Taxonomic revision of the genus *Geobacillus*: emendation of *Geobacillus*, *G. stearothermophilus*, *G. jurassicus*, *G. toebii*, *G. thermodenitrificans* and *G. thermoglucosidans* (nom. corrig., formerly '*thermoglucosidasius*'); transfer of *Bacillus thermantarcticus* to the genus as *G. thermantarcticus* comb. nov.; proposal of *Caldibacillus debilis* gen. nov., comb. nov.; transfer of *G. tepidamans* to *Anoxybacillus* as *A. tepidamans* comb. nov.; and proposal of *Anoxybacillus caldiproteolyticus* sp. nov.

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Sixty-two strains of thermophilic aerobic endospore-forming bacteria were subjected to polyphasic taxonomic study including 16S rRNA gene sequence analysis, polar lipid and fatty acid analysis, phenotypic characterization, and DNA–DNA hybridization experiments. Distinct clusters of the species *Geobacillus stearothermophilus*, *Geobacillus thermodenitrificans*, *Geobacillus toebii* and *Geobacillus thermoglucosidasius* were formed, allowing their descriptions to be emended, and the distinctiveness of the poorly represented species *Geobacillus jurassicus*, *Geobacillus subterraneus* and *Geobacillus caldxylosilyticus* was confirmed. It is proposed that the name *Geobacillus thermoglucosidasius* be corrected to *Geobacillus thermoglucosidans* nom. corrig. *Bacillus thermantarcticus* clustered between *Geobacillus* species on the basis of 16S rRNA gene sequence analysis, and its transfer to the genus *Geobacillus* as *Geobacillus thermantarcticus* comb. nov. (type strain LMG 23032^T=DSM 9572^T=strain M1^T=R-35644^T) is proposed. The above-mentioned species, together with *Geobacillus thermoleovorans* and *Geobacillus thermocatenulatus*, form a monophyletic cluster representing the genus *Geobacillus*. The distinctiveness of '*Geobacillus caldoproteolyticus*' was confirmed and it is proposed that it be accommodated, along with *Geobacillus tepidamans*, in the genus *Anoxybacillus* as *Anoxybacillus caldiproteolyticus* sp. nov. (type strain DSM 15730^T=ATCC BAA-818^T=LMG 26209^T=R-35652^T) and *Anoxybacillus tepidamans* comb. nov. (type strain LMG 26208^T=ATCC BAA-942^T=DSM 16325^T=R-35643^T), respectively. The type strain of *Geobacillus debilis* was not

Abbreviations: AL, aminolipid; APGL, aminophosphoglycolipid; DPG, diphosphatidylglycerol; GL, glycolipid; MSC, monophyletic subgeneric cluster; PAL, phosphoaminolipid; PE, phosphatidylethanolamine; PG, phosphatidylglycerol.

The GenBank/EMBL/DBJ accession numbers for the 16S rRNA gene sequences of all strains under study can be found in Table 1.

Two supplementary tables and three supplementary figures are available with the online version of this paper.

closely related to any members of the genera *Anoxybacillus* and *Geobacillus*, and it is proposed that this species be placed in the new genus *Caldibacillus* as *Caldibacillus debilis* gen. nov. comb. nov. The type strain of the type species, *Caldibacillus debilis*, is LMG 23386^T (=DSM 16016^T=NCIMB 13995^T=Tf^T=R-35653^T).

INTRODUCTION

For many years *Bacillus stearothermophilus* (Donk, 1920) was the only obligately thermophilic species of the genus *Bacillus* with a validly published name. After 1980, further thermophilic species were proposed on the basis of phenotypic analyses, and the subsequent application of 16S rRNA gene sequencing to members of the genus *Bacillus* indicated that *B. stearothermophilus*, *Bacillus kaustophilus* and *Bacillus thermoglucosidasius* formed a generic lineage that was distinct from *Bacillus sensu stricto* (Ash *et al.*, 1991). Nazina *et al.* (2001) proposed that the six species of that lineage, namely *B. stearothermophilus*, *B. kaustophilus*, *B. thermoglucosidasius*, *B. thermocatenulatus*, *B. thermoleovorans* and *B. thermodenitrificans*, should be accommodated in a new genus, *Geobacillus*, along with two novel species, *Geobacillus subterraneus* and *Geobacillus uzenensis*. Subsequently, *Bacillus pallidus* (Scholz *et al.*, 1987), *Saccharococcus caldxylosilyticus* (Ahmad *et al.*, 2000) and *Bacillus vulcani* (Caccamo *et al.*, 2000) were transferred to *Geobacillus* (Fortina *et al.*, 2001; Banat *et al.*, 2004; Nazina *et al.*, 2004) and then Miñana-Galbís *et al.* (2010) proposed the further transfer of *Geobacillus pallidus* to the new genus *Aeribacillus*. Subsequently, six further species, *Geobacillus toebii* (Sung *et al.*, 2002), *G. gargensis* (Nazina *et al.*, 2004), *G. debilis* (Banat *et al.*, 2004), *G. lituanicus* (Kuisiene *et al.*, 2004), *G. tepidamans* (Schäffer *et al.*, 2004) and *G. jurassicus* (Nazina *et al.*, 2005) have been proposed and the names '*Geobacillus caldoproteolyticus*' (Chen *et al.*, 2004) and the subspecies '*G. toebii* subsp. *decanicus*' (Poli *et al.*, 2006) await valid publication. The transfer of *Bacillus thermantarcticus* to the genus *Geobacillus*, recommended by Zeigler (2005), awaits formal proposal. In contrast to this expansion of the number of species in *Geobacillus*, Dinsdale *et al.* (2011) showed that *G. kaustophilus*, *G. lituanicus*, *G. vulcani*, and several other strains with invalidly published names, were all synonyms of *G. thermoleovorans* and that *G. gargensis* was a synonym of *G. thermocatenulatus*; these mergers leave 12 members of the genus *Geobacillus* with validly published names. The description of *G. stearothermophilus* – the type species of the genus – that was given by Nazina *et al.* (2001) was largely based on the one given by Claus & Berkeley (1986), who recognized the species to be heterogeneous because, for many years, most thermophilic *Bacillus* isolates were automatically assigned to it. *G. stearothermophilus* thus lacks a modern description based upon a polyphasic taxonomic study. We therefore subjected representatives of members of the genus *Geobacillus* and *B. thermantarcticus* to a polyphasic study in order to resolve their taxonomy and allow their descriptions to be emended where necessary.

METHODS

Bacterial strains. Strains were grown on trypticase soy agar (TSA; Oxoid), unless otherwise stated, and incubated at 50 or 60 °C for 18–24 h. Exceptions were *B. thermantarcticus*, which was grown on half-strength *Bacillus fumarioli* agar (Forsyth & Logan, 2000) or half-strength nutrient agar (NA; Oxoid), and *G. tepidamans*, which was grown on NA. All solid growth media were supplemented with 5 mg MnSO₄ l⁻¹ to encourage sporulation, and sporulated cultures were maintained at 3–5 °C. All strains used in this study are listed in Table 1, and were assigned a Research (R)-number.

Phenotypic characterization. Colonial and microscopic morphologies were observed, and the Gram-reaction, temperature and pH ranges, NaCl tolerance, production of catalase and oxidase, and starch and casein hydrolysis were tested according to Logan & De Vos (2009). Characterization tests for each strain were performed in duplicate and repeated if results were ambiguous. Species represented by single strains were tested in triplicate. Biochemical characters were tested following the methods of Logan & De Vos (2009) and Allan *et al.* (2005), and data were subjected to numerical taxonomic analysis using BioNumerics 4.61 software (Applied Maths, Belgium) using the general similarity coefficient of Gower (*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 found to be 0.25 %.

Chemotaxonomic characterization. For fatty acid methyl ester analysis, the method of Vauterin *et al.* (1991) was followed, including standardization of incubation temperature (52 °C), incubation time (24 h) and physiological age (overlap area of the second and third quadrant from a quadrant streak); profiles were identified and clustered using the Microbial Identification System software and MIDI TSBA database version 5.0. Polar lipids were extracted and separated as described by Dinsdale *et al.* (2011) after growth on TSA (Oxoid) at 52 °C for 24 h.

Genotypic characterization. DNA extraction and 16S rRNA gene sequencing was carried out as described by Coorevits *et al.* (2011). All sequences were aligned using the integrated aligner of ARB (Ludwig *et al.*, 2004) and manual refinement. Sequences were then exported to MEGA5 software (Tamura *et al.*, 2011) to construct a maximum-likelihood tree. Reliability of clusters was assessed by calculating bootstrap values based on 1000 replications. DNA–DNA hybridization experiments and determination of DNA G+C content were performed as described by Dinsdale *et al.* (2011).

RESULTS AND DISCUSSION

On the basis of 16S rRNA gene sequence analysis, the strains studied fell into four distinct clusters, corresponding with four different genera, namely *Geobacillus*, *Anoxybacillus*, *Aeribacillus* and the new genus *Caldibacillus* that is proposed herein (Fig. 1). These clusters are discussed individually below, and some differentiating features for the four genera are listed in Table 2.

