Emended descriptions of *Geobacillus thermoleovorans* and *Geobacillus thermocatenulatus*

Anna E. Dinsdale,¹ Gillian Halket,¹ An Coorevits,^{2,3}
Anita Van Landschoot,^{2,3} Hans-Jürgen Busse,⁴ Paul De Vos³
and Niall A. Logan¹

Correspondence

An Coorevits

an.coorevits@hogent.be

¹Department of Biological and Biomedical Sciences, Glasgow Caledonian University, Cowcaddens Road, Glasgow, G4 0BA, UK

²Department of Applied Engineering Sciences, Laboratory of Biochemistry and Brewing, University College Ghent, Schoonmeersstraat 52, B-9000 Ghent, Belgium

³Laboratory of Microbiology, Department of Biochemistry and Microbiology, Ghent University, K.L. Ledeganckstraat 35, B-9000 Ghent, Belgium

⁴Institut für Bakteriologie, Mykologie & Hygiene, Veterinärmedizinische Universität, Veterinärplatz 1, A-1210 Vienna, Austria

Nineteen thermophilic, aerobic, endospore-forming bacterial strains were subjected to 16S rRNA gene sequence analysis. Eight of these strains had been received as cultures of *Geobacillus kaustophilus*, *G. lituanicus*, *G. stearothermophilus*, '*G. thermoleovorans* subsp. *stromboliensis*', *G. vulcani*, '*Bacillus caldolyticus*', '*B. caldotenax*' and '*B. caldovelox*', but they showed close relationships with the type strain of *G. thermoleovorans*, as did two other strains received as *G. thermoleovorans*. All strains underwent further taxonomic analysis by API and other phenotypic tests and fatty acid methyl ester analysis, and selected strains were analysed for their polar lipids and for DNA relatedness. The 11 strains that formed the *G. thermoleovorans* 16S rRNA cluster also showed some phenotypic similarities, and DNA relatedness data support the reassignment of the strains received as *G. kaustophilus*, *G. lituanicus*, '*G. thermoleovorans* subsp. *stromboliensis*', *G. vulcani*, '*B. caldolyticus*', '*B. caldotenax*' and '*B. caldovelox*', and one of the *G. stearothermophilus* strains, as members of the species *G. thermoleovorans*. Four other strains received as *G. kaustophilus* were misnamed; two were identified as *G. stearothermophilus* and two appeared to be closely related to *Anoxybacillus rupiensis*. One strain received as *G. stearothermophilus* remained unidentified. On the basis of a single strain, *Geobacillus thermocatenulatus* was shown to represent a distinct species, but study of the type strain of *Geobacillus gargensis* showed this species to be a later heterotypic synonym of *Geobacillus thermocatenulatus*. Emended descriptions of *Geobacillus thermoleovorans* and *Geobacillus thermocatenulatus* are therefore presented.

Following the discovery of novel thermophilic, aerobic endospore-formers in petroleum reservoirs, Nazina *et al.* (2001) proposed that the six species belonging to 16S rRNA group 5 of Ash *et al.* (1991) should be accommodated in a

new genus, *Geobacillus*, along with two novel species, *Geobacillus subterraneus* and *G. uzenensis*. *Geobacillus* thus contained eight species: *Geobacillus stearothermophilus* (the type species), *G. kaustophilus*, *G. subterraneus*, *G. thermocatenulatus*, *G. thermodenitrificans*, *G. thermoglucosidasius*, *G. thermoleovorans* and *G. uzenensis*. For many years, the taxonomic positions of *G. kaustophilus* and *G. thermocatenulatus*, along with those of '*Bacillus caldolyticus*', '*B. caldotenax*' and '*B. caldovelox*', have been unclear. Metabolic studies and phage typing by Sharp *et al.* (1980) also revealed close relationships between these species. White *et al.* (1993) considered whether '*B. caldolyticus*', '*B. caldotenax*' and '*B. caldovelox*' (Heinen & Heinen, 1972)

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of *Geobacillus* strains under study are FN428637, FN428638, FN428642, FN428645, FN428646, FN428671, FN428672, FN428674, FN428675, FN428683, FN428684, FN428686, FN428693–FN428696, FN538989, AM988775 and AM988776, as detailed in Table 1.

Three supplementary figures are available with the online version of this paper.

were synonymous, and they recommended the revival of '*B. caldotenax*', but their DNA relatedness data were inconclusive. They also proposed an emended description of *Bacillus kaustophilus*. The 16S rRNA gene sequence studies of Rainey *et al.* (1994) showed that *B. kaustophilus*, *B. thermoleovorans*, '*B. caldolyticus*', '*B. caldotenax*' and '*B. caldovelox*' were closely related and that this group was related to *Bacillus thermocatenulatus*. Sunna *et al.* (1997) proposed the merger of all these species on the basis of 72–88 % DNA relatedness between representative strains. Nazina *et al.* (2004) did not support such a merger, however, as they found only 47–54 % DNA relatedness between strains of *G. kaustophilus*, *G. thermoleovorans* and *G. thermocatenulatus*. Nazina *et al.* (2004) also proposed the novel species *Geobacillus gargensis*, and the transfer of *Bacillus vulcani* (Caccamo *et al.*, 2000) to *Geobacillus* on the basis of 99.4 % 16S rRNA gene sequence similarity and 55 % DNA relatedness with the type strain of *G. kaustophilus*. Other taxa showing high 16S rRNA gene sequence similarities with *G. kaustophilus*, *G. thermocatenulatus* and *G. thermoleovorans* are *Geobacillus lituanicus* (Kuisiense *et al.*, 2004), '*G. thermoleovorans* subsp. *stromboliensis*' (Romano *et al.*, 2005) and '*G. zalihae*' (Abd Rahman *et al.*, 2007). The present study subjected strains of *G. gargensis*, *G. kaustophilus*, *G. lituanicus*, *G. stearothermophilus*, *G. thermocatenulatus*, *G. thermoleovorans*, '*G. thermoleovorans* subsp. *stromboliensis*', *G. vulcani*, '*B. caldolyticus*', '*B. caldotenax*' and '*B. caldovelox*' to a polyphasic taxonomic study, in order to resolve the taxonomic confusion.

