Geobacillus gargensis sp. nov., a novel thermophile from a hot spring, and the reclassification of *Bacillus vulcani* as *Geobacillus vulcani* comb. nov.

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A novel thermophilic spore-forming strain, Ga^T, was isolated from the Garga hot spring located in the northern part of the Transbaikalian region (Russia). Strain Ga^T was found to be an aerobic, Gram-positive, rod-shaped, thermophilic (optimum growth temperature is 60–65 °C), chemo-organotrophic bacterium that grows on various sugars, carboxylic acids and hydrocarbons. The G + C content of its DNA is 52.9 mol%. The 16S rRNA gene sequence similarity data show that strain Ga^T is closely related to members of the genus *Geobacillus*. Relevant chemotaxonomic data (in particular, the major fatty acid profile of strain Ga^T, which includes iso-C15:0, iso-C16:0 and iso-C17:0 acids) support the assignment of this strain to the genus *Geobacillus*. The physiological, biochemical and DNA–DNA hybridization studies of strain Ga^T showed that it differs both genotypically and phenotypically from the recognized *Geobacillus* species. Based on these data, strain Ga^T belongs to a novel species, *Geobacillus gargensis* sp. nov. (type strain, Ga^T = VKM B-2300^T = DSM 15378^T). The analysis of the phenotypic characteristics (additional to those given in the original description) of the type strain of *Bacillus vulcani* (DSM 13174^T) showed that they are very similar to the major phenotypic characteristics of the genus *Geobacillus*. The low DNA–DNA reassociation values of strain DSM 13174^T with various species of this genus (from 38 to 54 %) clearly demonstrate a sufficient genomic distinction of this strain and its taxonomic status as a species. The physiological characteristics, phylogenetic position and DNA–DNA reassociation values of *B. vulcani* allow this species to be reclassified as *Geobacillus vulcani* comb. nov. The main properties that differentiate *G. vulcani* from the other species of the genus are its ability to produce acids from glycerol, lactose and ribose.

INTRODUCTION

In 2001, thermophilic bacilli of phylogenetic group 5 (Ash *et al.*, 1991) were classified in a new genus, *Geobacillus* (Nazina *et al.*, 2001). The bacteria of this genus with growth temperatures ranging from 35 to 78 °C are widespread in various geographical areas on Earth (Claus & Berkeley, 1986; White *et al.*, 1993; Manachini *et al.*, 2000; Markossian *et al.*, 2000; Nazina *et al.*, 2000, 2001; Maugeri *et al.*, 2002). The genus includes the species *Geobacillus stearothermophilus*, *Geobacillus thermocatenulatus*, *Geobacillus thermoleovorans*, *Geobacillus kaustophilus*, *Geobacillus thermoglucosidarius*,

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of *Geobacillus gargensis* Ga^T is AY193888.

Electron micrographs showing the flagellation of strain Ga^T and thin sections of vegetative cells of this strain are available as supplementary material in IJSEM Online. Data pertaining to the cellular fatty acid composition of strain Ga^T, and G + C content and DNA–DNA hybridization values are also available in IJSEM Online.

Geobacillus thermodenitrificans, *Geobacillus subterraneus*, *Geobacillus uzenensis*, *Geobacillus caldohydrophilus* and *Geobacillus toebii* (Logan & Berkeley, 1984; Claus & Berkeley, 1986; Golovacheva *et al.*, 1975; Suzuki *et al.*, 1983; Priest *et al.*, 1988; White *et al.*, 1993; Zarilla & Perry, 1987; Manachini *et al.*, 2000; Ahmad *et al.*, 2000; Nazina *et al.*, 2001; Fortina *et al.*, 2001a; Sung *et al.*, 2002). Many thermophilic bacilli that have not yet been included in recognized species ('*Bacillus caldolyticus*', '*Bacillus caldoteanax*', '*Bacillus caldovelox*' and '*Geobacillus uralicus*'), and the recognized species *Bacillus thermantarcticus*, *Saccharococcus thermophilus* and *Bacillus vulcani* belong to genetic group 5, which is close to the genus *Geobacillus* both phenotypically and phylogenetically (Ash *et al.*, 1991; Rainey *et al.*, 1994; Caccamo *et al.*, 2000; Heinen & Heinen, 1972; Rainey & Stackebrandt, 1993; Nystrand, 1984; Nicolaus *et al.*, 1996; Popova *et al.*, 2002). Members of the genus *Geobacillus* contain iso-branched saturated fatty acids (iso-C15:0, iso-C16:0 and iso-C17:0) as the major fatty acids and exhibit high intrageneric similarity of their 16S rRNA gene sequences (96.5–99.2 %) (Nazina *et al.*, 2001; Fortina *et al.*, 2001a).