Table 1. List of strains included in this study

In the first column, 'R' refers to the Research collection of the Laboratory of Microbiology, Ghent University. Abbreviations: ATCC, American Type Culture Collection, USA; Banat, I. Banat, University of Ulster, UK; CECT, Coleccion Espanola de Cultivos Tipo, Spain; DSM, Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany; LMG, Belgian Co-ordinated Collections of Micro-organisms, Bacteria Collection, Laboratory of Microbiology, Ghent University, Belgium; LUDA, Leeds University Department of Agriculture, UK; Sharp, R. J. Sharp, CAMR, Porton Down, UK; Wolf, J. Wolf, Leeds University, UK.

Subculture used	Name and collection number as received	Identity based on this study (if changed)	Source/reference or comment	16S rRNA gene accession no.
R-32606	<i>Geobacillus stearothermophilus</i> DSM 231		Unknown	FN428658
R-32607	<i>Geobacillus stearothermophilus</i> DSM 2357		UV mutant from NCIMB 10278	FN428659
R-32765	<i>Geobacillus stearothermophilus</i> DSM 458		Sugar beet juice	FN428681
R-32504	<i>Geobacillus stearothermophilus</i> LUDA T28		Wolf	FN428642
R-32635	<i>Geobacillus stearothermophilus</i> Sharp 10007		Soil from Wales, UK	FN428672
R-32640	<i>Geobacillus stearothermophilus</i> DSM 457		Sugar beet juice	FN428673
R-32496	<i>Geobacillus stearothermophilus</i> CECT 43		Duplicate type strain	FN428633
R-32501	<i>Geobacillus stearothermophilus</i> ATCC 10149		Unknown, Iowa	FN428640
R-32605	<i>Geobacillus stearothermophilus</i> Sharp 11182		Unknown	FN428657
R-32604	<i>Geobacillus stearothermophilus</i> LMG 8193		Steam-sterilization control strain	FN428656
R-32513	<i>Geobacillus stearothermophilus</i> Sharp 12026		Unknown	FN428649
R-32603	<i>Geobacillus stearothermophilus</i> Sharp 12001		Sugar beet juice	FN428655
R-32602	<i>Geobacillus stearothermophilus</i> Sharp 12026		Unknown	FN428654
R-32601	<i>Geobacillus stearothermophilus</i> Sharp 11200		Unknown	FN428653
R-32600	<i>Geobacillus stearothermophilus</i> Sharp 12045		Deteriorated canned food	FN428652
R-32502	<i>Geobacillus stearothermophilus</i> LMG 17899		Flat sour spoilage of canned foods	FN428641
R-32512	<i>Geobacillus stearothermophilus</i> Wolf T210		Duplicate type strain	FN428648
R-35646 ^T	<i>Geobacillus stearothermophilus</i> DSM 22 ^T		Deteriorated canned food	FN428694
R-35648 ^T	<i>Geobacillus thermocatenulatus</i> DSM 730 ^T		Coating inside tube of hot gas well, USSR	FN538989
R-35636 ^T	<i>Geobacillus thermoleovorans</i> DSM 5366 ^T		Soil near hot water effluent, USA	FN428684
R-35651 ^T	<i>Geobacillus jurassicus</i> DSM 15726 ^T		Formation water, Dagang oilfield, China	FN428697
R-32597	<i>Geobacillus jurassicus</i> DSM 15727		Formation water, Dagang oilfield, China	FN428651
R-32641 ^T	<i>Geobacillus subterraneus</i> DSM 13552 ^T		Formation water, Liaohe oilfield, China	FN428689
R-35640 ^T	<i>Geobacillus uzenensis</i> DSM 13551 ^{T*}		Formation water, Uzen oilfield, Kazakhstan	FN428688
R-32500	<i>Geobacillus thermodenitrificans</i> Sharp 12643		Ayutthaya, Thailand	FN428639
R-32506	<i>Geobacillus thermodenitrificans</i> Sharp 11033		Atypical phenotype	FN428644
R-32616	<i>Geobacillus thermodenitrificans</i> Sharp 12823		Soil from Iceland	FN428660
R-32617	<i>Geobacillus thermodenitrificans</i> Sharp 11024		Atypical phenotype	FN428661
R-32618	<i>Geobacillus thermodenitrificans</i> Sharp 10097		River Cam, Cambridge, UK	FN428662
R-32511	<i>Geobacillus thermodenitrificans</i> Sharp 10078		Soil, France	FN428647
R-32619	<i>Geobacillus thermodenitrificans</i> Sharp 11025		Atypical phenotype	FN428663
R-32621	<i>Geobacillus thermodenitrificans</i> Sharp 12019		Sugar beet juice, Austria	FN428664
R-32622	<i>Geobacillus thermodenitrificans</i> Sharp 11022		Unknown	FN 428665
R-32623	<i>Geobacillus thermodenitrificans</i> DSM 13147		Soil, Italy	FN428666
R-32624	<i>Geobacillus thermodenitrificans</i> DSM 13148		Soil, Indonesia	FN428667
R-32614	<i>Geobacillus thermodenitrificans</i> Sharp 12628		Deli, China	FN538994
R-32615	<i>Geobacillus thermodenitrificans</i> Sharp 11020		Atypical phenotype	FN538995
R-32625	<i>Geobacillus thermodenitrificans</i> DSM 13149		Soil, Saudi Arabia	FN538990
R-35647 ^T	<i>Geobacillus thermodenitrificans</i> DSM 465 ^T		Sugar beet juice	FN538993
R-35644 ^T	<i>Bacillus thermantarcticus</i> DSM 9572 ^T	<i>Geobacillus thermantarcticus</i>	Mount Melbourne, Antarctica	FN428692
R-32639	<i>Geobacillus stearothermophilus</i> DSM 1550	<i>Geobacillus toebii</i>	Evaporated milk	FN538992
R-32652	<i>Geobacillus thermoglucosidasius</i> Sharp 11056	<i>Geobacillus toebii</i>	Unknown	FN538991
R-32651	<i>Geobacillus thermoglucosidasius</i> Sharp 12670	<i>Geobacillus toebii</i>	Soil, Thailand	FN428679

Table 1. cont.

Subculture used	Name and collection number as received	Identity based on this study (if changed)	Source/reference or comment	16S rRNA gene accession no.
R-32650	<i>Geobacillus thermoglucosidasius</i> Sharp 12633	<i>Geobacillus toebii</i>	Soil, Thailand	FN538988
R-32653	<i>Geobacillus thermoglucosidasius</i> Sharp 12668	<i>Geobacillus toebii</i>	Soil, Thailand	FN428680
R-35642 ^T	<i>Geobacillus toebii</i> DSM 14590 ^T		Hay compost, Korea	FN428690
R-35637 ^T	<i>Geobacillus thermoglucosidasius</i> DSM 2542 ^T	<i>Geobacillus thermoglucosidans</i>	Soil, Japan	FN428685
R-32515	<i>Geobacillus thermoglucosidasius</i> Sharp 12251	<i>Geobacillus thermoglucosidans</i>	Yellowstone National Park, USA	FN428650
R-32505	<i>Geobacillus thermoglucosidasius</i> Sharp 12641	<i>Geobacillus thermoglucosidans</i>	Ayttahya, Thailand	FN428643
R-32627	<i>Geobacillus thermoglucosidasius</i> Sharp 12678	<i>Geobacillus thermoglucosidans</i>	Soil, China	FN428668
R-32646	<i>Geobacillus thermoglucosidasius</i> Sharp 12656	<i>Geobacillus thermoglucosidans</i>	Soil, China	FN428676
R-32628	<i>Geobacillus thermoglucosidasius</i> Sharp 12673	<i>Geobacillus thermoglucosidans</i>	Soil, Thailand	FN428669
R-32648	<i>Geobacillus thermoglucosidasius</i> Sharp 11101	<i>Geobacillus thermoglucosidans</i>	Unknown	FN428677
R-32649	<i>Geobacillus thermoglucosidasius</i> Sharp 12620	<i>Geobacillus thermoglucosidans</i>	China	FN428678
R-32629	<i>Geobacillus thermoglucosidasius</i> DSM 2543	<i>Geobacillus thermoglucosidans</i>	Soil	FN428670
R-35639 ^T	<i>Geobacillus caldoxylosilyticus</i> DSM 12041 ^T		Soil, Australia	FN428687
R-35653 ^T	<i>Geobacillus debilis</i> Banat Tf ^T	<i>Caldibacillus debilis</i>	Undisturbed subsurface soil, Ireland	FN428699
R-35652 ^T	' <i>Geobacillus caldoproteolyticus</i> ' DSM 15730	<i>Anoxybacillus caldiproteolyticus</i>	Sewage sludge, Singapore	FN428698
R-35643 ^T	<i>Geobacillus tepidamans</i> DSM 16325 ^T	<i>Anoxybacillus tepidamans</i>	Sugar beet extraction juice	FN428691
R-32637	' <i>Geobacillus kaustophilus</i> ' Sharp 10332	<i>Anoxybacillus rupiensis</i>	Near Lake Taupo, New Zealand	AM988775
R-32636	' <i>Geobacillus kaustophilus</i> ' Sharp 10315	<i>Anoxybacillus rupiensis</i>	Near Lake Taupo, New Zealand	AM988776
R-35634 ^T	<i>Aeribacillus pallidus</i> LMG 19006 ^T		Yeast factory sewage	FN428682

*The type strain of *G. uzenensis* DSM 13551^T is a strain of *G. subterraneus* (data from this study).

