***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.**

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A polyphasic taxonomic study was performed on the type strain of *Bacillus thermosphaericus* DSM 10633^T and three related soil isolates. On the basis of phenotypic characteristics, chemotaxonomic profiles and phylogenetic data a new genus, *Ureibacillus* gen. nov., is proposed for the strains in the *Bacillus thermosphaericus* cluster. Strains of this cluster fall into two DNA–DNA similarity groups: while one group contains the type strain of *Ureibacillus thermosphaericus* comb. nov. and a single soil isolate, the other contains two soil isolates. The two groups differed in the composition of isoprenoid quinones and some phenotypic properties. These data support the description of a novel species of *Ureibacillus* for which the name *Ureibacillus terrenus* is proposed. The type strain of this new species is TH9A^T (= DSM 12654^T = LMG 19470^T).

Keywords: *Ureibacillus* gen. nov., *Ureibacillus terrenus* sp. nov., *Ureibacillus thermosphaericus*

INTRODUCTION

Recent taxonomic studies on thermophilic bacilli have shown that this biotechnologically important group of bacteria is still in need of further study to improve its classification and identification. Indeed, in recent years there has been a proliferation of new species described (Manachini *et al.*, 1985; Demharter & Hensel, 1989; Combet-Blanc *et al.*, 1995; Meier-Stauffler *et al.*, 1996; Pettersson *et al.*, 1996) and, through more detailed phylogenetic and chemosystematic studies, the proposal of new genera (Wisotzkey *et al.*, 1992; Shida *et al.*, 1996; Heyndrickx *et al.*, 1998; Wainø *et al.*, 1999).

In this framework *Bacillus thermosphaericus* represents a thermophilic *Bacillus* species which is not closely related to members of any of the newly described genera that accommodate former *Bacillus* species. This species was isolated in 1995 from urban air in Southern Finland (Andersson *et al.*, 1995), but its habitat is as yet unknown as no additional strains of this species

have been isolated. Among the thermophilic bacilli *Bacillus thermosphaericus* is rather unique as it stands phylogenetically isolated and possesses unique physiological properties. These features have already been stressed in the original description of the species (Andersson *et al.*, 1995) and the new taxon was tentatively assigned to the genus *Bacillus*.

As part of a study investigating genotypic diversity among thermophilic bacilli (Mora *et al.*, 1998), three strains were isolated from uncultivated soil in three different geographical areas. These strains showed morphological and phenotypic features typical of *Bacillus thermosphaericus*. Phylogenetic and chemotaxonomic data supported the affiliation but indicated that two of the soil isolates formed a novel species, the description of which is presented in this communication.

METHODS

Bacterial strains, growth conditions and phenotypic tests. The strains used in this study, designated TH9A^T, TH29A and TU1A, had been previously isolated from soil samples

The GenBank accession number for the 16S rDNA sequence of *Ureibacillus terrenus* is AJ276403.

from Italy, Egypt and Turkey, respectively (Mora *et al.*, 1998). *Bacillus thermosphaericus* DSM 10633^T was used as reference strain. Strains were routinely maintained at 4 °C after growth at 55 °C on CESP agar (casitone, 15 g; yeast extract, 5 g; soytone, 3 g; peptone, 2 g; MgSO₄, 0.015 g; FeCl₃, 0.007 g; MnCl₂, 0.002 g; made up to 1 l with distilled water, pH 7.2). For long-term maintenance, cell suspensions were stored at -80 °C in broth cultures supplemented with 15% (w/v) glycerol.

The morphological life-cycle was photographed in a phase-contrast microscope by using cells grown on slides coated with a thin layer of CESP agar. Gram staining was done as described by Salle (1961). Colony characteristics were determined from 24–36-h-old cultures grown at 55 °C. Anaerobic growth in glucose broth, anaerobic production of gas from nitrate, citrate utilization, starch and casein hydrolysis, indole, acetoin and catalase production were determined by the method described by Smith *et al.* (1952). Reduction of nitrate was examined using the method of Lanyi (1987); the urease test followed the method described by Atlas (1993). Utilization of different carbon sources was tested on agar plates containing (g l⁻¹) (NH₄)₂HPO₄ (1), KCl (0.2), MgSO₄ (0.2), yeast extract (0.2), bromocresol purple (0.004) (pH 7.0) supplemented with 0.5% (w/v) of each carbon source sterilized separately. The temperature range for growth was determined on CESP agar plates incubated at different temperatures over the range 35–70 °C. For pH studies, the pH of the medium was adjusted from 5.0 to 11.0. Tolerance to salinity was determined in CESP broth supplemented with 2–7% (w/v) NaCl.