When studying the microbial communities of the Garga hot spring located in the north-eastern part of the Transbaikalian region, we isolated several dominant aerobic, thermophilic, spore-forming bacteria in pure cultures. The Garga spring is characterized by a maximum temperature of 75 °C and pH 7.8–8.5. When the water temperature decreases to about 60 °C, the microbial mats that occur in this spring begin to develop. The colour of the mats varies from orange on the surface to green in deeper layers. The mats contain oxygenic cyanobacteria and non-oxygenic green filamentous photosynthetic bacteria, for example *Chloroflexus* (V. M. Gorlenko, personal communication). The material used for the isolation of bacilli was taken from the upper layer of a microbial mat. The preliminary phenotypic and genotypic characterization of one of the isolates (strain Ga^T) suggested its affiliation with the genus *Geobacillus*. In this study, we report the relevant characteristics of strain Ga^T and the phenotypic and genotypic characteristics of the species *B. vulcani* (Caccamo *et al.*, 2000), which allow strain Ga^T to be classified as a novel species, *Geobacillus gargensis* sp. nov., and *B. vulcani* to be reclassified as *Geobacillus vulcani* comb. nov.

METHODS

Bacterial strains, maintenance and characterization. Strain Ga^T was isolated from the upper layer of a microbial mat by using the technique of serial dilutions and inoculation of an agar medium containing sucrose (Adkins *et al.*, 1992). The agar plates were incubated at 60 °C. The colonies that grew within a few days were transferred to a liquid medium of the same composition. The purity of the isolate was tested microscopically.

Cell morphology, physiological characteristics, common DNA characteristics, 16S rRNA gene sequences, phylogenetic properties and cellular fatty acid profile were studied as described previously (Nazina *et al.*, 2001).

The reference strains used in this study were obtained from the DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen) (*G. uzenensis* DSM 13551^T, *G. subterraneus* DSM 13552^T, *Bacillus vulcani* DSM 13174^T and *G. kaustophilus* DSM 7263^T), from the All-Russian Collection of Microorganisms (*G. uzenensis* B-2228, '*G. uralicus*' B-2276, *G. thermocatenulatus* B-1259^T and *Bacillus subtilis* B-4537) and from Christian Jeanthon (Laboratory of Marine Microbiology, Station Biologique, CNRS UPR9042, Roscoff, France) (*G. stearothermophilus* DSM 22^T, *G. thermoleovorans* DSM 5366^T and *G. thermodenitrificans* DSM 466).

RESULTS AND DISCUSSION

The morphological and chemical characteristics of strain Ga^T are consistent with its affiliation with the genus *Geobacillus*. Strain Ga^T produced small, round, mucous, small, non-pigmented colonies with a diameter of about 1 mm. Vegetative cells were motile (in the exponential phase) peritrichously flagellated rods with rounded ends (Fig. A, available as supplementary material in IJSEM Online). The cells were 1.0–1.5 µm wide and 6–12 µm long. The endospore was terminal and ellipsoid-shaped, and slightly distended the mother cell. Cell division was usually symmetric. The electron microscopic examination of cells showed that they are likely to be Gram-positive (Fig. B, available as supplementary material in IJSEM Online), since their cell wall did not have the external lipoprotein layer. The Gram-positive staining of the cell wall was also demonstrated by light microscopy. The cell wall had a complex structure, namely, it was composed of three to five layers. The outermost layer exhibited the presence of regularly arranged structures that are typical of S layers. The immature spores located within the sporangium contained the cytoplasm, polyribosomes, the coat and the cortex.

The physiological characteristics of strain Ga^T are given in Table 1 and in the species description. Strain Ga^T was found to be a thermophilic bacterium, since it could grow within the temperature range 45–70 °C, with an optimum growth temperature of 60–65 °C. The isolate grew well within the pH range 5.5–8.5, with an optimum pH for growth of 6.5–7.0. The isolate was able to utilize a variety of sugars, carboxylic acids and hydrocarbons aerobically. The bacterium was able to grow in synthetic media and did not require any growth factors, vitamins, NaCl or KCl. Significant culture turbidity was observed in a medium with sucrose as the sole carbon and energy source, with the generation time being 2.5 h.

