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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*

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Five hydrocarbon-oxidizing strains were isolated from formation waters of oilfields in Russia, Kazakhstan and China. These strains were moderately thermophilic, neutrophilic, motile, spore-forming rods, aerobic or facultatively anaerobic. The G+C content of their DNA ranged from 49·7 to 52·3 mol%. The major isoprenoid quinone was menaquinone-7; cellular fatty acid profiles consisted of significant amounts of iso-15:0, iso-16:0 and iso-17:0 fatty acids (61·7–86·8% of the total). Based on data from 16S rDNA analysis and DNA–DNA hybridization, the subsurface isolates could be divided into two groups, one of which consisted of strains U^T and X and the other of which consisted of strains K, Sam and 34^T. The new strains exhibited a close phylogenetic relationship to thermophilic bacilli of 'Group 5' of Ash *et al*. [Ash, C., Farrow, J. A. E., Wallbanks, S. & Collins, M. D. (1991). *Lett Appl Microbiol* 13, 202–206] and a set of corresponding signature positions of 16S rRNA. Comparative analysis of the 16S rDNA sequences and fatty acid compositions of the novel isolates and established species of thermophilic bacilli indicated that the subsurface strains represent two new species within a new genus, for which the names *Geobacillus subterraneus* gen. nov., sp. nov., and *Geobacillus uzenensis* sp. nov. are proposed. It is also proposed that *Bacillus stearothermophilus, Bacillus thermoleovorans, Bacillus thermocatenulatus, Bacillus kaustophilus, Bacillus thermoglucosidasius* and *Bacillus thermodenitrificans* be transferred to this new genus, with *Geobacillus stearothermophilus* (formerly *Bacillus stearothermophilus*) as the type species.

Keywords: thermophilic *Bacillus* group 5, *Geobacillus subterraneus*, *Geobacillus uzenensis*, 16S rDNA analysis, fatty acids

The GenBank accession numbers for the 16S rDNA sequences of strains U^T, X, 34^T, K and Sam are AF276304–AF276308.

INTRODUCTION

The genus *Bacillus* is a large and diverse collection of aerobic and facultatively anaerobic, rod-shaped, Gram-positive (to Gram-variable), endospore-forming bacteria (Claus & Berkeley, 1986). The genus includes thermophilic and psychrophilic, acidophilic and alkalophilic, freshwater and halophilic bacteria that utilize a wide range of carbon sources for heterotrophic growth or grow autotrophically. 16S rRNA gene sequence analysis has revealed high phylogenetic heterogeneity in the genus *Bacillus* (Ash *et al*., 1991; Rainey *et al*., 1994). Seven phylogenetic groups have been reclassified as the new genera *Alicyclobacillus* (Wisotzkey *et al*., 1992), *Paenibacillus* (Ash *et al*., 1993; Heyndrickx *et al*., 1996), *Brevibacillus* (Shida *et al*., 1996), *Aneurinibacillus* (Shida *et al*., 1996; Heyndrickx *et al*., 1997), *Virgibacillus* (Heyndrickx *et al*., 1998), *Salibacillus* and *Gracilibacillus* (Wainø *et al*., 1999).

Currently, thermophilic aerobic spore-forming bacteria having growth optima in the temperature range 45 to > 70 °C are classified into the genera *Bacillus*, *Alicyclobacillus*, *Brevibacillus*, *Aneurinibacillus*, *Sulfobacillus*, *Thermoactinomyces* and *Thermobacillus* (Sneath, 1986; Wisotzkey *et al*., 1992; Dufresne *et al*., 1996; Heyndrickx *et al*., 1997; Touzel *et al*., 2000). The majority of the thermophilic species described belong to the genus *Bacillus* genetic groups 1 and 5, as judged from their 16S rRNA sequences (Ash *et al*., 1991; Rainey *et al*., 1994). The thermophilic species *Bacillus smithii* and *Bacillus coagulans* fall into group 1, along with the type species of the genus, *Bacillus subtilis*, and other mesophilic species. *Bacillus tusciae* is related to the genus *Alicyclobacillus* according to RDP grouping and *Bacillus thermocloaceae* and *Bacillus thermosphaericus* represent distinct lineages (Rainey *et al*., 1994; Andersson *et al*., 1995).

Group 5 is a phenotypically and phylogenetically coherent group of thermophilic bacilli displaying very high similarity among their 16S rRNA sequences (98·5–99·2 %). This group comprises established species of thermophilic bacilli (*Bacillus stearothermophilus*, *Bacillus thermocatenulatus*, *Bacillus thermoleovorans*, *Bacillus kaustophilus*, *Bacillus thermoglucosidasius* and *Bacillus thermodenitrificans*), species that have not been validly published ('*Bacillus caldolyticus*', '*Bacillus caldotenax*', '*Bacillus caldovelox*' and '*Bacillus thermoantarcticus*') and the asporogenous species *Saccharococcus thermophilus*, representing a separate line of descent (Ash *et al*., 1991; Rainey *et al*., 1994; Sunna *et al*., 1997; Nicolaus *et al*., 1996; Manachini *et al*., 2000). Phylogenetic analysis has revealed that the genus *Bacillus* and its thermophilic members require extensive taxonomic revision (Stackebrandt *et al*., 1987; Ash *et al*., 1991; Rainey *et al*., 1994).

We have studied high-temperature oilfields from different geographical areas (Nazina *et al*., 1992, 1995, 2000). An active and diverse microbial community, including hydrocarbon-oxidizing bacteria, was found in these deep subsurface ecosystems. Several pure cultures of dominant aerobic, thermophilic, hydrocarbon-oxidizing bacteria were isolated. A preliminary characterization of these bacteria based on phenotypic features indicated that they belong to the genus *Bacillus* (Nazina *et al*., 1993). Comparative 16S rDNA analysis revealed that five isolates clustered with group 5 of the genus *Bacillus* and might represent two novel species (Nazina *et al*., 2000).

In order to examine the taxonomic position of the five strains U^T, X, K, Sam and 34^T within the family *Bacillaceae*, we determined virtually complete sequences of their 16S rRNA genes and compared these sequences with those available for other members of the *Bacillaceae*. These isolates were characterized further by electron microscopy methods, including thin sectioning, by studying cultural and physiological features and by determination of fatty acid composition and DNA–DNA similarity to other thermophilic *Bacillus* species. In this paper, we describe these strains as members of two new species, *Geobacillus subterraneus* (strains 34^T, K, Sam) and *Geobacillus uzenensis* (strains U^T, X), of the new genus *Geobacillus*, which comprises group 5 of the genus *Bacillus* and contains the transferred species *Geobacillus stearothermophilus* comb. nov., *Geobacillus thermoleovorans* comb. nov., *Geobacillus thermocatenulatus* comb. nov., *Geobacillus kaustophilus* comb. nov., *Geobacillus thermoglucosidasius* comb. nov. and *Geobacillus thermodenitrificans* comb. nov.

METHODS

Source of bacterial strains and maintenance of cultures. The isolation of subsurface bacterial strains was carried out using tenfold serial dilutions of thermophilic hydrocarbon-oxidizing enrichments from different oilfields. The dilutions were inoculated on agar medium (Zarilla & Perry, 1987) containing *n*-hexadecane. Incubation was carried at 55–60 °C. Strain Sam was isolated after inoculation in agar medium (Adkins *et al*., 1992) containing nitrate and acetate. Colonies were formed after a few days and were then transferred into liquid medium of the same composition. The purity of isolates was checked microscopically. The designation and origin of the isolates as well as those of the reference strains used are compiled in Table 1. The temperature of the oilfields investigated was 50–80 °C and the formation water had total salinity in the range of 3·5–18·0 g l^{-1} and pH 6·2–7·8 (Nazina *et al*., 1992, 1995, 2000). Strains were cultured on nutrient agar (Difco), potato agar or mineral medium (Adkins *et al*., 1992) with *n*-hexadecane (0·1 %, v/v) as the substrate.