The genus *Geobacillus*

16S rRNA gene sequence analysis revealed a monophyletic cluster of 56 strains assigned to the genus *Geobacillus*. Although interspecies 16S rRNA gene sequence similarities are high in *Geobacillus*, ranging from 96.0 to 99.4 %, intraspecies similarities are generally higher, ranging from 99.3 to 100.0 %, allowing reliable differentiation of species, supported by high bootstrap values. Nevertheless, the resolution of the 16S rRNA gene is often considered insufficient and several other genes were investigated for their applicability to *Geobacillus* taxonomy. The most promising approach so far appears to be *recN* gene sequencing (Zeigler, 2005), and this resulted in exactly the same grouping of *Geobacillus* as obtained with 16S rRNA gene sequencing, but with higher resolution. The reliability of species clusters in Fig. 1 was therefore further confirmed by comparing all *Geobacillus* strains from Table 1 with *Geobacillus* strains identified by Zeigler (2005) based on 16S rRNA and *recN* gene sequencing (data not shown). Other housekeeping genes investigated for their usability were

spo0A (Kuisiene *et al.*, 2009), *recA* (Weng *et al.*, 2009), *rpoB* (Meintanis *et al.*, 2008; Weng *et al.*, 2009), and *gyrB* and *parE* (Tourova *et al.*, 2010). Although resolution of these genes was often higher than for 16S rRNA genes, some of them (such as *spo0A* and *rpoB*) failed to differentiate *Geobacillus* species and others (such as *gyrB*, *parE* and *recA*) were not universally applicable so that only a few species have been analysed. An overview of these housekeeping genes using Taxongap software v.2.4.1 (Slabbinck *et al.*, 2008), showing their resolutions and their abilities to differentiate *Geobacillus* species, is given in Fig. S1 (available in IJSEM Online). Although it has been criticized for poor resolution, it can be concluded that the 16S rRNA gene is a good taxonomic marker for *Geobacillus*, and our groupings and conclusion correlate well with other studies using the above-mentioned housekeeping genes. One monophyletic sub-generic cluster (MSC), harbouring the type species *G. stearothermophilus* together with *G. thermocatenulatus*, *G. thermoleovorans*, *G. jurassicus*, *G. subterraneus* and *G. thermodenitrificans* can be differentiated from other *Geobacillus* species and the former *B. thermantarcticus* based on 16S

Fig. 1. Maximum-likelihood phylogenetic tree based on 16S rRNA gene sequences of all strains under study and some representatives of closely related species. Bootstrap values above 69 % are given at the branch points. All type strains that have been dealt with in this paper are indicated in bold. The 16S rRNA gene sequence of *Bacillus subtilis* subsp. *subtilis* DSM 10^T was chosen as an outgroup to root the tree. Accession numbers not given in the tree are provided in Table 1. *The strain of *G. uzenensis* R-35640^T (=DSM 13551^T) is a strain of *G. subterraneus*.

rRNA gene sequencing (Fig. 1). This distinction was confirmed by some small chemotaxonomic differences in fatty acid and polar lipid composition, as shown in Table 2. In general, phenotypic profiles across the genus showed little diversity, so that there are few features characteristic of individual species; the groupings of White *et al.* (1993) also showed rather small margins of separation. Fatty acid methyl ester profiles were not found to be reliable for species differentiation and these data are only used in species descriptions. However, fatty acid methyl ester analysis does allow a clear distinction of members of the genus *Geobacillus* from those of the other three genera, *Anoxybacillus*, *Aeribacillus* and *Caldibacillus* (Table 2). Major polar lipids detected within *Geobacillus* species were diphosphatidylglycerol (DPG), phosphatidylglycerol (PG) and phosphatidylethanolamine (PE). For the minor polar lipids, differences were observed between profiles of MSC members and non-MSc members, as represented by *G. stearothermophilus* R-35646^T, *G. thermoleovorans* R-35636^T, *G. thermoglucosidans* (formerly *G. thermoglucosidasius*) R-35637^T and *G. toebii*

R-35642^T in Fig. S2. Whereas MSC members contain an aminophosphoglycolipid (APGL) and a phosphoaminolipid (PAL), the non-MSc members contain a glycolipid (GL). *Geobacillus* species can be easily differentiated from members of *Caldibacillus* by the presence of DPG and PG, from members of *Aeribacillus* by the presence of PE, and from *Anoxybacillus* by the presence of GL (non-MSc members) or PAL and APGL (MSc-members). DNA G + C content within the genus varies from 42.4 to 54.5 mol%. Other characteristics for each of the species within the genus are discussed individually below.

Geobacillus stearothermophilus

The original description for *G. stearothermophilus* (Donk, 1920) was based on an unstated number of strains for which few characters were given, and the methods used then are not comparable with current ones. Gordon *et al.* (1973) published a description of 31 strains that they believed to represent '*Bacillus stearothermophilus*', and they

Table 2. Characters useful for differentiating between the genera *Geobacillus*, *Caldibacillus*, *Aeribacillus* and *Anoxybacillus*

Genera: 1, *Geobacillus*; 2, *Aeribacillus*; 3, *Anoxybacillus*; 4, *Caldibacillus*. All data are from this study except where marked and strains used are listed in Table 1.

Character	1	2	3	4
Polar lipids*	DPG, PG, PE, AL1, PAL†, APGL†, AL2†, GL1‡, PL1–2	DPG, PE, PG, PGL, GLs	PE, ALs, Ls	APGL, PGLs, GLs, PLs
DNA G + C content (mol%)§	42–55	39–41 ^a	41–54 ^b	50
Fatty acids (% of total):				
C _{14:0}	1.3 (0.7)	3.2	2.6 (1.2)	3.2
anteiso-C _{15:0}	2.9 (1.1)	3.4	5.0 (3.2)	3.8
iso-C _{15:0}	35.6 (11.9)	22.3	55.1 (7.3)	4.7
C _{16:0}	12.4 (7.8)	25	9.8 (4.5)	50.4
iso-C _{16:0}	10.3 (4.5)	12.4	3.8 (1.1)	12.4
C _{16:1} ω11c		1.9		
C _{17:0}				3.4
anteiso-C _{17:0}	9.0 (4.1)	11.8	5.3 (2.1)	10.2
iso-C _{17:0}	22.3 (5.0)	17.9	15.0 (5.9)	8.9
C _{18:0}	1.3 (3.2)†			2.1
iso-C _{17:1} ω5c	1.2 (2.2)†			
Summed feature 3	1.7 (2.0)			

*AL, Aminolipid; APGL, aminophosphoglycolipid; DPG, diphosphatidylglycerol; GL, glycolipid; L, lipid; PAL, phosphoaminolipid; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PGL, phosphoglycolipid; PL, phospholipid.

†Only for monophyletic subgeneric cluster (MSc)-members.

‡Only for non-MSc members.

§Data from: *a*, Miñana-Galbis *et al.* (2010); *b*, Pikuta (2009).

||For fatty acid analysis, mean values of all profiles included are shown with their standard deviation values in parentheses. Summed feature 3 represents C_{16:1} iso I and/or C_{14:0} 3-OH. Trace amounts (<1 %) are not shown.

Table 3. DNA relatedness values between representatives of strains under study

Values represented here (%) are mean values of two reciprocals. Values above 70 %, and thus representing the same species, are indicated in bold.

Species/strain	1	2	3	4	5	6	7	8	9	10	11	12	13	14
<i>G. stearothermophilus</i>														
1. R-32640	100													
2. R-32765	99.4	100												
3. R-32635	85.8	87.3	100											
4. R-32504	71.3	70.1	67.2	100										
5. R-35646 ^T	77.7	72.3	72.4	66.4	100									
<i>G. thermoglucosidans</i>														
6. R-32649						100								
7. R-35637 ^T						89.2	100							
<i>G. toebii</i>														
8. R-32653								100						
9. R-35642 ^T								89.9	100					
<i>Anoxybacillus</i>														
10. R-35652 ^T										100				
11. DSM 17127 ^T										29.7	100			
12. R-32636										28.5	93.4	100		
13. R-32637										24.6	97.8	90.7	100	
14. R-35643										33.6	37.9	27.7	23.7	100

included '*B. calidolactis*', *B. kaustophilus* and '*B. thermoliquefaciens*' in the synonymy. Subsequently, the heterogeneity of *B. stearothermophilus* was indicated by wide ranges of DNA composition and differences in phenotypic properties among strains assigned to it (Walker & Wolf, 1971; Logan & Berkeley, 1984). In the present study, 19 strains were received as *G. stearothermophilus* and 18 of those were confirmed as belonging to that species on the basis of 16S rRNA gene sequence analysis (Table 1). The remaining strain, R-32639, was a member of *G. toebii* and showed high phenotypic similarities with *G. toebii* strains R-32650, R-32653 and R-32651 (Fig. S3). Thirteen strains showed high phenotypic similarities (91 % S_G) to the type strain R-35646^T, again supporting their allocation to *G. stearothermophilus*, yet in contrast to the original description of Donk (1920), they were found to hydrolyse gelatin but not produce acid from lactose. The remaining four strains (R-32640, R-32765, R-32635 and R-32504) were recovered in other parts of the phenogram (Fig. S3), but 16S rRNA gene sequence similarities above 99.4 % (Fig. 1) and DNA relatedness values above 70 % (Table 3) clearly showed that these were all strains of *G. stearothermophilus*; nonetheless, R-32504 lies at the margin of the species with a DNA relatedness value of only 66.4 % with the type strain R-35646^T.