16S rRNA gene sequence analysis

The analysis of the almost-complete 16S rRNA gene sequence (1459 nucleotides) of strain Ga^T, corresponding to *Escherichia coli* positions (Brosius *et al.*, 1978) 37–1479, showed that this strain has sequence similarity values of 94.6–99.3 % to the recognized species of the genus *Geobacillus* (Fig. 1). The strain formed a separate phylogenetic cluster, which was closely related to *G. kaustophilus* (99.3 % sequence similarity), *G. thermoleovorans* (99.1 % similarity), *G. thermocatenulatus* (99.0 % similarity) and

Table 1. Characteristics that differentiate *Geobacillus gargensis* sp. nov. from the closely related species of the genus *Geobacillus*

Taxa: 1, *G. gargensis* strain Ga^T; 2, *G. stearothermophilus*; 3, *G. thermocatenulatus*; 4, *G. thermoglucosidasius*; 5, *G. thermoleovorans*; 6, *G. kaustophilus*; 7, *G. thermodenitrificans*; 8, *G. uzenensis*; 9, *G. subterraneus*; 10, *G. caldioxysilyticus*; 11, *G. toebii*; 12, *G. vulcani*. Symbols: +, growth/activity observed; –, no growth/activity observed; W, weak growth; ND, not determined; V, variable within group. Data are from the present study (*G. gargensis*) or from Logan & Berkeley (1984), Claus & Berkeley (1986) and Priest *et al.* (1988) (*G. stearothermophilus*); Golovacheva *et al.* (1975), Nazina *et al.* (2001) and data from the present study (*G. thermocatenulatus*); Suzuki *et al.* (1983) (*G. thermoglucosidasius*); Zarilla & Perry (1987) and Nazina *et al.* (2001) (*G. thermoleovorans*); Priest *et al.* (1988) and White *et al.* (1993) (*G. kaustophilus*); Manachini *et al.* (2000) (*G. thermodenitrificans*); Nazina *et al.* (2001) (*G. uzenensis*; *G. subterraneus*); Ahmad *et al.* (2000) and Fortina *et al.* (2001a) (*G. caldioxysilyticus*); Sung *et al.* (2002) (*G. toebii*); Caccamo *et al.* (2000) and data from the present study (*B. vulcani*=*G. vulcani*). Strain Ga^T and all other strains are negative for indole production and are catalase-positive, and produce acid from glucose, fructose, maltose, mannose, sucrose and trehalose (in the description of *G. toebii*, most of these characteristics are absent).