Light and electron microscopy. Cell morphology was examined under a Zetopan phase-contrast microscope. Bacterial size was determined in living cell preparations from cultures grown on medium with sucrose (0·2 %, w/v) and mannitol (0·2 %, w/v) (Adkins *et al*., 1992) for 12–18 h. Gram staining was performed using a Merck kit. Negative staining of cells was achieved with 1 % phosphotungstic acid and cells were examined under a transmission electron microscope. The fine structure of cells was studied after fixation of cells with a 1 % (w/v) solution of OsO_4 in

Table 1 Strains isolated from oilfields and reference strains used in this study

Taxon	Origin/source
Strain U^T	New isolate from Uzen oilfield, Kazakhstan
Strain X	New isolate from Mykhpaiskoe oilfield, Western Siberia, Russia
Strain 34^T	New isolate from Liaohe oilfield, People's Republic of China
Strain Sam	New isolate from Samotlor oilfield, Western Siberia, Russia
Strain K	New isolate from Uzen oilfield, Kazakhstan
B. stearothermophilus 22^T	C. Jeanthon* (DSM 22^T)
B. thermoleovorans 5366^T	C. Jeanthon (DSM 5366^T)
B. thermodenitrificans 466	C. Jeanthon (DSM 466)
B. thermocatenulatus B-1259^T	All-Russian Collection of Microorganisms B-1259^T (= DSM 730^T)
B. subtilis B-4520	All-Russian Collection of Microorganisms B-4520 (= ATCC 15841)
B. subtilis B-4537	All-Russian Collection of Microorganisms B-4537 (= ATCC 6633)

* Isolates obtained from Christian Jeanthon, Laboratory of Marine Microbiology, Station Biologique, CNRS UPR9042, Roscoff, France.

acetate/veronal buffer (pH 6·2) by the method of Ryter & Kellenberger (1958). The cells were placed into agar, dehydrated with ethanol of increasing concentration (30, 50, 70, 80, 96 and 100 %) and then with acetone and embedded in Epon 812 (Fluka). Ultrathin sections were obtained on an LKB-4800 ultramicrotome and contrasted with a 3 % (w/v) aqueous solution of uranyl acetate with subsequent treatment with lead citrate (Reynolds, 1963). A JEM-100C electron microscope was used at magnifications of $\times$10000–30000.

Physiological characterization. Bacterial growth was monitored by measuring the OD_{600} (Spekol 11) in liquid media or by determination of protein content with a Merck kit. The effect of salinity (at 55 °C) or temperature on the growth of strains was tested in nutrient broth. The effect of pH on growth was determined in media adjusted to the appropriate pH with HCl or NaOH and recorded after 3 d at 55 °C.

Anaerobic growth was tested by incubation of the cultures in 100-ml rubber-sealed screw-cap bottles containing anoxic medium (Adkins *et al*., 1992) with 2 g acetate, peptone or glucose l^{-1} as potential substrates and 0·85 g KNO_3 l^{-1} or in acetate/ferric citrate medium (Lovley & Phillips, 1988). Pure argon was used as the gas phase. Occurrence of denitrification was confirmed using a GC equipped with a Porapak Super Q column and a flame-ionization detector. Iron reduction was monitored visually: positive cultures change their colour from green to clear during growth.

Gelatin liquefaction, starch hydrolysis, casein and tyrosine decomposition, phenylalanine deamination, the Voges–Proskauer reaction, nitrate and nitrite reduction, H_2S and indole production, catalase reaction, acid production from carbohydrates, citrate and propionate utilization were all examined by the methods described by Logan & Berkeley (1984). The organic acid utilization tests were performed in a basal medium (Adkins *et al*., 1992) supplemented with a separately sterilized solution of one of the substrates. Carbohydrates were added at 5 g l^{-1}, sodium salts of organic acids at 2·5 g l^{-1} and hydrocarbons at 5 ml l^{-1}. Strains were incubated at 55 °C. All assays were performed in duplicate and repeated in at least three consecutive culture transfers. Readings were made at days 1, 2 and 7.

DNA analysis. Bacterial DNA was prepared according to Marmur (1961). The G+C content of DNA was determined by the thermal denaturation method using the DNA of *Escherichia coli* K-12 as a standard (Owen *et al*., 1969). DNA–DNA hybridization was carried out as described by De Ley *et al*. (1970).

Sequencing of the 16S rRNA gene. The sets of primers used to amplify and sequence 16S rDNA were as described by Brosius *et al*. (1978) and Edwards *et al*. (1989). Amplification was performed in a Perkin-Elmer temperature controller. Fifty microlitres of the reaction buffer [170 mM $(NH_4)_2SO_4$, 60 mM Tris/HCl, 20 mM $MgCl_2$, pH 8·8] was supplemented with 50–100 ng genomic DNA as the template, 5 pmol of each primer and 5 U of *Taq* polymerase (BioMaster). Amplification involved 30 cycles of DNA denaturation at 94 °C for 1 min, primer annealing at 40 °C for 1 min and elongation at 72 °C for 2 min. The amplified fragment of the 16S rRNA gene was purified on agarose gel and sequenced on an ABI 373A automated DNA sequencer using a Ready Reaction Dye Terminator Sequencing kit with Amplitaq DNA polymerase FS (Applied Biosystems).

Phylogenetic analysis. The nucleotide sequences of the 16S rRNA genes from thermophilic bacilli were aligned manually against representatives of the genus *Bacillus* and related taxa available from the latest versions of the Ribosomal Database Project (Larsen *et al*., 1993) and GenBank databases. Sequence positions for which not all nucleotides had been determined were excluded from the analysis and a total of 1217 nucleotides were compared. Pairwise evolutionary distances (expressed as estimated changes per 100 nucleotides) were computed by use of the correction of Jukes & Cantor (1969). A phylogenetic tree was constructed by the neighbour-joining method (Saitou & Nei, 1987) with the bootstrap analysis of 100 trees by the TREECON software package (Van de Peer & De Wachter, 1994).

Analyses of cellular fatty acids and quinones. Whole-cell fatty acids and the main type of isoprenoid quinones were determined using biomass grown on nutrient agar at 55 °C for 18–24 h. Fatty acids were extracted from lyophilized cells. To 30 mg of dry biomass, 200 µl of a 5·4 M solution of anhydrous HCl in methanol was added and the mixture was heated at 70 °C for 2 h. The methyl esters of fatty acids and aldehyde derivatives obtained were extracted twice with 100 µl hexane. The extract was dried and silylated in 20 µl *N*,*O*-bis(trimethylsilyl)trifluoroacetamide for 15 min at

Table 2 Characteristics that differentiate the thermophilic bacilli from oilfields from thermophiles of *Bacillus* group 5

Taxa are indicated as: 1, *G. uzenensis* strain U^T; 2, *G. uzenensis* strain X; 3, *G. subterraneus* strain Sam; 4, *G. subterraneus* strain K; 5, *G. subterraneus* strain 34^T; 6, *B. stearothermophilus*; 7, *B. thermoglucosidasius*; 8, *B. thermocatenulatus*; 9, *B. thermoleovorans*; 10, *B. kaustophilus*; 11, *B. thermodenitrificans*. Characteristics are scored as: +, 90% or more of strains are positive; D, 11–89% of strains are positive; −, 90% or more of strains are negative. ND, Not determined. Data were obtained from the present study (*G. uzenensis*, *G. subterraneus*) or from Suzuki *et al.* (1983), White *et al.* (1993) and Priest *et al.* (1988) (*B. thermoglucosidasius*), Claus & Berkeley (1986) (*B. stearothermophilus*), Golovacheva *et al.* (1975) (*B. thermocatenulatus*), Zarilla & Perry (1987) (*B. thermoleovorans*), White *et al.* (1993) (*B. kaustophilus*) and Manachini *et al.* (2000) (*B. thermodenitrificans*) unless otherwise indicated.