Geobacillus thermodenitrificans

This species was described by Ambroz (1913) as '*Denitrobacterium thermophilum*', was renamed *Bacillus thermodenitrificans* by Mishustin (1950), and included in the Approved List of Bacterial Names (Skerman *et al.*, 1980). Manachini *et al.* (2000) emended the species description

based on 10 strains and Nazina *et al.* (2001) transferred it to *Geobacillus* on the basis of 16S rRNA gene sequence comparisons and DNA relatedness. Although all 15 of our strains received as *G. thermodenitrificans* could be confirmed in their identities, clustering with the type strain R-35647^T at 99.7 % 16S rRNA gene pairwise sequence similarity (S_P), only seven clustered closely with the type strain in the phenotypic analysis of 14 strains (Fig. S3). Of the remainder, two strains (R-32619 and R-32621) clustered at 87.5 % S_G with the type strain, three (R-32506, R-32617 and R-32625) joined with *G. jurassicus* at 86 % S_G and one (R-32615) remained unclustered. This implied that phenotypic description would be difficult, but careful examination of profiles showed variations in the strength of reaction rather than substantial pattern differences. All strains were found to be negative for acid production from galactose and lactose, in contrast to the results of Manachini *et al.* (2000).

Geobacillus uzenensis

This species was proposed in 2001 by Nazina *et al.* (2001) but it appears that the strain deposited as the type of *G. uzenensis* in the DSMZ (DSM 13551^T), and studied in the present work, is not the authentic strain U^T, but actually a strain of *G. subterraneus*. This was confirmed by 100 % S_P and complete DNA relatedness between strain R-35640^T (=DSM 13551^T) and strain R-32641^T (=DSM 13552^T).

Geobacillus subterraneus

This species was also proposed in 2001 by Nazina *et al.* (2001). We provide additional characters for the type strain of *G. subterraneus* in Table S2.

Geobacillus jurassicus

This species was proposed by Nazina *et al.* (2005) on the basis of two isolates, both of which (R-35651^T and R-32597) were included in our study. They showed similar but distinct phenotypic profiles (96 % S_G ; Fig. S3), and the closest phenotypic profile was that of three *G. thermodenitrificans* strains, but a number of characteristics allow distinction of these species (Table 4).

Geobacillus thermoglucosidasius

This species was proposed by Suzuki *et al.* (1983) on the basis of six strains and it was transferred to the genus *Geobacillus* by Nazina *et al.* (2001) without its description being emended. Of the thirteen strains received as *G. thermoglucosidasius*, only three (R-32627, R-32628 and R-32505) showed appreciable phenotypic similarity (84 % S_G) with the type strain R-35637^T (Fig. S3). These strains and R-32515, R-32646, R-32649, R-32629 and R-32648 clustered with the type strain at above 99.9 % S_p in 16S rRNA gene sequence comparisons (Fig. 1). One representative, R-32649, showed 89.2 % DNA relatedness to the type strain (Table 3), and so all eight strains may be identified as *G. thermoglucosidasius*. Strains R-32515, R-32649 and R-32646 differed from R-32505, R-32627, R-32628 and R-35637^T in giving negative results for aesculin hydrolysis and for acid production from amygdalin, arbutin, gentiobiose, salicin and sorbitol. In contrast with Suzuki *et al.* (1983), but in agreement with Nazina *et al.* (2001), all *G. thermoglucosidasius* strains in the present study hydrolysed gelatin. Four other strains received as *G. thermoglucosidasius*, R-32650, R-32651, R-32652 and R-32653, were identified as *G. toebii* (see below). We propose an emended description of *G. thermoglucosidasius* with correction of the name to *G. thermoglucosidans* nom. corrig.

Saccharococcus caldoxylosilyticus

Saccharococcus caldoxylosilyticus was described by Ahmad *et al.* (2000) and transferred to *Geobacillus* by Fortina *et al.* (2001). The type strain R-35639^T was the only strain available and had no near neighbours in the phenotypic analysis (Fig. S3); its closest relative in 16S rRNA gene sequence analysis was *G. toebii*, ranging from 98.5–99.2 % S_p . Our data confirm the distinction of the species and additional characters for the type strain are provided in Table S2.

Geobacillus toebii

The type strain (R-35642^T) of this single-strain species (Sung *et al.*, 2002) clustered at over 99.4 % S_p in 16S rRNA gene sequence comparisons with a strain (R-32639) received as *G. stearothermophilus*, and with four strains (R-32650, R-32651, R-32652 and R-32653) received as *G. thermoglucosidasius* (Fig. 1). DNA relatedness between R-35642^T and R-32653 was 89.9 % (Table 3), confirming that they are members of the same species. In the phenotypic analysis, R-35642^T only clustered loosely with these five other strains at

about 86 % S_G , and so the species does not have a distinct phenotypic profile (Fig. S3). The description of *G. toebii* is now based upon six strains and is emended accordingly. '*G. toebii* subsp. *decanicus*', isolated from compost, was proposed by Poli *et al.* (2006) but the name has not been validly published and it was not included in our study.

Bacillus thermantarcticus

B. thermantarcticus was proposed (with the name '*Bacillus thermoantarcticus*') by Nicolaus *et al.* (1996) on the basis of a single strain. Zeigler (2005) recommended, on the basis of *recN* and 16S rRNA gene sequences, that this species should be transferred to the genus *Geobacillus*, with further analysis being required to determine whether it be a novel species, or a subspecies of *G. thermoglucosidasius*. In our analysis, *B. thermantarcticus* R-35644^T did not show a close relationship with the type strain of *G. thermoglucosidasius* (now *G. thermoglucosidans*) in 16S rRNA gene sequence or phenotypic analyses, implying it should indeed be transferred to the genus *Geobacillus* as the separate species *Geobacillus thermantarcticus* comb. nov.

***Caldibacillus debilis* gen. nov., comb. nov.**

Only the type strain of *G. debilis* (R-35653^T) was available for study. It did not grow well below 60 °C and even at or above this temperature it often grew poorly, with cells appearing distorted and stressed. The phenotypic characters were therefore tested on several occasions, in order to be confident of consistent and accurate results. The phenotypic profile was distinct and this strain did not show any significant similarity with members of any other species in the study (Table 4, Fig. S3). The 16S rRNA gene sequence did not imply a strong relationship with other *Geobacillus* strains either, the closest being members of *G. thermoglucosidans* with similarities between 93.3 and 93.6 %. The polar lipid profile of strain R-35653^T was remarkably different to those of members of *Geobacillus* (Table 2, Fig. S2), as the major polar lipids DPG and PG were absent. Instead, two phosphoglycolipids, two GLs and two phospholipids were detected. The fatty acid pattern was also quite different from those of *Geobacillus* species (Table 2) with $C_{16:0}$ as the major fatty acid (50.4 %). We conclude that *G. debilis* is a member of a genus that is separate from *Geobacillus*, and we supplement the description given by Banat *et al.* (2004) in our proposal to transfer *G. debilis* to the novel, single-species genus *Caldibacillus* as *Caldibacillus debilis* gen. nov., comb. nov.

Aeribacillus pallidus

Our data support the proposal of Miñana-Galbés *et al.* (2010) that *Geobacillus pallidus* be reclassified as *Aeribacillus pallidus*, and we provide additional characters based on extensive analysis of strain R-35634^T (Table S2). The polar lipid profile of strain R-35634^T is provided in Fig. S2, and matches the profile described by Miñana-Galbés *et al.* (2010), with DPG and PG as major polar lipids. It can be

Table 4. Characters useful for differentiating between *Geobacillus* (G.) species, *Caldibacillus* (C.), *Aeribacillus* and some *Anoxybacillus* (A.) species

Species: 1, *G. stearothermophilus* (n=15); 2, *G. jurassicus* (n=2); 3, *G. subterraneus* (n=1); 4, *G. thermoleovorans* (n=11); 5, *G. thermocatenulatus* (n=2); 6, *G. thermodenitrificans* (n=9); 7, *G. uzenensis* [n=2; data were taken from Logan *et al.* (2009), because an authentic strain of this species was not available]; 8, *G. thermoglucosidans* (n=8); 9, *G. caldioxysilyticus* (n=1); 10, *G. thermantarcticus* (n=1); 11, *G. toebii* (n=6); 12, *A. caldiproteolyticus* (n=1); 13, *A. rupiensis* (n=2); 14, *A. tepidamans* (n=1); 15, *G. debilis* (n=1); 16, *Aeribacillus pallidus* (n=1). Except where stated otherwise, all data were obtained in the course of the present study or were taken from Dinsdale *et al.* (2011). No entry indicates that data are not available. All strains studied were motile, formed ellipsoidal spores, and produced acid from fructose, glucose, maltose and mannose. Symbols: +, >85 % strains give positive reaction; –, <15 % of the strains give positive reaction; +/w, positive or weakly positive reaction; w, weak reaction; w/–, weak or negative reaction; +/d, usually positive, but different strains give different reactions; d, different strains give different reactions; d (w), different strains give different reactions, but positive reactions are weak; v, result varies.

