. *Appl Environ Microbiol* **61**, 3756–3758.
- Simbahan, J., Drijber, R. & Blum, P. (2004). *Alicyclobacillus vulcanalis* sp. nov., a thermophilic, acidophilic bacterium isolated from Coso Hot Springs, California, USA. *Int J Syst Evol Microbiol* **54**, 1703–1707.
- Smibert, R. M. & Krieg, N. R. (1981). General characterization. In *Manual of Methods for General Bacteriology*, pp. 411–442. Edited by P. Gerhardt, R. G. E. Murray, R. N. Costilow, E. W. Nester, W. A. Wood, N. R. Krieg & G. B. Philips. Washington, DC: American Society for Microbiology.
- 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.
- Tsuruoka, N., Isono, Y., Shida, O., Hemmi, H., Nakayama, T. & Nishino, T. (2003). *Alicyclobacillus sendaiensis* sp. nov., a novel acidophilic, slightly thermophilic species isolated from soil in Sendai, Japan. *Int J Syst Evol Microbiol* **53**, 1081–1084.
- Van de Peer, Y. & De Wachter, R. (1997). Construction of evolutionary distance trees with TREECON for Windows: accounting for variation in nucleotide substitution rate among sites. *Comput Appl Biosci* **13**, 227–230.
- Viti, C. & Giovannetti, L. (2005). Characterization of cultivable heterotrophic bacterial communities in Cr-polluted and unpolluted soils using Biolog and ARDRA approaches. *Appl Soil Ecol* **28**, 101–112.
- Wisotzkey, J. D., Jurtshuk, P., Jr, Fox, G. E., Deinhard, G. & Poralla, K. (1992). Comparative sequence analyses on the 16S rRNA (rDNA) of *Bacillus acidocaldarius*, *Bacillus acidoterrestris*, and *Bacillus cycloheptanicus* and proposal for creation of a new genus, *Alicyclobacillus* gen. nov. *Int J Syst Bacteriol* **42**, 263–269.