Characteristic	1	2	3	4	5	6	7	8	9	10	11
Cell width (μm)	0·9–1·3	1·0–1·7	0·8–1·5	1·0–1·5	0·8–1·2	0·6–1	< 3	0·5–1·2	0·9	1·5	0·5–1·0
Cell length (μm)	4·7–8·0	5·5–8·5	5·5–8·0	4·7–7·0	4·6–6·6	2–3·5	< 0·9	3–7	6–8	3·5	1·5–2·5
Motility	+	+	+	+	+	+	ND	+	+	−	ND
Production of acid from:											
Adonitol	−	−	−	−	−	ND	+	−	ND	ND	ND
L-Arabinose	+	+	−	−	−	D	−	−	−	D	+
Cellobiose	+	+	+	+	+	−	+	+	+	+	+
Galactose	+	+	+	+	+	−	D	−	+	+	+
Ribose	+	+	+	+	+	ND	−	ND	ND	+	+
Glycerol	+	+	+	+	+	+	−	+	+	D	+
Inositol	−	−	−	−	−	−	+	−	−*	−*	ND
Lactose	−	−	−	−	−	−	−	−	−	−	+
Rhamnose	−	−	−	−	−	−	−	+	−	−	−
Sorbitol	−	−	−	−	−	−	−	+	ND	−	ND
D-Xylose	−	−	−	−	−	D	+	+	−	D	+
Hydrolysis of:											
Gelatin	+	+	−	−	−	D	+	−	−	ND	ND
Casein	−	−	−	−	−	D	+	+	ND	+	−
Starch	+	+	+	+	+	+	+	+	−	D	+
Aesculin	+	+	+	+	+	ND	−	+	ND	ND	ND
Utilization of:											
n-Alkanes	+	+	+	+	+	+*	ND	ND	+*	+	ND
Formate	−	−	+	+	+	−	D	ND	ND	ND	ND
Acetate	+	+	+	+	+	+	−	ND	ND	+	ND
Lactate	+	+	+	+	+	−	−	ND	ND	ND	ND
Citrate (Simmons)	−	−	−	−	−	D	+	D	+	ND	ND
Fermentation of glucose	−	−	−	−	−	D	−	−	+	−	ND
Denitrification	−	−	+	+	+	−	ND	−	+	ND	+
Methyl red test	−	−	+	+	+	D	−	D	ND	ND	ND
NaCl range (% w/v)	0–4	0–4	0–5	0–5	0–3	0–5	0–< 5	0–1·5	0–4	ND	0–3
pH range	6·2–7·8	6·2–7·8	6·0–7·8	6·2–7·6	6·2–7·6	6·0–8·0	6·0–8·0	6·5–8·5	6·2–7·8	6·2–7·5	6–8
Temperature range (°C)	45–65	45–65	45–70	48–70	45–65	37–65	37–68	42–69	35–78	40–75	45–70
DNA G+C content (mol%) (T_m)	50·4	51·5	52·3	49·7	52·3	51·9†	53·9	45–46	55·2*	52–58	48·2–52·3

* Data obtained in the present study.

† Data obtained from Fahmy *et al.* (1985).

65 °C. Aliquots of 1 μl of the reaction mixture were analysed with a model HP-5985B GC/MS system (Hewlett Packard) equipped with a fused-quartz capillary column (25 m × 0·25 mm) containing an Ultra-1 non-polar methylsilicone phase. The temperature program was run from 150 °C (2-min isotherm) to 250 °C at 5 °C min^{-1} and then from 250 to 300 °C at 10 °C min^{-1}. Data processing was carried out with an HP-1000 computer by using the standard programs of the GC/MS system (Hewlett Packard). A dendrogram based on principal-component analysis of whole fatty acid composition of the organisms was generated by treating the Euclidian distances of the fatty acids with the unweighted pair group method with arithmetic averages (UPGMA) algorithm.

Isoprenoid quinones were extracted by treating 100 mg lyophilized cells with chloroform–methanol (2:1, v/v) for 2 h, using a reciprocal shaker (120 strokes per min) at room temperature (Collins, 1985). The extracted solution was concentrated using a rotary vacuum evaporator and transferred by redissolving in acetone. The resulting solution was evaporated and separated by TLC using *n*-hexane–dimethyl ether (85:15, v/v) as the solvent. Quinones were detected at 269 nm, recovered from the TLC plate and dissolved in methanol. Quinones were analysed with a Finnigan Mat 8430 GC/MS system.

Nucleotide sequence accession numbers. The accession numbers of the reference strains used in the phylogenetic analyses are as follows: *B. stearothermophilus*, X60640; *B. thermocatenulatus*, Z26926; *B. thermodenitrificans*, Z26928; *B. thermoleovorans*, M77488; *B. kaustophilus*, X70430; '*B. caldolyticus*', M77484; '*B. caldotenax*', X62180; '*B. caldovelox*', M77485; *B. thermoglucosidasius*, X60641; '*Bacillus caldoxylolyticus*', AF067651; '*Bacillus thermoalkalophilus*', Z26931; *Bacillus pallidus*, Z26930; '*Bacillus flavothermus*', AF001960; *B. smithii*, Z26935; *Bacillus thermoamylovorans*, L27478; *Bacillus thermoaerophilus*, Z26934; *Bacillus licheniformis*, X60623; *B. coagulans*, X60614; *Bacillus infernus*, U20385; *B. subtilis*, X60646; *B. thermosphaericus*, X90640; *Bacillus schlegelii*, Z26934; *B. thermocloaceae*, Z26939; *B. tusciae*, Z26933; *Aneurinibacillus thermoaerophilus*, X94196; *Brevibacillus thermoruber*, Z26921; *Alicyclobacillus acidocaldarius*, X60742; *Saccharococcus thermophilus*, L09227;

Sulfobacillus thermosulfidooxidans, X91080; and *Thermobacillus xylanilyticus*, AJ005795.

RESULTS

As a result of microbiological analysis of the formation waters of high-temperature oilfields located in different geographical areas, five pure cultures (strains U^T, X, 34^T, K and Sam) of thermophilic, aerobic, hydrocarbon-oxidizing, spore-forming bacteria were obtained (Nazina *et al*., 1993, 1995, 2000).

Colony and cell morphology

On the surface of nutrient agar, all strains formed round, mucous, small, colourless colonies with a diameter of about 1 mm (strains U^T, X, 34^T and K) or about 5 mm (strain Sam). The vegetative cells of all strains were motile (in the exponential phase) peritrichous rods with rounded ends, sometimes occurring in chains (strain X). Many sporulating cells and separately lying refractive spores could be observed in old cultures. Endospores were terminal or subterminal (strain Sam) and ellipse-shaped and did not distend the mother cell or distended it slightly (strain U^T). Morphological and cultural characteristics are presented in Table 2.

Electron-microscopic examination showed a typical Gram-positive cell envelope profile (Fig. 1), which was confirmed by Gram staining. Only strain X stained Gram-negative. Terminally swollen (strain U^T) or non-swollen sporangia liberated ellipsoidal spores. No parasporal bodies were found. A multilayered cell