Character	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Swollen sporangia	v	v	–	–	–	–	d	d	+	+	+	–	–	+	+	+
Spores:																
Cylindrical	–	–	–	–	d	–	–	d	v	–	–	+	–	–	+	v
Subterminal	+	+	+	+	d	+	–	+	–	+	–	+	+	–	–	+
Terminal	+	–	v	+	+	+	+	+	+	+	+	+	+	+	+	v
Central/paracentral	–	–	–	–	d	–	–	–	+	–	–	–	–	–	–	+
Hydrolysis of:																
Aesculin	+ /w	+	+	+	+	+	+	+	+	+	–	+	–	+	+	+
Casein	+ /w	+	–	d	–	–	–	–	v	–	–	+	+	–	w	–
Gelatin	+	+	w	+	+	d	+	+	+	+	+	+	d	–	+	–
ONPG	–	–	–	d	–	–	–	–	–	–	–	–	–	–	+	–
Starch	+	+	+	+	–	+ /w	+	d (w)	+	–	+ /w	+ /w	+	+	–	w
Catalase	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+
Oxidase	–	+	–	+	+	–	–	w	+	+ /w	+	+	w	–	+	+
Nitrate reduction	d	+	+	+	d	+ /d	+	d	+	–	+	–	–	–	–	–
Voges–Proskauer	+	–	+	d (w)	+	+ /d	–	d	–	w	v	+	w/–	+	w/–	+ /w
Acid from:																
N-Acetylglucosamine	–	–	–	d	d	–	–	+	–	–	–	+	–	+	+	–
Amygdalin	–	–	–	–	+	–	–	d	+	–	–	–	–	+	+	+
L-Arabinose	–	+	–	–	–	d (w)	+	d	+	–	–	–	+	+	–	–
Arbutin	–	–	–	–	+	–	w	d	+	–	–	v	–	+	w	+
Cellobiose	–	+	+	d	+	+	+	+	+	+	–	+	–	+	+	–
Galactose	w	+	+	d	+	–	+	–	+	w	+	+	–	+	+	–
Gentiobiose	–	–	–	–	–	–	–	d	+	–	–	–	–	+	+	–
Glycerol	w	+	+	+	+	–	+	w	w	w	–	+	–	+	–	+
Glycogen	+	–	+	d	–	–	+	–	+	–	–	+	w	–	–	–
Lactose	–	–	–	d (w)	–	–	–	w	+	–	–	–	–	+	+	–
myo-Inositol	–	–	+	d	–	–	–	–	–	–	–	–	–	+	–	+
Mannitol	–	+	+	d	+	w	+	+	–	–	w	+	–	–	–	–
Melezitose	+	+	+	d (w)	+	d (w)	–	–	–	–	–	–	–	+	–	–
Melibiose	+	–	–	d	+	d	–	–	+	–	d	–	–	+	–	–
Methyl D-glucoside	+	+	–	d	d	–	+	+	w	+	–	–	–	+	–	+
Raffinose	+	–	–	d	+	–	–	–	+	–	–	–	–	+	–	–
Ribose	–	+	–	+	+	w	–	+	+	–	d	+	+	w	–	+
Salicin	–	–	+	w	+	d	+	+	+	+	–	+	–	+	+	+
Sucrose	+	+	+	d	+	d	+	+	+	+	d	+	w	+	–	+
Trehalose	+	+	+	d	+	d	+	+	+	+	+	+	+	+	+	+
Turanose	+	+	–	–	–	–	–	+	+	+	–	w	–	+	w	w
D-Xylose	–	+	+	d	–	w	–	+	+	+	–	+	+	+	w	–
Anaerobic growth	w/–	w	+	w	w	+	+	+	+	–	+	–	–	+	–	+
Growth at/in:																
pH 5	–	+	–	w	+	–	–	–	–	–	–	+	–	–	–	–
pH 9	+	+	–	w	+	+	–	w	+	+	+	+	–	+	+	+
1 % NaCl (w/v)	+	+	+	d	+	–	+	–	–	+	+	–	+	+	+	+
5 % NaCl (w/v)	–	+	–	–	+	–	–	–	–	–	–	–	–	–	–	–

Table 4. cont.

Character	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Growth temperature (°C)																
Minimum	30–45	45	37	37–40	37	50	45	40	50	37	37	37	35	40	50	37
Maximum	60–70	65	60	70	80	70	65	<60	70	80	70	70	67	65	70	65
Optimum	40–60	60	55–60	60	60	50	55–60	50	50–65	60	60	60	55	55	65	50

easily differentiated from members of the genera *Geobacillus*, *Caldibacillus* and *Anoxybacillus* by the absence of PE (Table 2). It should also be noted that the name *Bacillus pallidus* was recently proposed for a mesophilic soil organism (Zhou *et al.*, 2008), but as this is a homonym of *Bacillus pallidus*, the name of which is validly published (Scholz *et al.*, 1988), Zhou *et al.* (2009) renamed it *Falsibacillus pallidus*.

***Anoxybacillus tepidamans* comb. nov. and *Anoxybacillus caldiproteolyticus* sp. nov.**

The type strain of *Geobacillus tepidamans* (R-35643^T) and the strain received as '*Geobacillus caldiproteolyticus*' (R-35652) clustered together in 16S rRNA gene sequence analysis, and were recovered in a group harbouring *Anoxybacillus* species. Overall, they showed higher similarities with members of the genus *Anoxybacillus* than with any members of the genus *Geobacillus*. Their respective DNA G + C values of 43.2 and 40.2 mol% are consistent with the range (41.6–44.4 mol%) seen for most other species in *Anoxybacillus*. The first-reported DNA G + C values for *A. kestanbolensis*, *A. ayderensis* and *A. gonensis* appear to be rather high for this genus, at 50.0, 54.0 and 57.0 mol%, respectively, but Kevbrin *et al.* (2005) gave a re-determined value of 42.8 mol% for *A. gonensis*. Poli *et al.* (2009), however, reported a DNA G + C value of 53.5 mol% for *A. thermarum*, and this species clustered closely with strains of *A. kestanbolensis*, *A. ayderensis* and *A. gonensis* in their 16S rRNA gene sequence analysis. The polar lipid profiles for strains R-35643^T and R-35652 were very similar, with DPG, PG and PE as the major polar lipids (Fig. S2); two aminolipids (ALs), a phospholipid and three novel lipids were also detected. These findings correlate well with the profile given for *Anoxybacillus pushchinoensis* by Miñana-Galbís *et al.* (2010). Other polar lipid profiles for *Anoxybacillus* species also show DPG, PG and PE as the major polar lipids, although *A. thermarum* lacks PG (Gul-Guven *et al.*, 2008; Poli *et al.*, 2006, 2009). Fatty acid profiles of R-35643^T and R-35652 were consistent with the fatty acid composition of other *Anoxybacillus* species, with iso-C_{15:0}, iso-C_{17:0} and C_{16:0} as the major fatty acids (De Clerck *et al.*, 2004). The phenotypic profile of *G. tepidamans* R-35643^T was distinct (Table 4) and it did not cluster with any other of the type strains analysed in the phenotypic analysis (Fig. S3). We supplement the description given by Schäffer *et al.* (2004) with further phenotypic data in our description of the new combination *Anoxybacillus tepidamans* comb. nov. The name '*Geobacillus caldiproteolyticus*'

was proposed for a single, sewage sludge isolate by Chen *et al.* (2004), but was not validly published. The phenotypic profile (Table 4) of strain R-35652^T was distinct from that of *G. tepidamans* and other *Anoxybacillus* species and it showed low DNA relatedness with *A. rupiensis* DSM 17127^T (Table 3). These data demonstrate conclusively that this strain should be classified as a representative of a novel species in the genus *Anoxybacillus*, for which the name *Anoxybacillus caldiproteolyticus* sp. nov. is proposed. Dinsdale *et al.* (2011) reported that strains R-32636 and R-32637, isolated from near Lake Taupo, New Zealand and received as *Geobacillus kaustophilus*, most probably belonged to *Anoxybacillus rupiensis*. DNA relatedness values confirmed this (Table 3), and phenotypic data for these two strains for characters that were not included in the original description of this species are given in Table S2.

Descriptions

Characters useful for differentiating the species are shown in Table 4.

Emended description of *Geobacillus Nazina et al.* 2001

Geobacillus (Ge.o.ba.cil'lus. Gr. n. *Gê* the Earth; L. dim. n. *bacillus* small rod; N.L. masc. n. *Geobacillus* earth or soil small rod).