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12
Cell width (µm)	1.0–1.5	0.6–1	0.9	0.5–1.2	1.5	≤3	0.5–1.0	0.9–1.7	0.8–1.5	0.5–1.0	0.5–0.9	0.6–0.8
Cell length (µm)	6.0–12	2–3.5	6–8	3–7	3.5	≤0.9	1.5–2.5	4.7–8.5	4.6–8.0	4–6	2.0–3.5	4.0–7.0
Motility	+	+	+	+	–	ND	ND	+	+	+	+	+
Production of acid from:												
Adonitol	–	V	–*	–	V	+	ND	–	–	ND	–	–*
L-Arabinose	–	–	–	–	–	–	+	+	–	+	–	–*
Cellobiose	+	–	+	+	+	+	+	+	+	+	–	+
Galactose	+	–	+	–	+	V	+	+	+	+	–	+
Glycerol	+	+	+	+	V	–	+	+	+	+	–	+
Inositol	–	–	–	–	–	+	ND	–	–	ND	+	–*
Lactose	–	–	–	–	–	–	+	–	–	+	–	+
Mannitol	+	V	+	+	+	+	+	+	ND	+	–	–
Rhamnose	–	–	–	+	–	–	V	–	–	V	–	–*
Ribose	+	–	+	–	+	–	+	+	+	+	–	+
Sorbitol	–	–	+	+	–	–	ND	–	–	+	–	–*
D-Xylose	–	–	–	+	V	+	+	–	–	+	–	+
Hydrolysis of:												
Aesculin	+	V	+	+	ND	–	ND	+	+	ND	–	+
Casein	+	V	W	+	+	+	V	–	–	+	+	–
Gelatin	–	+	–	–	–	+	ND	+	–	ND	–	+
Starch	+	+	+	+	+	+	V	+	+	+	–	+
Utilization of:												
n-Alkanes	+	+	+	ND	+	ND	+	+	+	ND	+	+
Formate	–	–	–*	ND	ND	V	ND	–	+	ND	–	–*
Acetate	+	V	+	ND	+	–	ND	+	+	ND	–	–*
Lactate	+	–	+	ND	ND	–	ND	+	+	W	–	–*
Citrate (Simmons)	–	V	+	–	+	+	V	–	–	V	–	+
Gas from glucose	–	–	–	–	ND	–	V	–	–	–	–	–*
NO ₃ [–] → NO ₂ [–]	+	V	+	+	ND	+	+	+	+	+	+	–
Gas from nitrate	–	V	+	–	–	–	+	–	+	V	+	–
Methyl red test	–	V	–*	+	ND	+	ND	–	+	ND	–	–*
Voges-Proskauer reaction	–	–	–	–	–	–	–	–	–	–	+	–*
Urease	–	–	–	+	ND	–	–	–	–	–	ND	–
NaCl range (% w/v)	0–1	0–≤5	0–4	0–1.5	ND	0–≤5	0–3	0–4	0–≤5	0–<3	0–<5	0–3
pH range	5.5–8.5	6.0–8.0	ND	6.5–8.5	6.2–7.5	6–8	6–8	6.2–7.8	6.0–7.6	6.8–7.2	6.0–9.0	5.5–9.0
Temperature range (°C)	45–70	37–65	35–78	42–69	45–70	37–68	45–70	45–65	45–70	42–70	45–70	37–72
DNA G+C content (mol%) (T _m)	52.9	46–52	55.2	45–46	52–58	51–55	48.2–52.3	50.4–51.5	49.7–52.3	44–50.2	43.9†	53.0

*Data from the present study.

†Determined by HPLC.

Fig. 1. Phylogenetic tree showing the positions of strain Ga^T and *B. vulcani* among *Geobacillus* species. The scale bar represents the expected number of changes per sequence position (the Jukes–Cantor distance). Bootstrap values (expressed as percentage of 100 replications) are shown at branch points; values greater than 95 % were considered significant. *G.*, *Geobacillus*; *B.*, *Bacillus*; *S.*, *Saccharococcus*. The GenBank/EMBL/DDBJ accession numbers of the reference strains are shown in parentheses.

B. vulcani (98.8 % similarity). The analysis also showed that *B. vulcani* was closely related to *G. kaustophilus* (99.4 % sequence similarity). The phylogenetic clustering of *B. vulcani* with members of the genus *Geobacillus*, with 95.3–99.4 % sequence similarity values, suggested that *B. vulcani* may actually represent a species of the genus *Geobacillus*. In order to verify the taxonomic positions of strain Ga^T and *B. vulcani* DSM 13174^T, they were subjected to DNA–DNA hybridization analysis with the type strains of most of the known *Geobacillus* species.

DNA base composition and DNA–DNA similarity

The G + C content of the DNA of strain Ga^T was found to be 52.9 ± 0.2 mol%. The DNA–DNA reassociation values of strain Ga^T with *B. vulcani* DSM 13174^T and the nine type or reference strains of the genus *Geobacillus* varied from 31 to 55 % (see Table A available in IJSEM Online). These values are within the range that is typical of a genospecies (30–70 %) (Wayne *et al.*, 1987). The DNA–DNA reassociation values indicate that both strain Ga^T and *B. vulcani* DSM 13174^T belong to the genus *Geobacillus* as separate species. The DNA–DNA hybridization values between the

thermophilic *Geobacillus* strains and a mesophilic strain of *B. subtilis*, taken as an outgroup, varied from 3 to 10 %.

Our earlier (Nazina *et al.*, 2001) and present data contradict the results of the hybridization experiments of Sunna *et al.* (1997), who reported a very high value of DNA–DNA reassociation (more than 75 %) between *Bacillus thermocatenulatus*, *Bacillus kaustophilus* and *Bacillus thermoleovorans*. This disagreement may be due to the absence of adequate hybridization controls at inter- and intraspecies levels in the experiments of Sunna *et al.* (1997). The DNA–DNA reassociation values obtained by Caccamo *et al.* (2000) for *B. kaustophilus*, *Bacillus thermodenitrificans* and *Bacillus stearothermophilus* were within a range of 40.4–61.4 %, which is in agreement with our data.