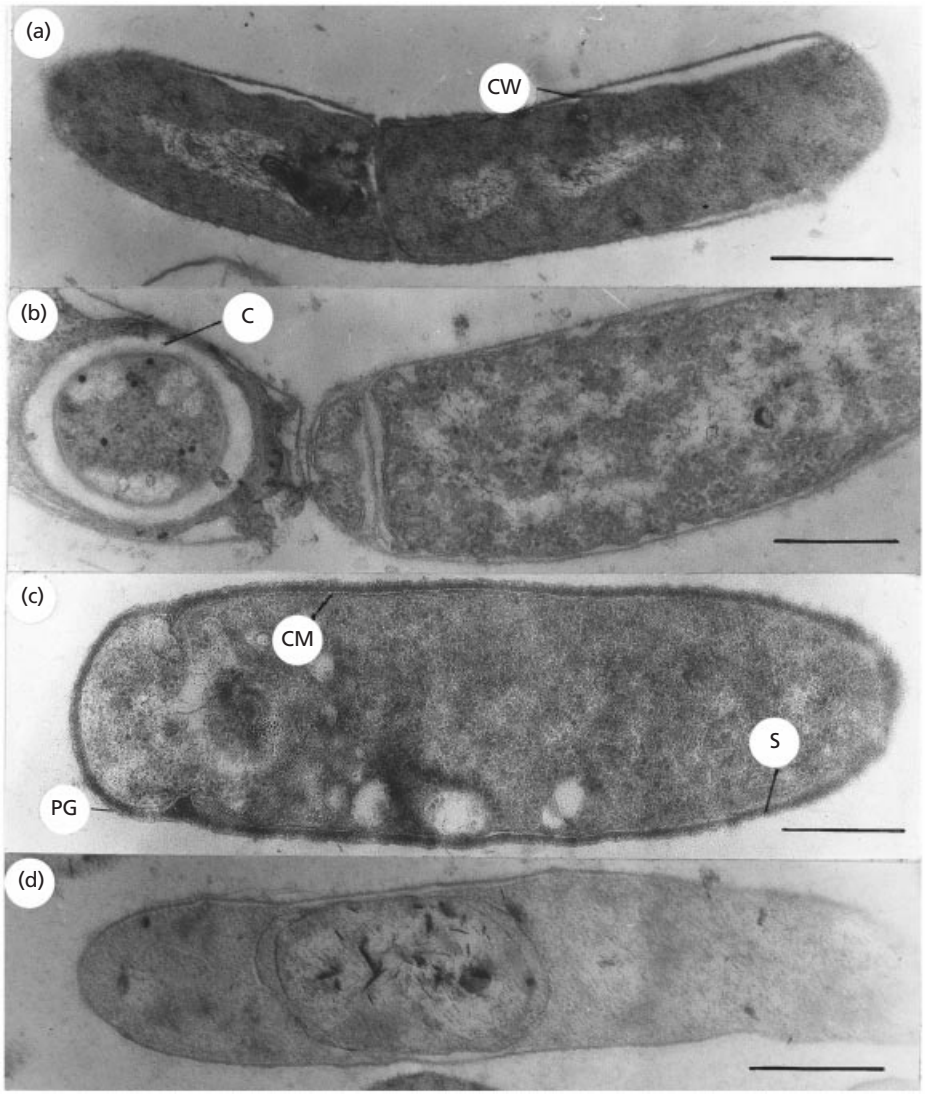

Fig. 1. Thin sections of 2-d-old vegetative and sporulating cells of strains U^T (a), X (b), K (c) and Sam (d), showing round to ovoid spores located terminally (b) or subterminally (d) within the sporangium. The cell envelope shows multiple layers, where the outermost layer exhibits the regularly arranged structure of the S layer. CW, Cell wall; CM, cytoplasmic membrane; PG, peptidoglycan; S, S layer; C, cortex. Bars, 0·5 μm.

Table 3 DNA G+C content and DNA–DNA homology (%) among the thermophilic isolates from oilfields and reference strains of the genus *Bacillus*

Taxon	DNA G+C content (mol%)		Reassociation (%) with DNA from:										
	Genome	16S rDNA	1	2	3	4	5	6	7	8	9	10	11
1. *B. stearothermophilus* 22^T	52·2	52·4	100										
2. *B. thermodenitrificans* 466	49·6	59·0	32	100									
3. *B. thermoleovorans* 5366^T	53·7	59·0	51	31	100								
4. *B. thermocatenulatus* B-1259^T	55·2	60·0	37	47	51	100							
5. Strain U^T	50·4	56·0	38	45	45	54	100						
6. Strain X	51·5	57·5	33	43	48	51	80	100					
7. Strain K	49·7	58·3	39	44	41	44	32	37	100				
8. Strain Sam	52·3	ND	37	47	45	48	42	44	93	100			
9. Strain 34^T	52·3	56·4	53	45	48	50	49		96	91	100		
10. *B. subtilis* B-4520	44·8	ND	3	5	7	4	7	9	4	5	5	100	
11. *B. subtilis* B-4537	44·1	ND	3	4	6	3	9	10	3	2	5	84	100
12. *E. coli* K-12	51·7	ND											

ND, Not determined.

envelope is seen in Fig. 1. The cytoplasmic membrane was surrounded by a thin peptidoglycan layer; an overlaid surface layer was separated from peptidoglycan by a zone of low contrast. Cell division was frequently asymmetric. In micrographs of immature spores within sporangia, the following structures were observed: mother cell wall, mother cell membrane, mother cell cytoplasm, spore coats and cortex (Fig. 1).

Nutritional requirements and physiology

The subsurface isolates were moderately thermophilic organisms able to utilize aerobically a large variety of sugars, carboxylic acids, alcohols, hydrocarbons and petroleum (Table 2). Strains 34^T, K and Sam could grow anaerobically, reducing nitrate to N_2; strains U^T and X reduced nitrate only to nitrite. Bacteria developed on synthetic media and did not require growth factors, vitamins, NaCl or KCl. Good growth was observed on potato agar and nutrient agar. Growth in liquid media with hydrocarbons was characterized by low biomass yields (300–500 mg of wet cells l^{-1}) compared with the yield of other thermophilic, hydrocarbon-oxidizing bacteria (Zarilla & Perry, 1987). Significant visible turbidity was observed in media with glucose or fructose as substrates, with a cell yield of 3–6 g l^{-1} (wet weight) and a doubling time (t_d) of 2–3 h.

All five strains produced acid but no gas from cellobiose, galactose, glucose, fructose, glycerol, maltose, mannose, ribose, sucrose and trehalose. No acid was formed from adonitol, inositol, lactose, raffinose, rhamnose, sorbitol or xylose. The substrates used by all strains as energy and carbon sources included hydrocarbons (C_{10}–C_{16}), methane–naphthenic and naphthenic–aromatic oils, peptone, tryptone, nutrient broth, yeast extract, acetate, butyrate, pyruvate, benzoate, lactate, fumarate, succinate, ethanol, phenol and phenylacetate. All five strains failed to grow autotrophically on H_2+CO_2 or to ferment glucose, were catalase-positive, hydrolysed starch and aesculin and produced NH_3 from peptone. Phenylalanine was not deaminated, Fe^{3+} was not reduced, casein was not hydrolysed and urea and tyrosine were not decomposed. H_2S, indole and dihydroxyacetone were not produced and the egg yolk lecithinase reaction and Voges–Proskauer reaction were negative.

Strains K, Sam and 34^T differed from strains U^T and X in their ability to grow on formate, to produce nitrogen from nitrate and to give a positive reaction in the methyl red test and by their failure to hydrolyse gelatin and to produce acid from arabinose.

The strains grew optimally at pH 6·8–7·0, 55–60 °C and with 0·5–1 % (w/v) NaCl in the medium. Thus, as a result of phenotypic investigation, the subsurface isolates could be divided into two groups: the first included strains U^T and X and the second included strains K, Sam and 34^T.