Obligate thermophiles. Vegetative cells are rod-shaped and produce one endospore per cell. Cells occur either singly or in short chains and are motile by means of peritrichous flagella or they are non-motile. The cell wall structure is Gram-positive, but the Gram-stain reaction may vary between positive and negative. Ellipsoidal endospores are located subterminally or terminally in non-swollen or slightly swollen sporangia. Colony morphology and size are variable. Chemo-organotrophs. Aerobic or facultatively anaerobic, but anaerobic growth may be weak. Oxygen is the terminal electron acceptor, replaceable in some species by nitrate. The temperature range for growth is 30 to 80 °C, with optimum growth at 50–60 °C. Neutrophilic. Growth occurs at pH 5.0–9.0, with optimum growth between pH 6.2 and 7.5. Growth factors, vitamins, NaCl and KCl are not required by most species. Many species can utilize n-alkanes as carbon and energy sources. Most species produce acid but no gas from fructose, glucose, maltose, mannose, sucrose and trehalose. Most species do not produce acid from lactose and gentiobiose. Produce catalase. Oxidase

reaction varies. Phenylalanine is not deaminated, tyrosine is not degraded, indole is not produced and the Voges–Proskauer reaction varies between species. Aesculin and gelatin are hydrolysed, starch is hydrolysed by most species, casein is hydrolysed by some species and ONPG is rarely hydrolysed. The major cellular fatty acids are iso-C_{15:0}, iso-C_{16:0} and iso-C_{17:0}, which make up more than 60 % of the total. Major polar lipids for members of this genus are DPG, PG, PE, ALs and APGL. The main menaquinone type is MK-7. The DNA G + C of the of the species ranges from 48.4 to 54.5 mol%. The type species is *Geobacillus stearothermophilus*.

Emended description of *Geobacillus stearothermophilus* (Donk 1920) Nazina et al. 2001 (*Bacillus stearothermophilus* Donk 1920)

Geobacillus stearothermophilus [ste.a.ro.ther.mo'phi.us. Gr. n. *stear* fat; Gr. n. *thermê* heat; N.L. adj. *philus* -a -um (from Gr. adj. *philos* -ê -on) friend, loving; N.L. masc. adj. *stearothermophilus* heat and fat loving].

Gram-positive, -negative and -variable, motile rods, 2.0–3.5 × 0.6–1.0 µm, occurring singly or in short chains. Ellipsoidal spores are located subterminally and/or terminally within sporangia that may be swollen. Colonies on TSA are circular and 0.5–2.0 mm in diameter after 24–48 h at 60 °C, usually convex, and may be smooth-surfaced and with crenate edges. Grows weakly if at all in the absence of oxygen. Minimum growth temperature is 30 to 45 °C, maximum 60 to 70 °C; most strains grow between 40 and 60 °C. Growth occurs at pH 6 and pH 9. Tolerates 1 % but not 5 % NaCl. Aesculin, casein, gelatin and starch are hydrolysed, but aesculin and casein reactions may be weak. Oxidase-negative. Gelatin is hydrolysed and the Voges–Proskauer test is positive; nitrate reduction is variable, and arginine dihydrolase, citrate utilization (Simmons'), hydrogen sulphide production, indole production, lysine decarboxylase, ornithine decarboxylase, ONPG reaction, tryptophan deaminase and urease production are negative. Acid without gas is produced from D-fructose, D-glucose, glycogen, maltose, D-mannose, melezitose, melibiose, methyl D-glucoside, raffinose, starch, sucrose, trehalose and turanose; acid is produced weakly from galactose and glycerol. Acid production is variable between strains for inulin and methyl D-mannoside; positive reactions are often weak. No acid is produced from N-acetylglucosamine, adonitol, amygdalin, D- and L-arabinose, D- and L-arabitol, arbutin, cellobiose, dulcitol, erythritol, D- and L-fucose, gentiobiose, gluconate, 2- and 5-keto-D-gluconate, lactose, D-lyxose, mannitol, myo-inositol, methyl xyloside, rhamnose, ribose, salicin, sorbitol, L-sorbose, D-tagatose, D-xylose, L-xylose or xylitol. In the variable reactions listed above, the type strain does not have swollen sporangia, it reduces nitrate, produces acid from methyl D-mannoside, and produces acid weakly from inulin. The polar lipid profile of the type strain consists of DPG, PG, PE, ALs and APGL. Hydrocarbons (C₁₀, C₁₁) may be used as carbon and energy sources (Nazina et al., 2001). The major fatty acids are iso-C_{15:0}, iso-C_{16:0} and iso-C_{17:0}, which comprise more than 60 % of the total fatty acids.

The type strain is LMG 6939^T (=DSM 22^T=ATCC 12980^T=R-35646^T), which was originally isolated from deteriorated canned corn and beans; it has also been isolated from evaporated milk, sugar beet juice from extraction installations, hot springs, hydrothermal vents, petroleum reservoirs and soil. The DNA G + C content of the type strain is 52.8 mol% (by HPLC).

Emended description of *Geobacillus jurassicus* Nazina et al. 2005

Geobacillus jurassicus (ju.ras.si'cus. N.L. masc. adj. *jurassicus* of Jurassic, referring to the geological period of the oil-bearing formation from where the strains were isolated).

Gram-positive rods, motile by peritrichous flagella, and producing subterminally located ellipsoidal spores in slightly swollen sporangia. Description is based upon two isolates. On NA, colonies are round, mucoid, colourless and glossy, and reach a diameter of about 2 mm after 24–48 h of incubation. Aerobic and chemo-organotrophic; anaerobic growth is weak. Oxidase-positive. Grows between 45 and 65 °C, with optimum growth at 58–60 °C. Grows at pH 5 and pH 9, with optimum growth at pH 7.0–7.2. Can tolerate 5.0–5.5 % (w/v) NaCl; grows weakly in the presence of up to 7 % NaCl (w/v). Can grow in nutrient broth and on potato agar. Produces NH₃ from peptone. Does not grow autotrophically on H₂ + CO₂. Aesculin, casein, gelatin and starch are hydrolysed. Reactions for ONPG, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, citrate utilization, hydrogen sulphide production, urease, tryptophan deaminase, indole, Voges–Proskauer, tyrosine degradation, dihydroxyacetone production, egg-yolk and the methyl red test are negative. Phenylalanine is not deaminated. Fe²⁺ is not used as an electron acceptor. Nitrate is reduced to nitrite. Acid but no gas is produced from the following: L-arabinose, cellobiose, D-fructose, galactose, D-glucose, glycerol, maltose, mannitol, D-mannose, melezitose, methyl D-glucoside, ribose, starch (weak), sucrose, trehalose, turanose and D-xylose. No acid is produced from: N-acetylglucosamine, adonitol, amygdalin, D-arabinose, D- and L-arabitol, arbutin, dulcitol, erythritol, D- and L-fucose, gentiobiose, gluconate, glycogen, myo-inositol, inulin, 2- and 5-keto-D-gluconate, D-lyxose, melibiose, methyl D-mannoside, methyl xyloside, raffinose, rhamnose, salicin, sorbitol, L-sorbose, D-tagatose, xylitol and L-xylose. The following are utilized as carbon and energy sources: hydrocarbons (C₆, C₁₀, C₁₁, C₁₄ and C₁₆), methane-naphthenic oil, acetate, butyrate, pyruvate, lactate, benzoate, fumarate, succinate, malate, ethanol, peptone, tryptone and yeast extract. Cannot grow on methanol, propanol, butanol, iso-butanol, phenol, phenylacetate, alanine, glutamate, serine, formate or propionate. Poor growth is observed on asparagine and glutamine. The major cellular fatty acids are iso-C_{15:0}, iso-C_{16:0} and iso-C_{17:0}. The DNA G + C content is 53.8–54.5 mol% (T_m).

The type strain is LMG 23069^T (=DSM 15726^T=VKM B-2301^T=R-35651^T), isolated from the formation water of the high-temperature Dagang oilfield, China. The DNA G+C content of the type strain is 54.5 mol% (T_m).

Emended description of *Geobacillus thermodenitrificans* (Manachini et al. 2000) Nazina et al. 2001 (*Bacillus thermodenitrificans* (ex Klaushofer and Hollaus 1970) Manachini et al. 2000; '*Denitrobacterium thermophilum*' Ambroz 1913; '*Bacillus thermodenitrificans*' Mishustin 1950; '*Bacillus thermodenitrificans*' Klaushofer and Hollaus 1970)

Geobacillus thermodenitrificans (ther.mo.de.ni.tri'fi.cans. Gr. n. *thermê* heat; N.L. part. adj. *denitrificans* denitrifying; N.L. part. adj. *thermodenitrificans* thermophilic denitrifying, referring to two of the organism's features).

Gram-positive, motile, straight rods 0.5–1.0 × 1.5–2.5 µm. Endospores are ellipsoidal, lie subterminally and/or terminally and do not distend the sporangium. Facultatively anaerobic. Colonies on TSA are flat with irregular margins, off-white to beige in colour, with non-glossy surfaces; they show some motility and are 1–4 mm in diameter after incubation at 60 °C for 24 h. Growth occurs between 50 and 70 °C, with optimum growth at 50 °C. Grows at pH 6 and 9 but not at pH 5; optimum is pH 7. Tolerates 0.5 % but not 1 % NaCl. Oxidase-negative. Aesculin and starch are hydrolysed, the latter sometimes only weakly, and casein is not hydrolysed. Nitrate reduction and the Voges–Proskauer test are variable, but usually positive, gelatin hydrolysis is variable, and arginine dihydrolase, citrate utilization, hydrogen sulphide production, indole production, lysine decarboxylase, ornithine decarboxylase, ONPG reaction, tryptophan deaminase and urease production are negative. Acid without gas is produced from cellobiose, D-fructose, D-glucose, maltose and D-mannose; acid is produced weakly from mannitol, ribose, starch and D-xylose. Acid production is variable between strains from melibiose, salicin, sucrose and trehalose, and variable, but weak when positive, from L-arabinose and melezitose. No acid is produced from N-acetylglucosamine, adonitol, amygdalin, arbutin, D-arabinose, D- and L-arabitol, dulcitol, erythritol, D- and L-fucose, galactose, gentiobiose, gluconate, glycerol, glycogen, inulin, 2- and 5-keto-D-gluconate, lactose, D-lyxose, myo-inositol, methyl D-glucoside, methyl D-mannoside, methyl xyloside, raffinose, rhamnose, D-tagatose, sorbitol, L-sorbose, turanose, L-xylose or xylitol. In the variable reactions listed above, the type strain gives positive reactions for gelatin hydrolysis, nitrate reduction and the Voges–Proskauer test; acid is produced from L-arabinose, melibiose, sucrose and trehalose, and produced weakly from salicin and melezitose. The main fatty acids are iso-C_{15:0} and iso-C_{17:0}, which account for over 66 to 69 % of the total; the minor fatty acids are C_{16:0}, iso-C_{16:0} and anteiso-C_{17:0}.