Fatty acids

The fatty acid profile of strain Ga^T (see Table B and Fig. C in IJSEM Online) was largely composed of iso-C15:0 (25.84 %), iso-C16:0 (25.91 %) and iso-C17:0 (19.79 %). In the fatty acid composition, strain Ga^T and the bacterium *B. vulcani* were clustered with the species of the genus *Geobacillus*, but not with the thermophilic species of the genera *Bacillus* and *Ureibacillus* or *Thermobacillus* and *Aneurinibacillus* (data not shown) (Kämpfer, 1994; Nazina *et al.*, 2001; Manachini *et al.*, 2000; Ahmad *et al.*, 2000; Fortina *et al.*, 2001a, b; Caccamo *et al.*, 2000; Andersson *et al.*, 1995).

Strain Ga^T differed both genotypically and phenotypically from the recognized species of the genus *Geobacillus*. The strain utilized acetate and lactate, but not citrate, and hydrolysed casein and aesculin, but not gelatin. The strain was also able to produce acids from glycerol and ribose, but not from inositol, lactose or xylose. The methyl red and the urea decomposition tests were negative. All these properties allowed us to differentiate strain Ga^T from *B. vulcani* and the representatives of the genus *Geobacillus*. Based on the results of the phenotypic and genotypic analyses, we conclude that strain Ga^T is a member of a novel *Geobacillus* species, for which we propose the name *Geobacillus gargensis* sp. nov.

Characteristics of reference strains

The phenotypic characteristics of *B. vulcani* DSM 13174^T and *G. thermocatenulatus* B-1259^T, additional to those available in the original description of these strains, are summarized in Table 1. The results of the Voges–Proskauer test and the test for the anaerobic growth of strain DSM 13174^T in glucose broth were negative (as with the other species of the genus *Geobacillus*). Strain DSM 13174^T grew on C₆ hydrocarbons, but not on C₁₄–C₁₆ hydrocarbons; no acids were produced from adonitol, arabinose, inositol, rhamnose or sorbitol. In contrast to their original descriptions (Golovacheva *et al.*, 1975; Caccamo *et al.*, 2000), strain B-1259^T hydrolysed starch, whereas strain DSM 13174^T was catalase-positive. A comparison of *B. vulcani* DSM 13174^T with the phylogenetically closest species *G. kaustophilus*

DSM 7263^T (99.4 % sequence similarity) indicated that they differ phenotypically in the ability to hydrolyse casein and to produce acids from glycerol, inositol, lactose and ribose. Thus, the species *B. vulcani* must be reclassified as *Geobacillus vulcani* comb. nov. on the basis of its physiological characteristics, phylogenetic position and DNA–DNA reassociation values.

Description of *Geobacillus gargensis* sp. nov.

Geobacillus gargensis [gar.g.en'sis. N.L. adj. *gargensis* of Garga, pertaining to the Garga hot spring located in Eastern Siberia (Russia), from which the type strain was isolated].

Cells are rod-shaped, motile by means of peritrichous flagella and produce terminally located ellipsoidal spores in slightly swollen sporangia. The cells are 1.0–1.5 µm wide and 6–12 µm long. Gram-positive, with the chemo-organotrophic type of metabolism. Aerobic. No acids are produced from raffinose. Utilizes hydrocarbons (C₁₂–C₁₆), butyrate, pyruvate, fumarate, succinate, peptone, tryptone, nutrient broth, potato agar and yeast extract as carbon and energy sources. No growth occurs on methanol, ethanol, propanol, butanol or isobutanol. Unable to grow autotrophically on H₂ + CO₂. Unable to produce NH₃ from peptone. H₂S is not produced. The egg-yolk lecithinase test is negative. Growth factors, vitamins, NaCl and KCl are not required for growth. The optimum temperature for growth is 60–65 °C, with an optimum pH of 6.5–7.0. Fatty acid profile consists of iso-C15:0 (25.84 %), iso-C16:0 (25.91 %), iso-C17:0 (19.79 %), anteiso-C17:0 (8.2 %), anteiso-C15:0 (1.99 %), C17:1 (4.21 %), iso-C17:1 (3.91 %) and iso-C18:0 (1.40 %). Carbon source utilization and hydrolysis of chromogenic substrates (including differentiating characters for all *Geobacillus* species) are indicated in Table 1.