Bacterial strains

The strains included in the study are listed in Table 1. Strains were grown on trypticase soy agar (TSA; Oxoid), unless otherwise stated, and incubated at 50 or 60 °C for 18–24 h. An exception was *G. lituanicus* R-35645^T, which was grown on nutrient agar. Both media were supplemented with 5 mg MnSO₄ l⁻¹ to encourage sporulation, and sporulated cultures were maintained at 3–5 °C.

Phenotypic characterization

Colonial characters were recorded, and cellular and sporangial morphologies were observed by phase-contrast microscopy. Gram reaction, temperature and pH ranges, NaCl tolerance, production of catalase and oxidase and starch and casein hydrolysis were tested by the methods of Logan & De Vos (2009). Characterization tests for each strain were performed in duplicate and repeated if the results were ambiguous. Species represented by single strains were tested in triplicate. Biochemical characters were tested following the methods of Logan & Berkeley (1984) and Allan *et al.* (2005), and data were subjected to numerical taxonomic analysis using BioNumerics 5.1 software (Applied Maths) using Gower's general similarity coefficient (*S_G*; Gower, 1971) and UPGMA cluster analysis. Test error was calculated from mean similarities of several duplicated strains and the probability of error (*P*) for an individual test was 0.25 %.

Fatty acid methyl ester (FAME) analysis

For FAME analysis, the method of Vauterin *et al.* (1991) was followed, and profiles were identified using the Microbial Identification System software version 3.8. Cells were grown on TSA at 52 °C for 24 h as recommended in the Microbial Identification System instructions (Microbiol ID).

Polar lipids

Polar lipids of type and reference strains were extracted and separated by using two-dimensional TLC according to Tindall (1990a, b). Total lipids were visualized by spraying with molybdotatphosphoric acid and further characterized by spraying with ninhydrin (specific for amino groups), molybdenum blue (specific for phosphates) and α -naphthol (specific for sugars).

16S rRNA gene sequencing

Partial 16S rRNA gene sequences were generated as described by Heyrman & Swings (2001). Sequencing products were purified with the Big Dye X Terminator Purification kit (Applied Biosystems) according to the manufacturer's instructions, using sequential pipetting and a MixMate (Eppendorf) shaking device. Sequence analysis was performed using a 3100 DNA Sequencer (Applied Biosystems) according to the manufacturer's guidelines. Sequences were assembled and compared using BioNumerics 5.1 software. Phylogenetic trees were constructed using the maximum-likelihood, maximum-parsimony and neighbour-joining methods, based on these partial 16S rRNA gene sequences, together with publicly available sequences of type strains within the genus *Geobacillus*. Alignment and clustering of sequences was performed using MEGA4 (Tamura *et al.*, 2007) and PhyML (Guindon & Gascuel, 2003). The maximum-likelihood tree is represented in Fig. 1, and is supported by the maximum-parsimony and neighbour-joining trees (Supplementary Figs S1 and S2, available in IJSEM Online).

DNA–DNA relatedness and DNA G+C content

DNA was prepared and purified as described by Logan *et al.* (2000). Hybridizations were performed using a modification (Willems *et al.*, 2001) of the microplate method described by Ezaki *et al.* (1989) with a reassociation temperature of 37 °C. The G+C contents of the DNAs were determined by HPLC (Mesbah *et al.*, 1989), using further specifications given by Logan *et al.* (2000).

Our findings supported earlier proposals for merging the species *G. kaustophilus*, *G. thermoleovorans*, '*B. caldolyticus*', '*B. caldotenax*' and '*B. caldovelox*'. The 16S rRNA gene sequences of the type strains *G. kaustophilus* R-35638^T, *G. lituanicus* R-35645^T and *G. thermoleovorans* R-35636^T and of '*G. thermoleovorans* subsp. *stromboliensis*' R-35650 clustered at 99.9 % *S_p* (pairwise similarity). '*B. caldolyticus*'

Table 1. Overview of strains included in this study

Culture collections: ATCC, American Type Culture Collection, Manassas, VA, USA; Atkinson, A. Atkinson, Centre for Applied Microbiology and Research (CAMR), Porton Down, UK; B, *Bacillus* Collection at Glasgow Caledonian University, Glasgow, UK; CIP, Collection de l'Institut Pasteur, Paris, France; DSM(Z), Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany; Kuisiene, N. Kuisiene, Vilnius University, Lithuania; LMG, Belgian Coordinated Collections of Microorganisms, Bacteria Collection, Laboratorium voor Microbiologie, Universiteit Gent, Belgium; LUDA, Leeds University Department of Agriculture, Leeds, UK; NCA, National Canners Association, Berkeley, CA, USA; Sharp, R. J. Sharp, CAMR, Porton Down, UK; R, Research Collection of the Laboratory of Microbiology, Universiteit Gent, Ghent, Belgium; VKM, All-Russian Collection of Microorganisms, Institute of Biochemistry and Physiology of Microorganisms, Russian Academy of Sciences, Pushchino, Russia.