The type strain is LMG 17532^T (=ATCC 29492^T=DSM 465^T=R-35647^T), which was originally isolated from soil; it

has also been isolated from sugar beet juice from extraction installations, hot compost and hydrothermal vents. The DNA G+C content of the type strain is 48.4 mol% (by HPLC).

Emended description of *Geobacillus thermoglucosidans* nom. corrig.

Geobacillus thermoglucosidans (ther.mo.glu.co'si.dans Gr. n. *thermê* heat; N.L. part. adj. *glucosidans* glucosidating, i.e. releasing glucoside units; N.L. part. adj. *thermoglucosidans* indicating the production of heat-stable glucosidase).

Basonym: *Bacillus thermoglucosidasius* Suzuki et al. 1983.

Gram-positive, motile rods 0.5–1.2 × 3.0–7.0 µm. Short ellipsoidal to cylindrical endospores are borne subterminally and terminally and occasionally swell the sporangia. Facultatively anaerobic. Colonies on TSA are 0.5 to 5 mm in diameter, flat, smooth, translucent, glossy, circular and entire, and faintly brown in colour after 50 °C incubation for 24–48 h. Viscid pellicles are formed in broth. Optimum growth at 50 °C; no growth at 37 or 60 °C. Optimum growth at pH 8, weak growth at pH 7 and 9, and no growth at pH 6. Tolerates up to 0.5 % NaCl. Catalase-positive, oxidase weakly positive. Aesculin is hydrolysed, casein is not. Starch hydrolysis is variable and weak when positive. Citrate is not utilized; arginine dihydrolase, hydrogen sulphide, indole, lysine decarboxylase, tryptophan deaminase and urease production are negative; and the Voges–Proskauer test and nitrate reduction are variable. Acid without gas is produced from cellobiose, D-glucose, D-fructose, maltose, mannitol, D-mannose, methyl D-glucoside, N-acetylglucosamine, ribose, salicin, sucrose, trehalose, turanose and D-xylose. Acid is produced weakly from glycerol and lactose. Acid production is variable from amygdalin, L-arabinose, arbutin, gentiobiose, rhamnose, sorbitol, L-sorbose (weak when positive) and starch. No acid is produced from adonitol, D-arabinose, D- and L-arabitol, dulcitol, erythritol, D- and L-fucose, galactose, gluconate, glycogen, inulin, 2- and 5-keto-D-gluconate, D-lyxose, melezitose, melibiose, myo-inositol, methyl D-mannoside, methyl xyloside, raffinose, D-tagatose, xylitol or L-xylose. In the variable reactions listed above, the type strain does not produce cylindrical spores or swollen sporangia; it hydrolyses starch weakly; is negative for nitrate reduction and the Voges–Proskauer test, and produces acid from L-arabinose, amygdalin, arbutin, gentiobiose, rhamnose, sorbitol, L-sorbose (weakly) and starch. Large amounts of exo-oligo-1,6-glucosidase are synthesized (Suzuki et al., 1983). The main cellular fatty acids are iso-C_{15:0}, iso-C_{16:0}, C_{16:0}, iso-C_{17:0} and anteiso-C_{17:0}, which make up 90 % of the total. The DNA G+C content is 43.1–43.4 mol% (HPLC).

The type strain is LMG 7137^T (=ATCC 43742^T=DSM 2542^T=NCIMB 11955^T=R-35637^T), isolated from soil. The DNA G+C content of the type strain is 43.4 mol% (by HPLC).

Description of *Geobacillus thermantarcticus* comb. nov.

Geobacillus thermantarcticus (therm.ant.arc'ti.cus. Gr. n. *thermê* heat; L. masc. adj. *antarcticus* southern, belonging to Antarctica; N.L. masc. adj. *thermantarcticus* a thermophile from Antarctica).

Basonym: *Bacillus thermantarcticus* Nicolaus *et al.* 2002 (*Bacillus thermoantarcticus* [sic] Nicolaus *et al.* 1996).

Description is based upon a single strain. Gram-positive, motile rods, $0.5\text{--}2.0 \times 3.0\text{--}5.0\text{ }\mu\text{m}$, with ellipsoidal endospores that are borne terminally or subterminally in swollen sporangia. Strictly aerobic. After 24 h incubation at $60\text{ }^{\circ}\text{C}$, colonies are whitish to cream in colour, opaque, flat and circular with entire margins, glossy surfaces and diameters of less than 0.5 mm. Grows at 37 and $80\text{ }^{\circ}\text{C}$, and optimally at $60\text{ }^{\circ}\text{C}$. Grows between pH 5.5 and 9.0; optimum is pH 6.0. Tolerates up to 3 % NaCl and is inhibited by 5 % NaCl. Oxidase-positive, but may be weak. Aesculin is hydrolysed, casein and starch are not. Growth occurs on yeast extract; glucose, trehalose and xylose can be utilized as sole carbon sources; propionate is not utilized; hippurate and tyrosine are not degraded. Gelatin hydrolysis is positive and the Voges–Proskauer reaction is weak, but citrate utilization, nitrate reduction, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, hydrogen sulphide production, indole production, tryptophan deaminase and urease production are negative. Acid without gas is produced from: cellobiose, D-fructose, galactose (weak), D-glucose, glycerol (weak), maltose, D-mannose, methyl D-glucoside, salicin, L-sorbose (weak), starch, sucrose, trehalose, turanose and D-xylose. No acid is produced from N-acetylglucosamine, adonitol, amygdalin, D- and L-arabinose, D- and L-arabitol, arbutin, dulcitol, erythritol, D- and L-fucose, gentiobiose, gluconate, glycogen, inulin, 2- and 5-keto-D-gluconate, lactose, D-lyxose, mannitol, melezitose, melibiose, *myo*-inositol, methyl D-mannoside, methyl xyloside, raffinose, rhamnose, ribose, sorbitol, D-tagatose, xylitol and L-xylose. In the stationary phase of growth, an exopolysaccharide, exo- and endocellular α -glucosidases, an intracellular alcohol dehydrogenase, and an exocellular xylanase are produced (Nicolaus *et al.*, 1996). The major fatty acids at $60\text{ }^{\circ}\text{C}$ are anteiso- $\text{C}_{17:0}$ (36 % of total), iso- $\text{C}_{17:0}$ (27 %), iso- $\text{C}_{15:0}$ (15 %) and iso- $\text{C}_{16:0}$ (13 %).

The type strain is LMG 23032^T (=DSM 9572^T=strain M1^T=R-35644^T), isolated from Antarctic geothermal soil. The DNA G+C content is 53.7 mol% for the type strain.

Emended description of *Geobacillus toebii* Sung *et al.* 2002

Geobacillus toebii (toe'bi.i. N.L. gen. n. *toebii* derived from toebi, a special, farmland compost in Korea, from which the organism was isolated).

Facultatively anaerobic, Gram-positive, motile rods, $0.5\text{--}0.9 \times 2.0\text{--}3.5\text{ }\mu\text{m}$. Ellipsoidal spores are located terminally

in swollen sporangia. Cells are motile and form colonies that are cream in colour, with smooth, glossy surfaces and diameters of $0.5\text{--}2.0\text{ mm}$ after 24–48 h at $60\text{ }^{\circ}\text{C}$. Grows at $37\text{ }^{\circ}\text{C}$ (weakly) and $70\text{ }^{\circ}\text{C}$; optimum growth at $60\text{ }^{\circ}\text{C}$. No growth at 30 or $80\text{ }^{\circ}\text{C}$. Grows at pH 6.0 and 9.0; optimum at pH 7.5. Tolerates up to 1 % NaCl. Oxidase-positive. Starch is hydrolysed (reaction may be weak), but aesculin and casein are not hydrolysed. Gelatin is hydrolysed and nitrate is reduced; the Voges–Proskauer test is variable; and citrate utilization, arginine dihydrolase, hydrogen sulphide, indole, lysine decarboxylase, ornithine decarboxylase, tryptophan deaminase and urease production are negative. Acid without gas is produced from D-fructose, galactose, D-glucose, maltose, mannitol (weak), D-mannose and trehalose. Acid production is variable from melibiose, ribose, starch and sucrose. Acid is not produced from N-acetylglucosamine, adonitol, amygdalin, D- and L-arabinose, D- and L-arabitol, arbutin, cellobiose, dulcitol, erythritol, D- and L-fucose, gentiobiose, gluconate, glycerol, glycogen, inulin, 2- and 5-keto-D-gluconate, lactose, D-lyxose, melezitose, *myo*-inositol, methyl D-glucoside, methyl D-mannoside, methyl xyloside, raffinose, rhamnose, salicin, sorbitol, L-sorbose, D-tagatose, turanose, xylitol, or D- and L-xylose. In the variable reactions listed above, the colonies of the type strain are motile, the Voges–Proskauer test and acid production from melibiose are positive, and acid production from ribose, starch and sucrose is negative. Sung *et al.* (2002) reported that the type strain utilizes n-alkanes but not acetate, formate or lactate. The cell wall peptidoglycan contains *meso*-diaminopimelic acid. The major cellular fatty acids are iso- $\text{C}_{15:0}$, iso- $\text{C}_{16:0}$ and iso- $\text{C}_{17:0}$, which comprise over 85 % of the total. The type strain is reported to produce factors that stimulate the growth of '*Symbiobacterium toebii*'. The DNA G+C content is 42.3–42.4 mol% (by HPLC).