G+C content and DNA–DNA homologies

For DNA analysis, we used the subsurface isolates and reference strains of the thermophilic species *B. stearothermophilus* 22^T, *B. thermoleovorans* 5366^T, *B. thermocatenulatus* B-1259^T and *B. thermodenitrificans* 466, which grow at 50–60 °C and have G+C contents of 50 mol% or above (Fahmy *et al.*, 1985; Claus & Berkeley, 1986). Our data on base composition calculated from the thermal melting points (T_m) (Table 3) differed from published data by less than 0·6 mol% for *B. stearothermophilus* 22^T and *B. thermodenitrificans* 466 (Fahmy *et al.*, 1985; Claus & Berkeley, 1986). A greater difference of 4·3 mol% was observed for *B.*

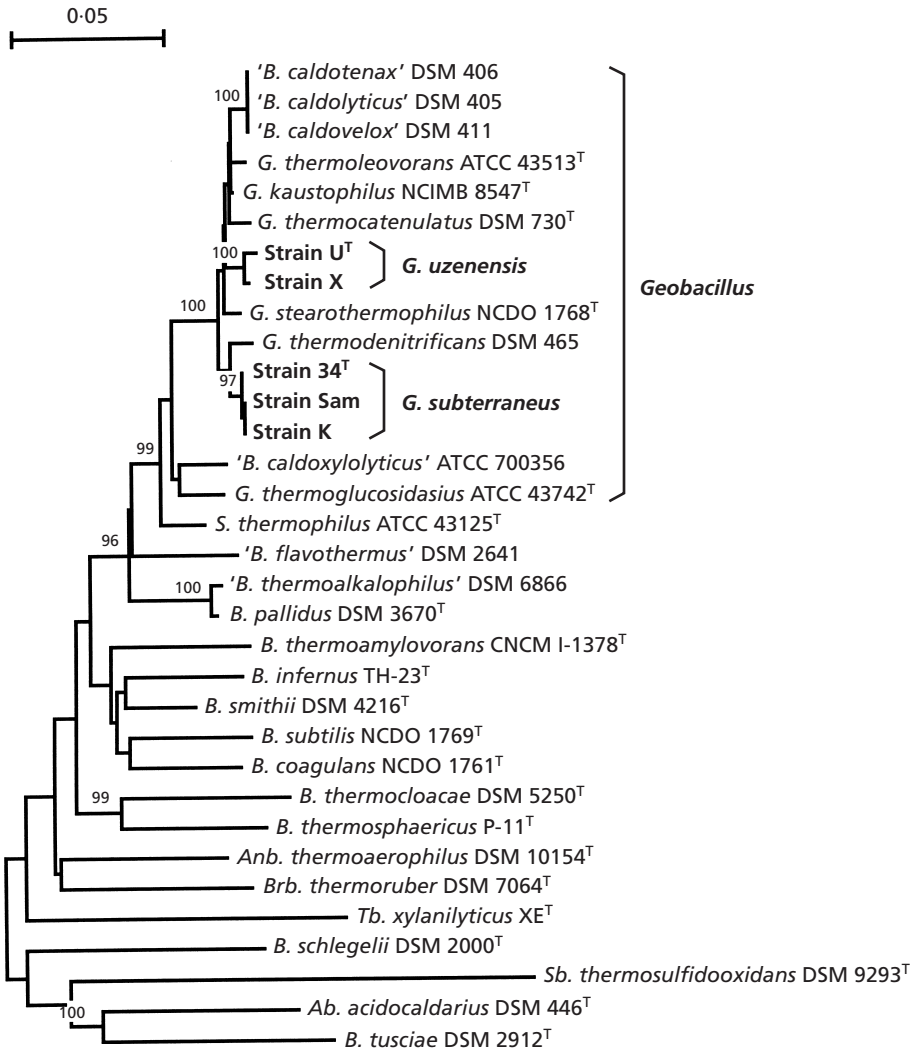

Fig. 2. Phylogenetic positions of the subsurface isolates (strains U^T, K, X, 34^T and Sam) among other thermophilic members of family *Bacillaceae*. Bootstrap values (expressed as percentages of 100 replications) are shown at branch points; values greater than 85% were considered significant. The bar indicates the Jukes–Cantor distance. Abbreviations: *G., Geobacillus; B., Bacillus; Anb., Aneurinibacillus; Brb., Brevibacillus; Sb., Sulfobacillus; Ab., Alicyclobacillus; Tb., Thermobacillus.*

thermoleovorans 5366^T (Zarilla & Perry, 1987). The G+C content of DNA of *B. thermocatenulatus* B-1259^T was determined to be 55·2 mol%, which was lower than the value reported previously for this strain (69 mol%; Golovacheva *et al.*, 1975). The five strains from oilfields had DNA G+C contents in the range 49·7–52·3 mol%, similar to those of other thermophiles of *Bacillus* rRNA group 5.

The level of DNA–DNA homology between strains U^T and X was 80%, and that between strains K, Sam and 34^T was 91–96%. The levels of homology between the two groups of strains were significantly lower (32–49%) and corresponded to those observed between the known thermophilic species of group 5 bacilli (31–51%). The levels of DNA homology between all nine thermophilic strains and two mesophilic strains, *B. subtilis* B-4520 and B-4537, were 10%.

16S rDNA sequence analysis

Almost complete 16S rDNA sequences of strains U^T, X, K and 34^T (more than 1500 nucleotides) and a partial sequence of strain Sam (430 nucleotides between positions 84 and 502 of the *Escherichia coli* nomenclature) were determined. These strains had G+C contents of 16S rDNA in the range 55·8–58·3 mol%. 16S rDNA sequence analysis showed that the new strains fall within group 5 of the genus *Bacillus* of the Gram-positive subdivision of the *Bacteria* (Ash *et al.*, 1991; Rainey *et al.*, 1994; Nazina *et al.*, 2000).

Table 4 Cellular fatty acid composition (% w/w) of the thermophilic isolates from oilfields and the recognized species of thermophilic bacilli

Taxa are identified as: 1, strain U^T; 2, strain X; 3, strain Sam; 4, strain K; 5, strain 34^T; 6, *B. thermodenitrificans* 466; 7, *B. thermoleovorans* 15366^T; 8, *B. thermocatenulatus* B-1259^T; 9, *B. stearothermophilus*; 10, '*B. caldotenax*' DSM 406; 11, '*B. caldovelox*' DSM 411; 12, '*B. caldolyticus*' DSM 405; 13, *B. thermoglucosidasius*; 14, *B. licheniformis*; 15, *B. smithii* DSM 459^T; 16, *B. thermosphaericus* P11^T; 17, *Aneurinibacillus thermoaerophilus* DSM 10154^T. Fatty acid abbreviations: 10:0, decanoic acid; 12:0, dodecanoic acid; a13:0, 10-methyl dodecanoic acid; i14:0, 12-methyl tridecanoic acid; 14:0, tetradecanoic acid; i15:0, 13-methyl tetradecanoic acid; a15:0, 12-methyl tetradecanoic acid; 15:0, pentadecanoic acid; i16:0, 14-methyl pentadecanoic acid; 16:0, hexadecanoic acid; i17:0, 15-methyl hexadecanoic acid; a17:0, 14-methyl hexadecanoic acid; 17:0, heptadecanoic acid; i18:0, 16-methyl heptadecanoic acid; 18:0, octadecanoic acid. Data were taken from this study (columns 1–8), Kämpfer (1994) (9, 13 and 14), Andersson *et al.* (1995) (10–12 and 15–16) and Meier-Stauffer *et al.* (1996) (17). Major components are shown in bold.

Fatty acid	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
10:0							2·7										
a13:0									5·1								
i14:0		2·3	3·3	5·2	2·9	0·4	1·0	1·3	0·1					0·5			0·2
14:0		1·1	0·3	0·5		1·8	1·4	0·6	1·5				0·6				0·2
i15:0	**20·7**	**14·5**	**39·1**	**26·3**	**37·8**	**33·6**	**22·6**	**25·5**	**39·8**	**29·0**	**27·0**	**22·0**	**22·0**	**38·0**	**19·0**	**13·0**	**54·3**
a15:0	2·0	1·7	2·2	2·1	2·3	1·8	1·3	0·6	6·4	2·0	1·0	1·0	1·6	30·4	12·0		0·6
15:0	0·4	4·1	1·6	1·3	1·4	2·3	2·1	1·3	0·5	3·0	2·0	1·0				3·0	0·6
i16:0	**16·6**	**38·9**	**27·4**	**41·7**	**29·2**	**9·5**	**21·0**	**31·8**	**6·2**	**31·0**	**26·0**	**37·0**	**10·4**	**2·0**	**6·0**	**61·0**	**2·3**
16:0	3·7	10·5	2·0	3·4	1·7	11·0	11·2	8·3	9·2	3·0	3·0	5·0	11·6	2·0	8·0	6·0	3·5
i17:0	**36·7**	**7·3**	**18·2**	**12·1**	**18·5**	**26·6**	**18·5**	**21·0**	**17·1**	**21·0**	**27·0**	**22·0**	**30·3**	**10·0**	**13·0**	**11·0**	**32·8**
a17:0	18·7	8·4	4·9	4·9	5·8	7·3	4·6	3·1	13·3	7·0	11·0	8·0	16·6	10·2	42·0	1·0	0·8
17:0	0·4	6·1	0·5	0·5	0·4	2·9	1·3	2·3		2·0	1·0	1·0	0·8			1·0	0·2
i18:0	0·6	0·8	0·3	1·4		0·2	0·9	1·3		1·0		2·0				1·0	
18:1		0·4		0·1		1·3	1·2	0·7									
18:0		1·4	0·2	0·5		1·3	3·4	2·2					0·5				0·3
Unsaturated C16		2·5					6·6							1·7		3·0	
Other	0·2						0·2		0·8	1·0	2·0	1·0	5·6	5·2			4·2
Total	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100