Name as received	Identity based on this study	Subculture used	Other designations	Source	16S rRNA gene sequence accession no.
' <i>B. caldolyticus</i> '	<i>G. thermoleovorans</i>	R-32508	LMG 17975, DSM 405, B0211, Heinen YT-P	Superheated pool water, USA; Atkinson	FN428646
' <i>B. caldotenax</i> '	<i>G. thermoleovorans</i>	R-32507	LMG 17974, DSM 406, B0212, Heinen YT-G	Superheated pool water, USA; Atkinson	FN428645
' <i>B. caldovelox</i> '	<i>G. thermoleovorans</i>	R-32499	LMG 14463, DSM 411, B0213, Heinen YT-F	Superheated pool water, USA; Atkinson	FN428638
<i>G. gargensis</i>	<i>G. thermocatenulatus</i>	R-35649 ^T	DSM 15378 ^T , LMG 23385 ^T , VKM B-2300 ^T , B3134 ^T	Garga hot spring, Eastern Siberia, Russia; DSMZ	FN428695
<i>G. kaustophilus</i>	<i>G. thermoleovorans</i>	R-35638 ^T	LMG 9819 ^T , DSM 7263 ^T , ATCC 8005 ^T , B3061 ^T	Pasteurized milk; DSMZ	FN428686
<i>G. kaustophilus</i>	<i>G. stearothermophilus</i>	R-32635	B1552, Sharp 10007	Soil from Wales, UK; Sharp	FN428672
<i>G. kaustophilus</i>	<i>G. stearothermophilus</i>	R-32504	B1555, LUDA T28	Wolf	FN428642
<i>G. kaustophilus</i>	<i>Anoxybacillus rupiensis</i>	R-32636	B2598, Sharp 10332	Near Lake Taupo, New Zealand; Sharp	AM988775
<i>G. kaustophilus</i>	<i>Anoxybacillus rupiensis</i>	R-32637	B2599, Sharp 10315	Near Lake Taupo, New Zealand; Sharp	AM988776
<i>G. lituanicus</i>	<i>G. thermoleovorans</i>	R-35645 ^T	DSM 15325 ^T ; LMG 23033 ^T , VKM B-2294 ^T , B3094 ^T	Lithuanian oilfield; Kuisiene	FN428693
<i>G. stearothermophilus</i>		R-35646 ^T	LMG 6939 ^T ; DSM 22 ^T ; ATCC 12980 ^T , B3117 ^T	Deteriorated canned food; DSMZ	FN428694
<i>G. stearothermophilus</i>	<i>G. thermoleovorans</i>	R-32642	DSM 3299, ATCC 15952, B3122, NCA 5520	DSMZ	FN428674
<i>G. stearothermophilus</i>	<i>Geobacillus</i> sp.	R-32643	DSM 13240, CIP 106956, B3124	Hot springs, Yellowstone National Park, USA; DSMZ	FN428675
<i>G. thermocatenulatus</i>		R-35648 ^T	DSM 730 ^T , LMG 19007 ^T , VKM B-1259 ^T , B3129 ^T	Coating inside tube of hot-gas well, Southern Urals, USSR; DSMZ	FN538989
<i>G. thermoleovorans</i>		R-35636 ^T	LMG 9823 ^T , DSM 5366 ^T , ATCC 43513 ^T , B3059 ^T	Soil near hot water effluent, Bethlehem, USA; DSMZ	FN428684
<i>G. thermoleovorans</i>		R-32630	DSM 11667, B3131, Sunna HSR	Soil, Jordan; DSMZ	FN428671
<i>G. thermoleovorans</i>		R-32498	DSM 11668, B3132, Antranikian K-3d	Hot spring, Japan; DSMZ	FN428637
' <i>G. thermoleovorans</i> subsp. <i>stromboliensis</i> '	<i>G. thermoleovorans</i>	R-35650	DSM 15392, ATCC BAA-979, B3135, Pizzo	Soil close to crater of Stromboli, Italy; DSMZ	FN428696
<i>G. vulcani</i>	<i>G. thermoleovorans</i>	R-35635 ^T	DSM 13174 ^T , LMG 19852 ^T , CIP 106305 ^T , B3058 ^T , Caccamo 3S-1 ^T	Sediment, Vulcano-La Roya marine thermal vent, Eolian Islands, Italy; DSMZ	FN428683

R-32508, '*B. caldotenax*' R-32507 and '*B. caldovelox*' R-32499 clustered together at 99.8 % S_p and joined the *G. thermoleovorans* cluster at 99.8 % S_p (Fig. 1), with *G. vulcani* R-35635^T joining the cluster at 99.7 % S_p and R-32642 (received as *G. stearothermophilus*) joining at 99.6 % S_p . Reference strains for species within the group showed over 71.5 % DNA relatedness: values for hybridization with

G. thermoleovorans R-35636^T for the type strains of *G. kaustophilus*, *G. lituanicus* and *G. vulcani* and for strains of '*B. caldolyticus*', '*B. caldotenax*', '*B. caldovelox*' and '*G. thermoleovorans* subsp. *stromboliensis*' were 82.4, 81.0, 79.4, 80.7, 75.8, 71.5 and 76.6 %, respectively. These DNA relatedness data indicate that *Geobacillus kaustophilus* (Priest *et al.* 1989) Nazina *et al.* 2001, *Geobacillus lituanicus*

Fig. 1. Maximum-likelihood tree based on partial 16S rRNA gene sequences (1363 bp) of 19 *Bacillus* and *Geobacillus* strains. The 16S rRNA gene sequence of *Bacillus aeolius* 4-1^T (GenBank accession no. AJ504797) was selected as an outgroup to root the tree. Alignment and clustering of sequences was performed using MEGA4 and PhyML. aLRT values are shown at branch points. Accession numbers are given in parentheses. All strains within the tree are represented by their names as received. The type strains, as studied, of *G. stearothermophilus*, *G. thermocatenulatus* and *G. thermoleovorans* are indicated in bold. Bar, 0.01 substitutions per nucleotide position.

Kuisiene *et al.* 2004 and *Geobacillus vulcani* (Caccamo *et al.* 2000) Nazina *et al.* 2004 are later heterotypic synonyms of *Geobacillus thermoleovorans* (Zarilla and Perry 1988) Nazina *et al.* 2001, and that '*B. caldolyticus*', '*B. caldovenax*', '*B. caldovelox*' and '*G. thermoleovorans* subsp. *stromboliensis*' are also members of *G. thermoleovorans*. These conclusions are consistent with the phylogenetic data of Nazina *et al.* (2005) and Zeigler (2005).

Only '*B. caldolyticus*' R-32508, '*B. caldovenax*' R-32507 and '*B. caldovelox*' R-32499 showed significant phenotypic similarities, clustering at 90 % S_G (not shown), while joining with *G. thermoleovorans* R-35636^T at less than 85 % S_G . *G. kaustophilus* R-35638^T and '*G. thermoleovorans* subsp. *stromboliensis*' R-35650 showed rather few phenotypic similarities with the *G. thermoleovorans* type strain. The considerable phenotypic diversity between these organisms makes a practically useful description difficult to write; indeed, Sunna *et al.* (1997) said that an emended *Bacillus thermoleovorans* would be 'characterized by its phenotypic heterogeneity and its genotypic homogeneity'. Further work, with more isolates, might allow biotypes to be discerned within the species.