The type strain is LMG 23037^T (=DSM 14590^T=KCTC 0306BP^T=SK-1^T=R-35642^T), isolated from farmland hay compost in Kongju, Korea, from soil in Thailand, and evaporated milk. The DNA G+C content of the type strain is 42.4 mol%.

Description of *Caldibacillus* gen. nov.

Caldibacillus (Cal.di.ba.cil'lus. L. adj. *caldus* warm, hot; L. masc. n. *bacillus* a small staff or rod; N.L. masc. n. *Caldibacillus* warm bacillus, referring to the organism's thermophily).

Gram-negative, motile rods $0.5\text{--}1.0 \times 1.0\text{--}14.0\text{ }\mu\text{m}$ that produce one endospore per cell. Ellipsoidal or cylindrical endospores are located terminally in sporangia that may be swollen. Obligately thermophilic and strictly aerobic. Colonies on TSA are flat and cream-coloured with smooth margins, and are approximately 0.5 mm in diameter after 24 h at $60\text{ }^{\circ}\text{C}$. The temperature range for growth is 50 to $70\text{ }^{\circ}\text{C}$, with optimum at $65\text{ }^{\circ}\text{C}$. Growth occurs within a pH range of 7.0 to 9.5. Grows in the presence of 0 % to 2.5 % NaCl. Catalase is weakly positive and oxidase-positive. Aesculin, arginine, casein, gelatin and ONPG are hydrolysed by some strains, but starch is not hydrolysed. The

Voges–Proskauer reaction is negative or weak. The polar lipid profile consists of PE, two phosphoglycerolipids, two GLs, an APGL, two phospholipids and two ALs. The major cellular fatty acids, which comprise more than 60 % of the total, are iso-C_{15:0}, iso-C_{16:0} and iso-C_{17:0}. The main menaquinone type is MK-7. The type species is *Caldibacillus debilis*.

Description of *Caldibacillus debilis* comb. nov.

Caldibacillus debilis (de'bil.is. L. masc. adj. *debilis* weak or feeble, referring to the restricted substrate range for this species).

Basonym: *Geobacillus debilis* Banat *et al.* 2004.

The type strain exhibits the following properties in addition to those given in the genus description. Reactions for arginine dihydrolase, gelatin hydrolysis and ONPG are positive; those for hydrogen sulphide, indole, lysine decarboxylase, ornithine decarboxylase, tryptophan deaminase and urease production, nitrate reduction and citrate utilization are negative. Acid but no gas is produced from the following: amygdalin, cellobiose, D-fructose, galactose, gentiobiose, D-glucose, lactose, maltose, D-mannose, N-acetylglucosamine, raffinose, salicin and starch; and weakly produced from arbutin, D-tagatose, trehalose, turanose and D-xylose. Acid is not produced from: adonitol, D- and L-arabinose, D- and L-arabitol, dulcitol, erythritol, gluconate, glycerol, glycogen, inulin, D- and L-fucose, 2- and 5-keto-D-gluconate, D-lyxose, mannitol, melezitose, melibiose, myo-inositol, methyl D-glucoside, methyl D-mannoside, methyl xyloside, raffinose, rhamnose, ribose, sorbitol, L-sorbose, sucrose, xylitol or L-xylose.

The type strain is LMG 23386^T (=DSM 16016^T=NCIMB 13995^T=Tf^T=R-35653^T), isolated from undisturbed sub-surface soil in Northern Ireland. The DNA G+C content of the type strain is 49.9 mol% (by HPLC).

Description of *Anoxybacillus caldiproteolyticus* sp. nov.

Anoxybacillus caldiproteolyticus (cal.di.pro.te.o.ly'ti.cus. L. adj. *caldus* hot; N.L. masc. adj. *proteolyticus* proteolytic; N.L. masc. adj. *caldiproteolyticus* hot and protein degrading).

Synonym: '*Geobacillus caldoproteolyticus*' Chen *et al.* 2004.

Description is based upon study of a single isolate. Gram-positive, motile rods. Strictly aerobic. Endospores are formed within 24 h of incubation at 60 °C on NA containing 5 mg MnSO₄ l⁻¹; they are ellipsoidal and sometimes cylindrical, lie subterminally and/or terminally and do not swell the sporangia. After 24 h incubation at 60 °C on NA, colonies are circular in shape with smooth edges, glossy surfaces and diameters of approximately 5 mm. Grows between 37 and 70 °C (at 48 h), with optimum at 60 °C. Grows at pH 5.0 and 9.0 with optimum growth at pH 6.5–7.0. Tolerates up to 0.5 % NaCl. Hydrolyses aesculin, casein and (sometimes weakly) starch. Catalase and oxidase are

positive. Positive for gelatin hydrolysis and the Voges–Proskauer test; negative for citrate utilization, arginine dihydrolase, hydrogen sulphide production, indole production, lysine decarboxylase production, nitrate reduction, ornithine decarboxylase, ONPG, tryptophan deaminase and urease production. Acid without gas is produced from cellobiose, D-fructose, galactose, D-glucose, glycerol, glycogen, maltose, mannitol, D-mannose, N-acetylglucosamine, ribose, salicin, starch, sucrose, trehalose, turanose (weak) and D-xylose. Acid production from arbutin is variable. No acid is produced from adonitol, amygdalin, D- and L-arabinose, D- and L-arabitol, dulcitol, erythritol, D- and L-fucose, gentiobiose, gluconate, inulin, 2- and 5-keto-D-gluconate, lactose, D-lyxose, melezitose, melibiose, myo-inositol, methyl D-glucoside, methyl D-mannoside, methyl xyloside, raffinose, rhamnose, sorbitol, L-sorbose, D-tagatose, xylitol or L-xylose.

The type strain is DSM 15730^T (=ATCC BAA-818^T=LMG 26209^T=R-35652^T), isolated from sewage sludge from a water reclamation plant in Singapore. The DNA G+C content of the type strain is 40.2 mol% (by HPLC).

Description of *Anoxybacillus tepidamans* comb. nov.

Anoxybacillus tepidamans (te.pid.a'mans. L. adj. *tepidus* moderately warm, lukewarm; L. part adj. *amans* loving; N.L. part adj. *tepidamans* loving warm conditions).

Basonym: *Geobacillus tepidamans* Schäffer *et al.* 2004.

Data are from study of the type strain. Facultatively anaerobic, Gram-positive rods 3.0–5.0 × 0.9–1.2 µm, producing ellipsoidal endospores that lie terminally and swell the sporangia. After 24 h at 60 °C on NA, colonies are opaque and white in colour, circular, with smooth edges and glossy surfaces, and diameters of approximately 0.5 mm. Grows at 40–65 °C, with an optimum at 55 °C. Grows between pH 6 and 9; no growth at pH 5. Tolerates up to 2 % NaCl. Aesculin and starch are hydrolysed, but casein is not. Catalase-positive and oxidase-negative. ONPG and the Voges–Proskauer test (this test was recorded as negative by Schäffer *et al.*, 2004) are positive; reactions for arginine dihydrolase production, citrate utilization, gelatin hydrolysis, hydrogen sulphide, indole, lysine decarboxylase, nitrate reduction (this test was recorded as positive by Schäffer *et al.*, 2004), ornithine decarboxylase, tryptophan deaminase and urease production are negative. Acid without gas is produced from amygdalin, L-arabinose, arbutin, cellobiose, D-fructose, galactose, gentiobiose, D-glucose, glycerol, lactose, maltose, D-mannose, melezitose, melibiose, myo-inositol, methyl D-glucoside, N-acetylglucosamine, raffinose, ribose (weak), salicin, starch, sucrose, trehalose, turanose and D-xylose. No acid is produced from: adonitol, D-arabinose, D- and L-arabitol, dulcitol, erythritol, D- and L-fucose, gluconate, glycogen, inulin, 2- and 5-keto-D-gluconate, D-lyxose, mannitol, methyl D-mannoside, methyl xyloside, rhamnose, sorbitol, L-sorbose, D-tagatose, L-xylose

and xylitol. Major polar lipids are DPG, PG and PE, together with two ALs, a phospholipid and some new lipids.

The type strain is LMG 26208^T (=ATCC BAA-942^T =DSM 16325^T=R-35643^T), isolated from a beet sugar factory in Austria. The DNA G+C content of the type strain (by HPLC) is 43.2 mol%.

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