The sequences of 16S rDNA of strains 34^T, K and Sam were identical; the 16S rDNAs of strains U^T and X were similar (99·4% sequence similarity). The new isolates formed a very compact phylogenetic cluster with the species of group 5: *B. thermoleovorans*, *B. kaustophilus*, *B. thermocatenulatus*, *B. stearothermophilus*, *B. thermodenitrificans*, '*B. caldolyticus*', '*B. caldotenax*' and '*B. caldovelox*' (97·3–99·5% sequence similarity). More distant relatedness was found between the new isolates and two other representatives of group 5, *B. thermoglucosidasius* and *Saccharococcus thermophilus* (96·9–97·9% sequence similarity). The unrooted phylogenetic tree in Fig. 2 shows the relationship of the subsurface strains to each other and to other organisms listed in Methods.

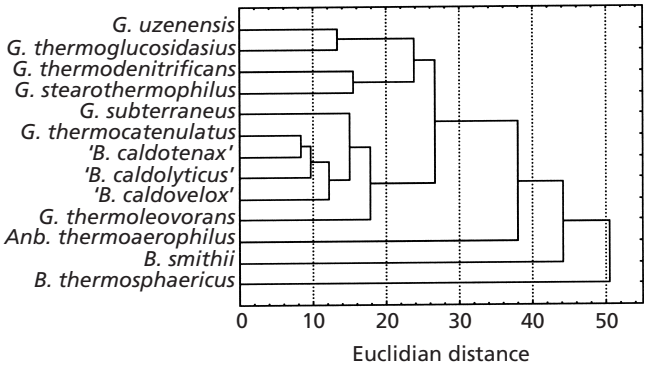

Fig. 3. Dendrogram of fatty acid similarities (Euclidian distance) based on data for strains shown in Table 4. See legend to Fig. 2 for abbreviations for genera.

Fatty acids and quinones

The fatty acid compositions of the subsurface isolates and thermophiles of group 5 (*B. thermodenitrificans* 466, *B. thermoleovorans* 5366^T and *B. thermocatenulatus* B-1259^T) were determined. The major fatty acids present in all strains were iso-fatty acids. Among them, iso-15:0, iso-16:0 and iso-17:0 accounted for about 61·7–86·8% of the total fatty acids (Table 4). All strains exhibited anteiso-15:0 and anteiso-17:0 as minor components (3·7–20·7% of total). However, a difference could be seen in the content of anteiso-17:0: it was present in smaller amounts in strains K, Sam and 34^T than in strains U^T and X.

The dendrogram in Fig. 3 summarizes the relatedness of the whole-cell fatty acid compositions of two subsurface isolates and the 11 thermophilic strains. The subsurface isolates and bacilli of group 5 formed one cluster, at a Euclidean distance of about 30. Other thermophilic bacilli did not cluster tightly with the subsurface isolates and strains of group 5, indicating that members of the latter may not belong to the same genus.

The major menaquinone detected in strains 34^T and U^T was menaquinone-7. Both strains revealed a

maximum band with an m/z value of 225, characteristic of the naphthoquinone fragment of menaquinones, and an intense band at m/z 648, corresponding to menaquinone-7, having the composition $C_{46}H_{64}O_2$.

Physiological characteristics of reference strains

We studied the utilization of saturated hydrocarbons by reference strains of group 5. Cultures were incubated without shaking at 55 °C for 4 d. *B. stearothermophilus* 22^T grew on C_{10} and C_{11} hydrocarbons (OD 0·14) and *B. thermodenitrificans* 466 and *B. thermocatenulatus* B-1259^T grew on C_{10}–C_{16} hydrocarbons (OD 0·13–0·22). Thus, the ability to utilize hydrocarbons is a widely distributed property in this group. *B. thermocatenulatus* B-1259^T and *B. thermoleovorans* 5366^T produced acid but no gas from mannose and maltose; no acid was formed from inositol. The Voges–Proskauer reaction was negative.

DISCUSSION

Geothermally heated oil reservoirs with a temperature of 50–60 °C or higher, in which liquid hydrocarbons are the prevailing organic matter, are a unique ecological niche for thermophilic, hydrocarbon-oxidizing bacteria. We have studied the microbiological characteristics of a range of high-temperature oilfields in Russia, Kazakhstan and China and isolated five strains that have a number of ecologically beneficial features that demonstrate their adaptation to the habitat (Nazina *et al*., 1992, 1993, 1995). All five isolates were moderately thermophilic, neutrophilic, aerobic or facultatively anaerobic, motile, spore-forming rods that utilize a range of hydrocarbons, oil, aromatic compounds, lower alcohols, organic acids and carbohydrates.

Phylogenetic position

According to both DNA–DNA reassociation studies and 16S rDNA sequence analysis, the subsurface strains formed two related groups. Within each of these groups, the strains had almost identical 16S rRNA sequences (99·4–100 %) and exhibited high DNA homology (80–96 %). These results suggest an interspecies level of relatedness (Stackebrandt & Goebel, 1994; Wayne *et al*., 1987) between the groups.

Examination of the 16S rDNA sequences of the subsurface isolates showed that these organisms are members of the phyletic group 5 of the genus *Bacillus*, exhibiting 96·5–99·2 % sequence similarity to other members of this group. The 16S rDNA sequence similarity of this coherent cluster to the type species of the genus *Bacillus*, *B. subtilis*, was less than 91 %; the sequence similarity to other thermophilic members of *Bacillus* ranged from 80·3 to 94·7 % and to thermophiles of the genera *Aneurinibacillus*, *Brevibacillus*, *Alicyclobacillus*, *Thermobacillus* and *Sulfobacillus* it was 81·4–91·3 %. These values indicate that the subsurface isolates and bacilli of group 5 are less closely related to the six genera above and represent the core of a new genus. The levels of 16S rDNA sequence similarity of strains U^T, X, 34^T, K and Sam to bacilli of group 5 (98·2–99·1 %) were approximately the same as those between established species of this group (97·9–99·5 %).

Strains Sam, K and 34^T were clearly distinct from strains U^T and X and from the type strains of the group 5 bacilli by levels of DNA homology, which were greater than 30 % but less than 70 %. This means that new strains and all of the group 5 bacilli examined in this work belong to one genus. The subsurface isolates exhibited low DNA homology to strains of the type species of the genus *Bacillus*, *B. subtilis* (10 %). The low level of relatedness (less than 20 % DNA homology for *B. stearothermophilus* and *B. thermoleovorans* to other mesophilic and thermophilic members of the genus *Bacillus*, *Bacillus amyloliquefaciens*, *Bacillus cereus*, *B. licheniformis*, *Bacillus megaterium*, *B. subtilis*, *Bacillus thuringiensis* and *B. smithii*) was demonstrated previously (Sharp *et al*., 1980; Priest, 1981; Zarilla & Perry, 1987; Nakamura *et al*., 1988).