Polar lipid data were analysed for the type strains *G. kaustophilus* R-35638^T, *G. lituanicus* R-35645^T, *G. stearothermophilus* R-35646^T, *G. thermoleovorans* R-35636^T and *G. vulcani* R-35635^T and for '*B. caldolyticus*' R-32508, '*B. caldovenax*' R-32507 and '*B. caldovelox*' R-32499. Polar lipid profiles were very similar for all strains, and displayed diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, an unknown aminophosphoglycolipid and some unknown aminolipids, a phosphoaminolipid, probably phosphatidylserine, and a lipid in minor amounts. Our polar lipid profiles for *Geobacillus* strains, including that of the type species *G. stearothermophilus*,

included phosphatidylglycerol, whereas Miñana-Galbís *et al.* (2010) reported its absence from the type strain of *G. stearothermophilus*. The polar lipid profile of the type strain of *G. thermoleovorans* is shown in Supplementary Fig. S3. The polar lipid profiles of the species of *Geobacillus* here examined are clearly distinguished from those of other endospore-forming taxa that show *meso*-diaminopimelic acid in the peptidoglycan and a quinone system with the predominant compound menaquinone MK-7, such as *Bacillus*, *Paenibacillus*, *Cohnella* (Kämpfer *et al.*, 2006) and *Falsibacillus* (Zhou *et al.*, 2009). *Bacillus subtilis* and *Paenibacillus polymyxa*, the type species of their respective genera, exhibit glycolipids not detectable in *Geobacillus* species but lack the aminophosphoglycolipid found in *Geobacillus*. *Cohnella* species can be distinguished from *Geobacillus* based on the absence of the aminophosphoglycolipid and the presence of two aminophospholipids (PN3 and PN4) and phospholipid PL2 (Kämpfer *et al.*, 2006). Whether the unidentified aminolipid AL2 of *Geobacillus* corresponds to lysyl-phosphatidylglycerol detected in *Cohnella* is still to be examined. In contrast to *Geobacillus*, *Falsibacillus* (Zhou *et al.*, 2009) exhibits two other aminophosphoglycolipids, two phosphoglycolipids and a glycolipid. An emended description of *G. thermoleovorans* is given below, and characters that differentiate the species from other members of the genus are shown in Table 2.

In the study of Abd Rahman *et al.* (2007), the proposed type strain of '*G. zalihae*' clustered with members of the *G. thermoleovorans* group and showed 68.2 and 73.6 % DNA relatedness, respectively, with the type strains of *G. thermoleovorans* and *G. kaustophilus*, so this strain appears to be yet another representative of *G. thermoleovorans*. This was confirmed by a 16S rRNA gene sequence similarity of

Table 2. Differentiation table

Species: 1, *G. stearothermophilus* (n=15); 2, *G. caldoproteolyticus* (n=1); 3, *G. caldoxylosilyticus* (n=1); 4, *G. debilis* (n=1); 5, *G. jurassicus* (n=2); 6, *G. subterraneus* (n=1); 7, *G. tepidamans* (n=1); 8, *G. thermocatenulatus* (n=2); 9, *G. thermodenitrificans* (n=9); 10, *G. thermoglucosidasius* (n=8); 11, *G. thermoleovorans* (n=11); 12, *G. toebii* (n=6); 13, *G. uzenensis* (n=2). Data for *G. uzenensis* were taken from Logan *et al.* (2009); all other data were obtained in the course of the present study. +, >85 % strains positive; –, 0–15 % strains positive; +/w, positive or weakly positive; w, weak reaction; w/–, weak or negative; d, different strains give different reactions; d (w), different strains give different reactions, but positive reactions are weak; v, result varies within strains; ND, no data available.

Character	1	2	3	4	5	6	7	8	9	10	11	12	13
Sporangia													
Cylindrical spores	–	+	v	+	–	–	–	d	–	d	–	–	–
Sporangia swollen	v	–	+	+	v	–	+	–	–	d	–	+	d
Spores subterminal	+	+	–	–	+	+	–	d	+	+	+	–	–
Spores terminal	+	+	+	+	–	v	+	+	+	+	+	+	+
Spores central/paracentral	–	–	+	–	–	–	–	d	–	–	–	–	–
Hydrolysis of:													
Aesculin	+/w	+	+	+	+	+	+	+	+	+	+	–	+
Casein	+/w	+	v	v	+	–	–	–	–	–	d	–	–
Gelatin	+	+	+	+	+	+	–	+	d	+	+	+	+
ONPG	–	–	–	+	–	–	+	–	–	–	d	–	–
Starch	+	+/w	+	–	+	+	+	–	+/w	d (w)	+	+/w	+
Catalase	+	+	+	–	–	+	+	+	+	+	+	+	ND
Oxidase	–	+	+	+	+	–	–	+	–	w	+	+	ND
Nitrate reduction	d	–	+	–	+	+	–	d	d	d	+	+	+
Voges–Proskauer reaction	+	+	–	–	–	–	–	+	d	d	d (w)	+	–
Acid from:													
N-Acetylglucosamine	–	+	–	+	–	–	+	d	–	+	d	–	–
Amygdalin	–	–	+	+	–	–	+	+	–	d	–	–	–
L-Arabinose	–	–	+	–	+	–	+	–	d (w)	d	–	–	+
Arbutin	–	v	+	w	–	–	+	+	–	d	–	–	w
Cellobiose	–	+	+	+	+	+	+	+	+	+	d	–	+
Galactose	w	+	+	+	+	+	+	+	–	–	d	+	+
Gentiobiose	–	–	+	+	–	–	+	–	–	d	–	–	–
Glycerol	w	+	w	–	+	+	+	+	–	w	+	–	+
Glycogen	+	+	+	–	–	+	–	–	–	–	d	–	+
Lactose	–	–	+	+	–	–	+	–	–	w	d (w)	–	–
myo-Inositol	–	–	–	–	–	+	+	–	–	–	d	–	–
Mannitol	–	+	–	–	+	+	–	+	w	+	d	w	+
Melezitose	+	–	–	–	+	+	+	+	d (w)	–	d (w)	–	–
Melibiose	+	–	+	–	–	–	+	+	d	–	d	d	–
Methyl D-glucoside	+	–	w	–	+	–	+	d	–	+	d	–	+
Raffinose	+	–	+	–	–	–	+	+	–	–	d	–	–
Ribose	–	+	+	–	+	–	w	+	w	+	+	d	–
Salicin	–	+	+	+	–	+	+	+	d	+	w	–	+
Sucrose	+	+	+	–	+	+	+	+	d	+	d	d	+
Trehalose	+	+	+	ND	+	+	+	+	d	+	d	+	+
Turanose	+	w	+	w	+	–	+	–	–	+	–	–	–
D-Xylose	–	+	+	w	+	+	+	–	w	+	d	–	–
Anaerobic growth	w/–	–	+/w	–	w	+	+	w	+	+	w	+	+
Growth at/in:													
pH 5	–	+	+	–	+	–	–	+	–	–	w	–	–
pH 9	+	+	+	+	+	–	+	+	+	w	w	+	–
1 % NaCl (w/v)	+	–	+	–	+	+	+	+	–	–	d	+	+
5 % NaCl (w/v)	–	–	–	–	+	–	–	+	–	–	–	–	–
37 °C	w	+	–	–	–	+	–	+	–	–	d	–	–
70 °C	d	+	+	+	+	+	–	+	+	–	d	+	–
Optimum temperature (°C)	40–60	60	50–65	>60	60	55–60	55	60	50	50	60	60	55–60