The type strain is Ga^T (= VKM B-2300^T = DSM 15378^T). Isolated from the Garga hot spring.

Description of *Geobacillus vulcani* comb. nov.

Basonym *Bacillus vulcani* Caccamo *et al.* 2000.

Geobacillus vulcani (vul.ca'ni. L. gen. m. n. *vulcani* of the volcano, pertaining to the Aeolian Island volcano with a shallow marine hydrothermal vent, from which the organism was isolated).

Phylogenetic and chemotaxonomic data indicate that *Bacillus vulcani* is more closely related to species of the genus *Geobacillus* than to any species of the genus *Bacillus*. The cultural, morphological and physiological characteristics of the species, described by Caccamo *et al.* (2000), are valid, except that strain DSM 13174^T is catalase-positive. Fatty acid profile consists of iso-C15:0 (16.6 %), iso-C16:0 (14.6 %), iso-C17:0 (21 %), anteiso-C17:0 (11.4 %) and C18:0 (13 %). Carbon source utilization and hydrolysis of chromogenic substrates are indicated in Table 1.

The type strain is 3s-1^T (= DSM 13174^T = CIP 106305^T). Isolated from sediment of a shallow hydrothermal vent.

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REFERENCES

- Adkins, J. P., Cornell, L. A. & Tanner, R. S. (1992). Microbial composition of carbonate petroleum reservoir fluids. *Geomicrobiol J* 10, 87–97.
- Ahmad, S., Scopes, R. K., Rees, G. N. & Patel, B. K. C. (2000). *Saccharococcus caldoxylosilyticus* sp. nov., an obligately thermophilic, xylose-utilizing, endospore-forming bacterium. *Int J Syst Evol Microbiol* 50, 517–523.
- Andersson, M., Laukkanen, M., Nurmiaho-Lassila, E.-L., Rainey, F. A., Niemelä, S. I. & Salkinoja-Salonen, M. (1995). *Bacillus thermosphaericus* sp. nov., a new thermophilic ureolytic bacillus isolated from air. *Syst Appl Microbiol* 18, 203–220.
- Ash, C., Farrow, A. E., Wallbanks, S. & Collins, M. D. (1991). Phylogenetic heterogeneity of the genus *Bacillus* revealed by comparative analysis of small-subunit-ribosomal RNA sequences. *Lett Appl Microbiol* 13, 202–206.
- Brosius, J., Palmer, M. L., Kennedy, P. J. & Noller, H. F. (1978). Complete nucleotide sequence of a 16S ribosomal RNA gene from *Escherichia coli*. *Proc Natl Acad Sci U S A* 75, 4801–4805.
- 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.
- Fortina, M. G., Mora, D., Schumann, P., Parini, C., Manachini, P. L. & Stackebrandt, E. (2001a). Reclassification of *Saccharococcus caldoxylosilyticus* as *Geobacillus caldoxylosilyticus* (Ahmad *et al.* 2000) comb. nov. *Int J Syst Evol Microbiol* 51, 2063–2071.
- Fortina, M. G., Pukall, R., Schumann, P., Mora, D., Parini, C., Manachini, P. L. & Stackebrandt, E. (2001b). *Ureibacillus* gen. nov., a new genus to accommodate *Bacillus thermosphaericus* (Andersson *et al.* 1995), emendation of *Ureibacillus thermosphaericus* and description of *Ureibacillus terrenus* sp. nov. *Int J Syst Evol Microbiol* 51, 447–455.
- Golovacheva, R. S., Loginova, L. G., Salikhov, T. A., Kolesnikov, A. A. & Zaitseva, G. N. (1975). A new thermophilic species *Bacillus thermocatenulatus* nov. sp. *Microbiology* (English translation of *Mikrobiologiya*) 44, 230–233.
- Heinen, U. J. & Heinen, W. (1972). Characteristics and properties of a caldo-active bacterium producing extracellular enzymes and two related strains. *Arch Microbiol* 82, 1–23.
- Kämpfer, P. (1994). Limits and possibilities of total fatty acid analysis for classification and identification of *Bacillus* species. *Syst Appl Microbiol* 17, 86–98.