Morphology and physiology

The affiliation of the subsurface isolates to two new species is supported by a number of morphological and physiological characters. The new strains were motile, spore-forming, thermophilic rods. Strains K, Sam and 34^T differed from strains U^T and X by the ability to utilize arabinose and formate and to grow anaerobically as denitrifiers, by the reaction in the methyl red test and by gelatin hydrolysis. The subsurface isolates could be distinguished from bacilli of group 5 by a number of morphological and biochemical features, nutritional traits (utilization of arabinose, cellobiose, galactose, lactose, rhamnose and xylose), production of gas from nitrate and temperature and NaCl growth ranges (Table 2).

Chemotaxonomy

On the basis of 16S rDNA sequence analysis and DNA–DNA hybridization data, chemotaxonomic studies were undertaken with the new strains and reference species of the group 5 bacilli in order to test the hypothesis that they belonged within the same higher taxon.

The presence of MK-7 in strains U^T and 34^T does not allow them to be distinguished from thermophilic bacilli of the genera *Bacillus*, *Alicyclobacillus*, *Paenibacillus*, *Brevibacillus*, *Aneurinibacillus*, *Salibacillus*, *Gracilibacillus* and *Thermobacillus*, in which MK-7 is the major menaquinone.

The genus *Bacillus* has been extensively studied with respect to fatty acid profiles and *B. stearothermophilus* was assigned to a separate group in all studies (Kaneda, 1977, 1991; Vaisanen & Salkinoja-Salonen, 1989; Kämpfer, 1994). According to our data and data

Table 5 Salient characters of genera containing thermophilic, aerobic, endospore-forming rods

Data were taken from Claus & Berkeley (1986), Golovacheva *et al.* (1975), Suzuki *et al.* (1983), Zarilla & Perry (1987), White *et al.* (1993) and Manachini *et al.* (2000) (*Geobacillus* and *Bacillus*); this study (*Geobacillus*); Shida *et al.* (1996, 1997) (*Brevibacillus*); Wisotzkey *et al.* (1992) (*Alicyclobacillus*); Heyndrickx *et al.* (1997) (*Aneurinibacillus*); Golovacheva & Karavaiko (1978), Dufresne *et al.* (1996) and Norris *et al.* (1996) (*Sulfobacillus*); and Touzel *et al.* (2000) (*Thermobacillus*). Abbreviations: MK-7, menaquinone 7; +, positive reaction; V, variable reaction; −, negative reaction; NT, not tested; ND, no data available; NA, not applicable.

Character	*Geobacillus*	*Bacillus*	*Brevibacillus*	*Aneurinibacillus*	*Alicyclobacillus*	*Sulfobacillus*	*Thermobacillus*
No. of species	8	> 60	10	3	3	3	1
Spore shape*	E, C	E, C, S, B	E	E	E	O, S	E
Sporangia swollen	+/−	+/−	+	+	+/−	+	+
Anaerobic growth	+/−	+/−	+/−	−	+/−	−	−
Catalase	+	+	+	+	+	ND	+
Optimum growth conditions:							
Temperature (°C)	55–65	V (15–55)	30–48	37–55	65	35–50	55
pH	6·5–7·0	V (7·0–9·5)	7·0	7·0	3·0	1·5–2·5	7·8
Main isoprenoid quinone	MK-7	MK-7	MK-7	MK-7	MK-7	ND	MK-7
Major cellular fatty acids	Iso-C15:0, iso-C16:0, iso-C17:0	V	Anteiso-C15:0 and iso-C15:0 or iso-C15:0	Iso-15:0, C16:0, iso-C16:0	ω-Alicyclic acids	ω-Alicyclic acids	Iso-C16:0, C16:0, anteiso-C17:0
Intragenic similarity (%) of 16S rRNA gene sequences	> 96·5	NT	> 93·2	98·6?	> 92·7	95·2	NA
DNA G+C content (mol %)	49–58	32–69	43–57	41–43	52–60	46–57	57·5

*E, Ellipsoid; S, spherical; O, oval; C, cylindrical; B, banana-shaped.

available in the literature, the subsurface isolates and all other representatives of group 5, *B. stearothermophilus*, *B. thermoglucosidasius*, *B. thermoleovorans*, *B. thermocatenulatus*, *B. thermodenitrificans*, '*B. caldolyticus*', '*B. caldotenax*' and '*B. caldovelox*', contain iso-branched saturated acids, iso-15:0, iso-16:0 and iso-17:0, as the main fatty acids (61·7–86·8% of total; Table 4) (Kämpfer, 1994; Andersson *et al.*, 1995). Thus, the fatty acid profile is a useful characteristic that distinguishes this group of bacilli clearly from other mesophiles and thermophiles of the genera *Bacillus*, *Alicyclobacillus*, *Brevibacillus*, *Aneurinibacillus*, *Sulfobacillus* and *Thermobacillus* (Fig. 3; Tables 4 and 5).

The subsurface isolates and thermophiles of group 5 differ essentially from thermoacidophilic *Alicyclobacillus* species and acidophilic *Sulfobacillus* species, which contain the rarely encountered ω-alicyclic fatty acid as the major membranous lipid component (Wisotzkey *et al.*, 1992; Norris *et al.*, 1996). They differ from *Aneurinibacillus thermoaerophilus*, which also contains iso-15:0 and iso-17:0 as dominant fatty acids, by a higher level of iso-16:0 (6·2–42 versus 2·6%) (Andersson *et al.*, 1995). They differ from the thermophilic *B. smithii* and thermotolerant *B. licheniformis* by a lower level of anteiso-15:0 plus anteiso-17:0 fatty acids (3·7–20·7% versus > 40%) and from *B. thermosphaericus*, which has an unusually high content of iso-16:0 (61%) (Andersson *et al.*, 1995; Kämpfer, 1994).

All these data show that the subsurface isolates are biochemically and genetically unique and support the proposal that they should be classified as two new species of a new genus.

Taxonomy

On the basis of physiological characteristics, the results of fatty acid analysis, DNA–DNA hybridization studies and 16S rRNA gene sequence analysis, we propose to create a new genus, *Geobacillus* gen. nov., containing the subsurface isolates as two new species, *Geobacillus subterraneus* sp. nov. (strains K, Sam and 34^T) and *Geobacillus uzenensis* sp. nov. (strains X and U^T). We propose the transfer of the validly described species of group 5, *B. stearothermophilus*, *B. thermoleovorans*, *B. thermocatenulatus*, *B. kaustophilus*, *B. thermoglucosidasius* and *B. thermodenitrificans*, to this new genus, with *Geobacillus stearothermophilus* (formerly *Bacillus stearothermophilus* DSM 22^T) as the type species.

The taxonomic position of '*B. caldotenax*', '*B. caldovelox*', '*B. caldolyticus*', '*B. caldoxylolyticus*', '*B. thermoantarcticus*' and *Saccharococcus thermophilus*, included in group 5, should be investigated (Sharp *et al.*, 1980; Claus & Berkeley, 1986; Rainey *et al.*, 1994; Nicolaus *et al.*, 1996).

Description of *Geobacillus* Nazina *et al.* gen. nov.

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

The description below is based on our observations, as well as on previous descriptions of obligately thermophilic species of the group 5 bacilli (Claus & Berkeley, 1986; Fahmy *et al.*, 1985; Suzuki *et al.*, 1983; Zarilla & Perry, 1987; Priest *et al.*, 1988; Golovacheva *et al.*, 1975, White *et al.*, 1993; Manachini *et al.*, 2000).

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 non-motile. The cell wall structure is Gram-positive, but the Gram-stain reaction may vary between positive and negative. Ellipsoidal or cylindrical endospores are located terminally or subterminally in slightly swollen or non-swollen sporangia. Colony morphology and size are variable; pigments may be produced on certain media. Chemo-organotrophs. Aerobic or facultatively anaerobic. Oxygen is the terminal electron acceptor, replaceable in some species by nitrate. Obligately thermophilic. The temperature range for growth is 37 to 75 °C, with an optimum at 55–65 °C. Neutrophilic. Growth occurs in a pH range of 6·0 to 8·5, with an optimum at pH 6·2–7·5. Growth factors, vitamins, NaCl and KCl are not required by most species. Acid but no gas is produced from glucose, fructose, maltose, mannose and sucrose. Most species do not produce acid from lactose. Most species form catalase. Phenylalanine is not deaminated, tyrosine is not degraded, indole is not produced, the Voges–Proskauer reaction is negative. Oxidase-positive or negative. The major cellular fatty acids are iso-15:0, iso-16:0 and iso-17:0, which make up more than 60% of the total. The main menaquinone type is MK-7. The G+C content of DNA is 48·2–58 mol% (thermal denaturation method). The levels of 16S rRNA gene sequence similarity are higher than 96·5% for the members of this genus. Most species are widely distributed in nature.