99.5 % between '*G. zalihae*' T1 and *G. thermoleovorans* R-35636^T (Fig. 1).

B. thermocatenulatus was described by Golovacheva *et al.* (1975), but later was classed as *species incertae sedis* by Claus & Berkeley (1986). Nazina *et al.* (2001) transferred it to *Geobacillus* on the basis of 16S rRNA gene sequence and DNA relatedness data. Subsequently, Nazina *et al.* (2004) proposed *G. gargensis* to accommodate a novel isolate from a hot spring. They found 99.0 % 16S rRNA gene sequence similarity between the type strains of *G. thermocatenulatus* and *G. gargensis*, similar FAME profiles and many shared phenotypic characteristics; however, a DNA relatedness value of 43 % for the two type strains supported their proposal of a novel species. In the present work, *G. gargensis* R-35649^T and *G. thermocatenulatus* R-35648^T clustered at 94.5 % S_G on the basis of phenotypic characters and at 98.9 % S_p on the basis of 16S rRNA gene sequence analysis (Fig. 1) and showed 85.6 % DNA relatedness. The phylogenetic tree (Fig. 1) shows slight differences between the 16S rRNA gene sequences of *G. gargensis* strains Ga^T (GenBank accession no. AY193888) and R-35649^T (FN428695); however, close examination of the sequence represented by AY193888 reveals a long stretch of undefined bases (positions 874–907), whereas these positions have been determined in sequence FN428695. We therefore conclude that *Geobacillus gargensis* Nazina *et al.* 2004 is a later heterotypic synonym of *Geobacillus thermocatenulatus* (Golovacheva *et al.* 1991) Nazina *et al.* 2001, and an emended description is given below; characters that differentiate the species from other members of the genus are shown in Table 2.

Strain R-32643, received as *G. stearothermophilus*, showed 98.9 and 99.2 % S_p in 16S rRNA gene sequence, respectively, with the type strains of *G. stearothermophilus* and *G. thermoleovorans*. Despite these high levels of similarity, the strain could not be identified, since its position in the phylogenetic tree indicates a clear separation between the *G. thermoleovorans* group and strain R-32643 (Fig. 1). Four cultures received as *G. kaustophilus* were believed to be misnamed. Strains R-32635 and R-32504 showed 98.7 and 99.2 % S_p 16S rRNA gene sequence similarity, respectively, with the type strain of *G. stearothermophilus* (Fig. 1). DNA relatedness between R-32635 and R-32504 was 67.1 %, and the DNA–DNA relatedness with *G. stearothermophilus* R-35646^T was 72.4 and 66.4 %, respectively. Thus R-32504 is clearly a member of *G. stearothermophilus*, while R-32635 lies at the margin of this species. Strains R-32636 and R-32637, both isolated from the Lake Taupo volcanic region in New Zealand and received as *G. kaustophilus*, showed high phenotypic (96 % S_G) and genotypic (99.9 % S_p) similarity to each other, but were distinct from all the other taxa studied; however, R-32637 showed 99.2 % S_p with the type strain of *Anoxybacillus rupiensis* (Derekova *et al.*, 2007) in 16S rRNA gene sequence analysis, so these Lake Taupo isolates probably belong to *A. rupiensis*.

Emended description of *Geobacillus thermoleovorans* (Zarilla and Perry 1988) Nazina *et al.* 2001

Basonym: *Bacillus thermoleovorans* Zarilla and Perry 1988. Effective publication: Zarilla & Perry (1987).

Geobacillus thermoleovorans [ther.mo'le.o.vo'rans. Gr. n. *therme* heat; L. n. *oleum* oil; L. v. *vorare* to devour; N.L. pres. part. *thermoleovorans* indicating heat-requiring bacteria capable of utilizing oil (hydrocarbons)].