- Logan, N. A. & Berkeley, R. C. W. (1984). Identification of *Bacillus* strains using the API system. *J Gen Microbiol* **130**, 1871–1882.
- Manachini, P. L., Mora, D., Nicastro, G., Parini, C., Stackebrandt, E., Pukall, R. & Fortina, M. G. (2000). *Bacillus thermodenitrificans* sp. nov., nom. rev. *Int J Syst Evol Microbiol* **50**, 1331–1337.
- Markossian, S., Becker, P., Märkl, H. & Antranikian, G. (2000). Isolation and characterization of lipid-degrading *Bacillus thermoleovorans* IHI-91 from an icelandic hot spring. *Extremophiles* **4**, 365–371.
- Maugeri, T. L., Gugliandolo, C., Caccamo, D. & Stackebrandt, E. (2002). Three novel halotolerant and thermophilic *Geobacillus* strains from shallow marine vents. *Syst Appl Microbiol* **25**, 450–455.
- Nazina, T. N., Tourova, T. P., Poltarau, A. B., Novikova, E. V., Ivanova, A. E., Grigoryan, A. A., Lysenko, A. M. & Belyaev, S. S. (2000). Physiological and phylogenetic diversity of the thermophilic spore-forming hydrocarbon-oxidizing bacteria from oil fields. *Microbiology* (English translation of *Mikrobiologiya*) **69**, 96–102.
- Nazina, T. N., Tourova, T. P., Poltarau, A. B. & 8 other authors (2001). Taxonomic study of aerobic thermophilic bacilli: descriptions of *Geobacillus subterraneus* gen. nov., sp. nov. and *Geobacillus uzenensis* 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.
- Nicolaus, B., Lama, L., Esposito, E., Manca, M. C., di Prisco, G. & Gambacorta, A. (1996). “*Bacillus thermoantarcticus*” sp. nov., from Mount Melbourne, Antarctica: a novel thermophilic species. *Polar Biol* **16**, 101–104.
- Nystrand, R. (1984). *Saccharococcus thermophilus* gen. nov., sp. nov. isolated from beet sugar extraction. *Syst Appl Microbiol* **5**, 204–219.
- Popova, N. A., Nikolaev Yu, A., Tourova, T. P., Lysenko, A. M., Osipov, G. A., Verkhovtseva, N. V. & Panikov, N. S. (2002). *Geobacillus uralicus*, a new species of thermophilic bacteria. *Microbiology* (English translation of *Mikrobiologiya*) **71**, 335–341.
- Priest, F. G., Goodfellow, M. & Todd, C. (1988). A numerical classification of the genus *Bacillus*. *J Gen Microbiol* **134**, 1847–1882.
- Rainey, F. A. & Stackebrandt, E. (1993). Phylogenetic evidence for the relationship of *Saccharococcus thermophilus* to *Bacillus stearothermophilus*. *Syst Appl Microbiol* **16**, 224–226.
- 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.
- Sung, M.-H., Kim, H., Bae, J.-W. & 9 other authors (2002). *Geobacillus toebii* sp. nov., a novel thermophilic bacterium isolated from hay compost. *Int J Syst Evol Microbiol* **52**, 2251–2255.
- Sunna, A., Tokajian, S., Burghardt, J., Rainey, F., Antranikian, G. & Hashwa, F. (1997). Identification of *Bacillus kaustophilus*, *Bacillus thermocatenulatus* and *Bacillus* strain HSP as members of *Bacillus thermoleovorans*. *Syst Appl Microbiol* **20**, 232–237.
- Suzuki, Y., Kishigami, T., Inoue, K., Mizoguchi, Y., Eto, N., Takagi, M. & Abe, S. (1983). *Bacillus thermoglucosidasius* sp. nov., a new species of obligately thermophilic bacilli. *Syst Appl Microbiol* **4**, 487–495.
- Wayne, L. G., Brenner, D. J., Colwell, R. R. & 9 other authors (1987). International Committee on Systematic Bacteriology. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int J Syst Bacteriol* **37**, 463–464.
- White, D., Sharp, R. J. & Priest, F. G. (1993). A polyphasic taxonomic study of thermophilic bacilli from a wide geographical area. *Antonie Leeuwenhoek* **64**, 357–386.
- 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.