The type species is *Geobacillus stearothermophilus* (basonym *Bacillus stearothermophilus* Donk 1920); the type strain of this species is strain DSM 22^T.

Description of *Geobacillus subterraneus* sp. nov.

Geobacillus subterraneus (sub.ter.ra′ne.us. L. adj. *subterraneus* subterranean, below the Earth's surface).

In addition to the description given above for the genus, the following features are characteristic for *G. subterraneus*. Cells are rod-shaped, motile by means of peritrichous flagella and produce subterminally or terminally located ellipsoidal spores in non-swollen sporangia. Gram staining is positive. Colonies are round, mucous and colourless. Acid but no gas is produced from cellobiose, galactose, glycerol, mannose and ribose. No acid is produced from adonitol, arabinose, inositol, raffinose, rhamnose, sorbitol or xylose. Utilizes as carbon and energy sources hydrocarbons (C_{10}–C_{16}), methane–naphthenic and naphthenic–aromatic oil, phenylacetate, formate, acetate, butyrate, pyruvate, benzoate, fumarate, succinate, peptone, tryptone, nutrient broth, potato agar, yeast extract, phenol, ethanol, butanol and lactate. Nitrate is reduced to dinitrogen. Does not grow autotrophically on $H_2 + CO_2$. Gelatin is not hydrolysed. Starch and aesculin are degraded. Phenylalanine is not deaminated, Fe^{3+} is not reduced, casein is not hydrolysed, urea and tyrosine are not decomposed and H_2S, indole and dihydroxyacetone are not produced. The egg-yolk lecithinase reaction and Voges–Proskauer reaction are negative. The methyl red test is positive. Growth occurs both in the absence of NaCl and at 5% (w/v) NaCl. The G+C content of DNA is 49·7–52·3 mol%.

Isolated from formation waters of high-temperature oilfields. The type strain is 34^T, which has been deposited in the Russian Collection of Microorganisms as VKM B-2226^T, in the DSMZ as DSM 13552^T and at the China General Microbiological Culture Collection Centre as AS 12673^T, and reference strains are K (= VKM B-2225) and Sam (= VKM B-2227).

Description of *Geobacillus uzenensis* sp. nov.

Geobacillus uzenensis (u.ze.nen′sis. N.L. adj. *uzenensis* of Uzen, referring to the Uzen oilfield, Kazakhstan, from where the type strain was isolated).

In addition to the characteristics given above for the genus, the following features are characteristic for *G. uzenensis*. Cells are rod-shaped, motile by means of peritrichous flagella and produce terminally located ellipsoidal spores in swollen or non-swollen sporangia. Gram staining is positive or negative. Colonies are round, mucous, small and colourless. Acid but no gas is produced from arabinose, cellobiose, galactose, glycerol, maltose, mannitol, mannose, ribose and trehalose. No acid is produced from adonitol, inositol, raffinose, rhamnose, sorbitol or xylose. Utilizes as carbon and energy sources hydrocarbons (C_{10}–C_{16}), methane–naphthenic and naphthenic–aromatic oil, acetate, propionate, butyrate, pyruvate, benzoate, phenylacetate, phenol, ethanol, butanol, malate, lactate, fumarate, succinate, peptone, tryptone, nutrient broth, potato agar and yeast extract. Nitrate is reduced to nitrite. Does not grow autotrophically on $H_2 + CO_2$. Gelatin, starch and aesculin are hydrolysed. Phenylalanine is not deaminated, Fe^{3+} is not reduced, casein is not hydrolysed and urea and tyrosine are not decomposed. H_2S, indole and dihydroxyacetone are not produced. The egg-yolk lecithinase reaction and Voges–Proskauer reaction are negative. The methyl red test is negative. Growth occurs both in the absence of NaCl and at 4% (w/v) NaCl. The G+C content of DNA is 50·4–51·5 mol%.

Isolated from formation waters of high-temperature oilfields. The type strain is U^T (= VKM B-2229^T = DSM 13551^T = AS 12674^T) and strain X (= VKM B-2228) is a reference strain.

Description of *Geobacillus stearothermophilus* Nazina *et al.* comb. nov. [basonym *Bacillus stearothermophilus* Donk 1920 (Approved Lists 1980, 27)]

In addition to the characteristics given for the new genus and those given for this species by Claus & Berkeley (1986), the following feature is characteristic

for *G. stearothermophilus*: it utilizes maltose and hydrocarbons (C_{10},C_{11}) as carbon and energy sources. An additional character found by Kämpfer (1994) is that the sum of iso-15:0, iso-16:0 and iso-17:0 fatty acids makes up more than 60% of the total fatty acids. The type strain of this species is strain DSM 22^T.

Description of *Geobacillus thermoleovorans* Nazina *et al.* comb. nov. [basonym *Bacillus thermoleovorans* Zarilla and Perry 1987 (Validation List no. 25, 1988)]

The description of *Geobacillus thermoleovorans* comb. nov. is identical to the description given by Zarilla & Perry (1987). Additional characters found in this study are as follows: utilizes mannose; no acid is formed from inositol; Voges–Proskauer reaction is negative; the major cellular fatty acids are iso-15:0, iso-16:0 and iso-17:0, making up more than 60% of the total fatty acids. The type strain is strain DSM 5366^T.

Description of *Geobacillus thermocatenulatus* Nazina *et al.* comb. nov. [basonym *Bacillus thermocatenulatus* Golovacheva *et al.* 1975 (Validation List no. 36, 1991)]

The original description of this species was given by Golovacheva *et al.* (1975). In addition to the characteristics given for the new genus and for this species, the following features are characteristic for *G. thermocatenulatus*: it utilizes maltose, mannose and a number of hydrocarbons (C_{10}–C_{16}) as carbon and energy sources; no acid is formed from inositol; Voges–Proskauer reaction is negative; the major cellular fatty acids are iso-15:0, iso-16:0 and iso-17:0, making up more than 60% of the total fatty acids. The revised value for the G+C content of DNA is 55·2 mol%. The type strain is strain DSM 730^T (= VKM B-1259^T).

Description of *Geobacillus kaustophilus* Nazina *et al.* comb. nov. [basonym *Bacillus kaustophilus* nom. rev. Priest *et al.* 1988 (Validation List no. 28, 1989)]

The description of *Geobacillus kaustophilus* comb. nov. is identical to that given for the new genus and to the description given by White *et al.* (1993). The type strain is strain ATCC 8005^T.

Description of *Geobacillus thermoglucosidasius* Nazina *et al.* comb. nov. [basonym *Bacillus thermoglucosidasius* Suzuki *et al.* 1983 (Validation List no. 14, 1984)]

The description of *Geobacillus thermoglucosidasius* comb. nov. is identical to that given for the new genus and to the description given by Suzuki *et al.* (1983). An additional character found by Kämpfer (1994) is that the main cellular fatty acids are iso-15:0, iso-16:0 and iso-17:0, making up more than 60% of the total fatty acids. The type strain is strain DSM 2542^T.

Description of *Geobacillus thermodenitrificans* Nazina *et al.* comb. nov. (basonym *Bacillus thermodenitrificans* nom. rev. Manachini *et al.* 2000)

The description of *Geobacillus thermodenitrificans* comb. nov. is identical to that given for the new genus and to the description given by Manachini *et al.* (2000). The type strain is strain DSM 465^T.

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