Gram-positive, rod-shaped cells, 0.7–1.5 × 2–6 µm. Some strains are peritrichously motile. Ellipsoidal endospores are borne terminally and subterminally and do not swell the sporangia. Facultatively anaerobic, but growth is weak in the absence of oxygen. Colonies are circular, creamy white in colour, with smooth and glossy or rough surfaces, entire or irregular margins, 0.5–5 mm in diameter, following incubation at 60 °C for 18–24 h. Grows between 37–40 °C and 65–70 °C, with optimum growth at 60 °C; no growth at 30 or 80 °C. Grows sparsely at pH 5 and 9; optimum growth at pH 6–7. Tolerates 0.5–1 % NaCl. Catalase and oxidase are positive. Aesculin and starch are hydrolysed. Casein hydrolysis is variable. In the API 20E strip, gelatin is hydrolysed and nitrate is reduced; the ONPG test is variable, the Voges–Proskauer test is variable and weak when positive and citrate utilization (Simmons'), arginine dihydrolase, hydrogen sulfide production, indole production, lysine decarboxylase, ornithine decarboxylase, tryptophan deaminase and urease production are negative. In the API 50CHB gallery, acid without gas is produced from D-fructose, D-glucose, glycerol, maltose, D-mannose and ribose. Acid is produced weakly from salicin and acid production is variable from cellobiose, galactose, glycogen, mannitol, melezitose (weak when positive), melibiose, myo-inositol, lactose (weak when positive), methyl D-glucoside, N-acetylglucosamine, raffinose, starch, sucrose, trehalose and D-xylose. No acid is produced from adonitol, amygdalin, D- or L-arabinose, D- or L-arabitol, arbutin, dulcitol, erythritol, D- or L-fucose, gentiobiose, gluconate, inulin, 2- or 5-keto-D-gluconate, D-lyxose, methyl D-mannoside, methyl xyloside, rhamnose, sorbitol, L-sorbose, D-tagatose, turanose, xylitol or L-xylose. The following carbohydrates are assimilated: cellobiose, D-fructose, D-galactose, gentiobiose, D-glucose, lactose, maltitol, maltose, maltotriose, mannitol, D-mannose, melezitose, melibiose, 1-O-methyl β-D-glucopyranoside, palatinose, D-ribose, sucrose, trehalose and turanose; assimilation is variable from aesculin, glycerol, myo-inositol, lactulose, D-lyxose, 1-O- and 3-O-methyl α-D-glucopyranoside, 1-O-methyl α-galactopyranoside (weak when positive), 1-O-methyl β-glucopyranoside, raffinose and D-xylose and negative for adonitol, L-arabinose, D- and L-arabitol, dulcitol, erythritol, L-fucose, L-rhamnose, D-sorbitol, L-sorbose, D-tagatose and xylitol. The following organic acids are assimilated: N-acetylglucosamine, fumarate, D-gluconate, DL-glycerate, DL-β-hydroxybutyrate (3-hydroxybutyrate), L-malate and succinate; assimilation is variable for 2-keto-D-gluconate

(weak when positive), DL-lactate, D-malate (weak when positive), phenylacetate and quinate and negative for *cis*- and *trans*-aconitate, 4-aminobutyrate, 4-aminovalerate, benzoate, caprate, caprylate, citrate, *m*-coumarate, D-galacturonate, gentisate, D-glucuronate, glutarate, 3- and 4-hydroxybenzoate, itaconate, α -ketoglutarate, 5-keto-D-gluconate, malonate, mucate, 3-phenylpropionate, propionate, protocatechuate, D-saccharate, D-, L- and *meso*-tartrate and tricarballoylate. The following amino acids are assimilated: D-glucosamine, L-glutamate and L-histidine; assimilation is variable for D- and L-alanine, histamine (weak when positive), L-proline (weak when positive) and L-serine and negative for L-aspartate, betaine, ethanolamine, putrescine, trigonelline, tryptamine, L-tryptophan and L-tyrosine. In the variable reactions listed above, the type strain displays the following reactions. Positive for hydrolysis of casein, negative for ONPG and positive for the Voges–Proskauer reaction. Acid is produced from galactose, glycogen, mannitol, melezitose (weakly), melibiose, *myo*-inositol, raffinose, starch, sucrose, trehalose and D-xylose; acid is not produced from cellobiose, lactose, methyl D-glucoside or *N*-acetylglucosamine. Positive for assimilation of aesculin, glycerol, *myo*-inositol, lactulose (weak), 1-*O*-methyl β -glucopyranoside, raffinose, D-xylose, DL-lactate, phenylacetate, quinate, D- and L-alanine, L-proline and L-serine and negative for assimilation of D-lyxose, 1-*O*- and 3-*O*-methyl α -D-glucopyranoside, 1-*O*-methyl α -galactopyranoside, 2-keto-D-gluconate, D-malate and histamine. The major cellular fatty acids are iso-C_{15:0}, iso-C_{16:0} and iso-C_{17:0}. Strains have been isolated from pasteurized milk, geothermally heated pools and springs and associated soil, marine sediment of a hydrothermal vent and crude oil. The DNA G+C content is 52–53 mol% (52.4 mol% for the type strain; HPLC). The EMBL/GenBank/DBJ accession number for the 16S rRNA gene sequence of the type strain (DSM 5366^T) is Z26923.

Type strain: LEH-1^T (=LMG 9823^T =ATCC 43513^T =DSM 5366^T).

Emended description of *Geobacillus thermocatenulatus* (Golovacheva *et al.* 1991) Nazina *et al.* 2001

Basonym: *Bacillus thermocatenulatus* Golovacheva *et al.* 1991. Effective publication: Golovacheva *et al.* (1975).

Geobacillus thermocatenulatus (ther.mo.ca.te'nu.la.tus. Gr. n. *therme* heat; N.L. adj. *catenulatus* chain-like; N.L. masc. adj. *thermocatenulatus* thermophilic and chain-like, referring to two of the organism's features).

Gram-positive, peritrichously motile rods, 0.9–1.5 × 6–12 µm. The description is based on two strains. Facultatively anaerobic, but growth is weak in the absence of oxygen. Cells occur singly or in long chains. Ellipsoidal or cylindrical spores are located terminally, occasionally subterminally and paracentrally, and do not swell the sporangia. Colonies on TSA are butyrous, cream or

yellowish in colour, round and raised with entire margins; may become partially heaped-up and viscid. Growth can occur at 37 and 80 °C, with optimum growth at 60 °C. Tolerates 5 % but not 7 % NaCl. Grows at pH 5 and 9, and optimally at pH 7. Aesculin is hydrolysed; casein and starch are not hydrolysed. Catalase and oxidase are positive. In the API 20E strip, gelatin is hydrolysed and the Voges–Proskauer test is positive; citrate utilization (Simmons') and nitrate reduction are variable and arginine dihydrolase, hydrogen sulfide, indole, lysine decarboxylase, ornithine decarboxylase, ONPG, tryptophan deaminase and urease production are negative. In the API 50CHB gallery, acid without gas is produced from amygdalin, arbutin, cellobiose, D-fructose, galactose, D-glucose, glycerol, maltose, mannitol, D-mannose, melezitose, melibiose, raffinose, ribose, salicin, starch, sucrose and trehalose. Acid production is variable from methyl D-glucoside and *N*-acetylglucosamine. Acid production is negative from adonitol, D- and L-arabinose, D- and L-arabitol, dulcitol, erythritol, D- and L-fucose, gentiobiose, gluconate, glycogen, inulin, 2- and 5-keto-D-gluconate, lactose, D-lyxose, *myo*-inositol, methyl D-mannoside, methyl xyloside, rhamnose, sorbitol, L-sorbose, D-tagatose, turanose, xylitol and D- and L-xylose. In the variable reactions listed above, the type strain produces acid from methyl D-glucoside and *N*-acetylglucosamine and does not utilize citrate or reduce nitrate. A number of hydrocarbons (C₁₀–C₁₆) may be utilized as carbon and energy sources. The major cellular fatty acids are iso-C_{15:0}, iso-C_{16:0} and iso-C_{17:0}, making up more than 60 % of the total fatty acids (Nazina *et al.*, 2001). The DNA G+C content of the type strain is 51.17 mol% (HPLC). The EMBL/GenBank/DBJ accession number for the 16S rRNA gene sequence of the type strain (BGSC 93^T) is AY608935.

The type strain is strain 178^T (=LMG 19007^T =DSM 730^T =VKM B-1259^T =BGSC 93^T), isolated from a slime layer inside a hot-gas borehole pipe; another strain (Ga =DSM 15378 =VKM B-2300) was isolated from the Garga hot spring in the Transbaikalian region, Russia.

Acknowledgements

The type strain of *G. lituanicus* was kindly donated by Dr N. Kuisienu (Dept of Plant Physiology and Microbiology, Vilnius University, Lithuania). A. E. D. and G. H. gratefully acknowledge the support of bioMérieux, Inc.

References

- Abd Rahman, R. N., Leow, T. C., Salleh, A. B. & Basri, M. (2007). *Geobacillus zalihai* sp. nov., a thermophilic lipolytic bacterium isolated from palm oil mill effluent in Malaysia. *BMC Microbiol* 7, 77.
- 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.

- Ash, C., Farrow, J. A. E., Wallbanks, S. & Collins, M. D. (1991). Phylogenetic heterogeneity of the genus *Bacillus* revealed by comparative analyses of small subunit-ribosomal RNA sequences. *Lett Appl Microbiol* **13**, 202–206.
- Caccamo, D., Gugliandolo, C., Stackebrandt, E. & Maugeri, T. L. (2000). *Bacillus vulcani* sp. nov., a novel thermophilic species isolated from a shallow marine hydrothermal vent. *Int J Syst Evol Microbiol* **50**, 2009–2012.
- Claus, D. & Berkeley, R. C. W. (1986). Genus *Bacillus* Cohn 1872. In *Bergey's Manual of Systematic Bacteriology*, vol. 2, pp. 1105–1139. Edited by P. H. A. Sneath, N. S. Mair, M. E. Sharpe & J. G. Holt. Baltimore: Williams & Wilkins.
- Derekova, A., Sjöholm, C., Mandeve, R. & Kambourova, M. (2007). *Anoxybacillus rupiensis* sp. nov., a novel thermophilic bacterium isolated from Rupi basin (Bulgaria). *Extremophiles* **11**, 577–583.
- 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.
- Golovacheva, R. S., Loginova, L. G., Salikhov, T. A., Kolesnikov, A. A. & Zaitseva, G. N. (1975). A new thermophilic species, *Bacillus thermocatenulatus* nov. spec. *Microbiology* (English translation of *Mikrobiologiya*) **44**, 230–233.
- Golovacheva, R. S., Loginova, L. G., Salikhov, T. A., Kolesnikov, A. A. & Zaitseva, G. N. (1991). *Bacillus thermocatenulatus* sp. nov. In *Validation of the Publication of New Names and New Combinations Previously Effectively Published Outside the IJSB*, List no. 36. *Int J Syst Bacteriol* **41**, 178–179.
- Gower, J. C. (1971). A general coefficient of similarity and some of its properties. *Biometrics* **27**, 857–874.
- Guindon, S. & Gascuel, O. (2003). A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Syst Biol* **52**, 696–704.
- Heinen, U. J. & Heinen, W. (1972). Characteristics and properties of a caldo-active bacterium producing extracellular enzymes and two related strains. *Arch Mikrobiol* **82**, 1–23.
- Heyrman, J. & Swings, J. (2001). 16S rDNA sequence analysis of bacterial isolates from biodeteriorated mural paintings in the Servilia tomb (Necropolis of Carmona, Seville, Spain). *Syst Appl Microbiol* **24**, 417–422.
- Kämpfer, P., Rosselló-Mora, R., Falsen, E., Busse, H.-J. & Tindall, B. J. (2006). *Cohnella thermotolerans* gen. nov., sp. nov., and classification of '*Paenibacillus hongkongensis*' as *Cohnella hongkongensis* sp. nov. *Int J Syst Evol Microbiol* **56**, 781–786.
- Kuisiene, N., Raugalas, J. & Chitavichius, D. (2004). *Geobacillus lituanicus* sp. nov. *Int J Syst Evol Microbiol* **54**, 1991–1995.
- Logan, N. A. & Berkeley, R. C. W. (1984). Identification of *Bacillus* strains using the API system. *J Gen Microbiol* **130**, 1871–1882.
- Logan, N. A. & De Vos, P. (2009). Genus I. *Bacillus* Cohn 1872, 174^{AL}. In *Bergey's Manual of Systematic Bacteriology*, 2nd edn, vol. 3, pp. 21–128. Edited by P. De Vos, G. M. Garrity, D. Jones, N. R. Krieg, W. Ludwig, F. A. Rainey, K.-H. Schleifer & W. B. Whitman. New York: Springer.
- Logan, N. A., Lebbe, L., Hoste, B., Goris, J., Forsyth, G., Heyndrickx, 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.
- Logan, N. A., De Vos, P. & Dinsdale, A. E. (2009). Genus VII. *Geobacillus* Nazina, Tourova, Poltarau, Novikova, Grigoryan, Ivanova, Lysenko, Petrunka, Osipov, Belyaev and Ivanov 2001, 442^{VP}. In *Bergey's Manual of Systematic Bacteriology*, 2nd edn, vol. 3, pp. 144–160. Edited by P. De Vos, G. M. Garrity, D. Jones, N. R. Krieg, W. Ludwig, F. A. Rainey, K.-H. Schleifer & W. B. Whitman. New York: Springer.
- 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.
- Miñana-Galbis, D., Pinzón, D. L., Lorén, J. G., Manresa, Á. & Oliart-Ros, R. M. (2010). Reclassification of *Geobacillus pallidus* (Scholz et al. 1988) Banat et al. 2004 as *Aeribacillus pallidus* gen. nov., comb. nov. *Int J Syst Evol Microbiol* **60**, 1600–1604.
- Nazina, T. N., Tourova, T. P., Poltarau, A. B., Novikova, E. V., Grigoryan, A. A., Ivanova, A. E., Lysenko, A. M., Petrunka, V. V., Osipov, G. A. & other authors (2001). Taxonomic study of aerobic thermophilic bacilli: descriptions of *Geobacillus subterraneus* gen. nov., sp. nov. and *Geobacillus uzensis* sp. nov. from petroleum reservoirs and transfer of *Bacillus stearothermophilus*, *Bacillus thermocatenulatus*, *Bacillus thermoleovorans*, *Bacillus kaustophilus*, *Bacillus thermoglucosidasius* and *Bacillus thermodenitrificans* to *Geobacillus* as the new combinations *G. stearothermophilus*, *G. thermocatenulatus*, *G. thermoleovorans*, *G. kaustophilus*, *G. thermoglucosidasius* and *G. thermodenitrificans*. *Int J Syst Evol Microbiol* **51**, 433–446.
- Nazina, T. N., Lebedeva, E. V., Poltarau, A. B., Tourova, T. P., Grigoryan, A. A., Sokolova, D. Sh., Lysenko, A. M. & Osipov, G. A. (2004). *Geobacillus gargensis* sp. nov., a novel thermophile from a hot spring, and the reclassification of *Bacillus vulcani* as *Geobacillus vulcani* comb. nov. *Int J Syst Evol Microbiol* **54**, 2019–2024.
- Nazina, T. N., Sokolova, D. Sh., Grigoryan, A. A., Shestakova, N. M., Mikhailova, E. M., Poltarau, A. B., Tourova, T. P., Lysenko, A. M., Osipov, G. A. & Belyaev, S. S. (2005). *Geobacillus jurassicus* sp. nov., a new thermophilic bacterium isolated from a high-temperature petroleum reservoir, and the validation of the *Geobacillus* species. *Syst Appl Microbiol* **28**, 43–53.
- Priest, F. G., Goodfellow, M. & Todd, C. (1989). *Bacillus kaustophilus* sp. nov. In *Validation of the Publication of New Names and New Combinations Previously Effectively Published Outside the IJSB*, List no. 28. *Int J Syst Bacteriol* **39**, 93–94.
- Rainey, F. A., Fritze, D. & Stackebrandt, E. (1994). The phylogenetic diversity of thermophilic members of the genus *Bacillus* as revealed by 16S rDNA analysis. *FEMS Microbiol Lett* **115**, 205–211.
- Romano, I., Poli, A., Lama, L., Gambacorta, A. & Nicolaus, B. (2005). *Geobacillus thermoleovorans* subsp. *stromboliensis* subsp. nov., isolated from the geothermal volcanic environment. *J Gen Appl Microbiol* **51**, 183–189.
- Sharp, R. J., Bown, K. J. & Atkinson, A. (1980). Phenotypic and genotypic characterization of some thermophilic species of *Bacillus*. *J Gen Microbiol* **117**, 201–210.
- Sunna, A., Tokajian, S., Burghardt, J., Rainey, F., Antranikian, G. & Hashwa, F. (1997). Identification of *Bacillus kaustophilus*, *Bacillus thermocatenulatus* and *Bacillus* strain HSR as members of *Bacillus thermoleovorans*. *Syst Appl Microbiol* **20**, 232–237.
- Tamura, K., Dudley, J., Nei, M. & Kumar, S. (2007). MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. *Mol Biol Evol* **24**, 1596–1599.
- 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.
- Vauterin, L., Yang, P., Hoste, B., Vancanneyt, M., Civerolo, E. L., Swings, J. & Kersters, K. (1991). Differentiation of *Xanthomonas campestris* pv. *citri* strains by sodium dodecyl sulfate-polyacrylamide gel electrophoresis of proteins, fatty acid analysis, and DNA-DNA hybridization. *Int J Syst Bacteriol* **41**, 535–542.

White, D., Sharp, R. J. & Priest, F. G. (1993). A polyphasic taxonomic study of thermophilic bacilli from a wide geographical area. *Antonie van Leeuwenhoek* **64**, 357–386.

Willems, A., Doignon-Bourcier, F., Goris, J., Coopman, R., de Lajudie, P., De Vos, P. & Gillis, M. (2001). DNA–DNA hybridization study of *Bradyrhizobium* strains. *Int J Syst Evol Microbiol* **51**, 1315–1322.

Zarilla, K. A. & Perry, J. J. (1987). *Bacillus thermoleovorans*, sp. nov., a species of obligately thermophilic hydrocarbon utilizing endospore-forming bacteria. *Syst Appl Microbiol* **9**, 258–264.

Zarilla, K. A. & Perry, J. J. (1988). *Bacillus thermoleovorans* sp. nov. In *Validation of the Publication of New Names and New Combinations Previously Effectively Published Outside the IJSB*, List no. 25. *Int J Syst Bacteriol* **38**, 220–222.

Zeigler, D. R. (2005). Application of a *recN* sequence similarity analysis to the identification of species within the bacterial genus *Geobacillus*. *Int J Syst Evol Microbiol* **55**, 1171–1179.

Zhou, Y., Xu, J., Xu, L. & Tindall, B. J. (2009). *Falsibacillus pallidus* to replace the homonym *Bacillus pallidus* Zhou *et al.* 2008. *Int J Syst Evol Microbiol* **59**, 3176–3180.