Chemotaxonomy. Peptidoglycan structure was elucidated by analyses of cell-wall hydrolysates employing the following methods: qualitative analysis of amino acids and peptides by two-dimensional TLC on cellulose plates using described solvent systems (Schleifer & Kandler, 1972), quantitative amino acid analysis by GC and GC/MS (MacKenzie, 1987; Groth *et al.*, 1996), dinitrophenylation of N-terminal amino acids of the interpeptide bridge (Schleifer, 1985) and enantiomeric amino acid analysis using a chiral gas chromatographic column (Groth *et al.*, 1997). Menaquinones were analysed by HPLC as described previously (Groth *et al.*, 1996). GC determination of cellular fatty acid profiles was performed as described by Schumann *et al.* (1997). Polar lipids extracted by the method of Minnikin *et al.* (1979) were identified by two-dimensional TLC on silica gel and spraying with specific reagents (Collins & Jones, 1980).

Determination of G + C content of DNA. DNA base composition was determined by the thermal denaturation method described by Marmur & Doty (1962), using the equation of Owen & Hill (1979). DNA from *Escherichia coli* strain B (Sigma) was used as internal standard.

DNA–DNA hybridization method. DNA was isolated and purified as described previously (Manachini *et al.*, 1985). DNA–DNA homology was determined by the optical renaturation rates method (Kurtzman *et al.*, 1979) with a model Response spectrophotometer equipped with Advance Kinetics Graphic Version 1.3 thermoprogrammer (Gilford System; Ciba Corning Diagnostic Corp.). For all samples tested the melting temperature (T_m) was calculated in 5 × SSC, the same salt concentration used for the determination of DNA–DNA similarities. The temperature of hybridization was 25 °C below the calculated T_m . The equation of Seidler & Mandel (1971) was used to calculate the extent of DNA–DNA reassociation.

Plasmid detection. Detection of plasmid DNA followed the alkaline extraction procedure described by Sambrook *et al.* (1989).

16S rDNA sequence determination and phylogenetic analysis. Genomic DNA extraction, PCR-mediated amplification of the 16S rDNA and sequencing of the PCR products was carried out as described previously (Rainey *et al.*, 1994). The sequence reactions were electrophoresed using a model 373A automatic DNA sequencer (Applied Biosystems). The 16S rDNA sequences were manually aligned with those of members of thermophilic bacilli and related organisms, deposited in the 16S rDNA database of the DSMZ. Evolutionary distances were calculated by the method of Jukes & Cantor (1969). Phylogenetic dendrograms were reconstructed using the treeing algorithm of DeSoete (1983) and Felsenstein (1993).

Nucleotide sequence accession numbers. The accession numbers of sequences used in the construction of the phylogenetic tree are: *Bacillus thermosphaericus* DSM 10633^T, X90640; *Bacillus thermocloaceae* DSM 5250^T, Z26939; *Bacillus pasteurii* NCIMB 884^T, X60631; *Bacillus globisporus* DSM 4^T, X68415; *Bacillus insolitus* DSM 5^T, X60642; *Bacillus subtilis* NCDO 1769, X60646; *Bacillus smithii* DSM 4216^T, Z26935; *Bacillus coagulans* IAM 12463^T, *Bacillus fastidiosus* DSM 91^T, X60615; D16267; *Brevibacillus brevis* NCIMB 9372^T, X60612; *Paenibacillus polymyxa* DSM 36^T, X57308; *Exiguobacterium aurantiacum* NCDO 2321^T, X70316; *Planococcus citreus* NCIMB 1493^T, X62172; *Kurthia zopfii* NCIMB 9878^T, X70321; and *Caryophanon latum* NCIMB 9533, X70314.

Ribotyping. Ribotyping was performed as described by Allerberger & Fritschel (1999). Analysis was done with the RiboPrinter Microbial Characterization System (Qualicon) (Bruce, 1996). Each sample lane was normalized to a standard marker set, characterized and identified using similarity measurements to previously run strains and reference patterns. Binary similarity values of riboprint patterns were used to generate a mean linkage clustering dendrogram (Sneath & Sokal, 1973).

RESULTS

Phenotypic properties

The three isolates could not be distinguished from one another nor from *Bacillus thermosphaericus* DSM 10633^T by any of the physiological, morphological or metabolic characteristics tested. Cells were rod-shaped, varying in length from 1 to 6 µm and approximately 0.5–0.7 µm in diameter, motile and occurred singly or in chains. Round spores were located terminally or subterminally within a swollen sporangium (Fig. 1). The Gram reaction was negative. Circular, entire, flat, transparent and swarming colonies were produced. The three isolates grew optimally between 50 and 60 °C. No growth was observed at temperatures above 68 °C, even though at 65 °C good growth was still observed for isolates TH9A^T and TU1A. No growth was observed below 40 °C, contrary to the type strain that also grew at 35–37 °C. According to the description of *Bacillus thermosphaericus* species, our isolates and strain DSM 10633^T were negative for hydrolysis of starch and gelatin, assimilation of arabinose, ribose, xylose, glucose, maltose, mannose, rhamnose, trehalose, sucrose and citrate, anaerobic

Fig. 1. Phase-contrast micrographs of the type strain of *Ureibacillus terrenus* grown at 55 °C on CESP agar. (a) Cells in the vegetative state after 18 h; (b) cells in the forespore state after 36 h; (c) sporulating cells after 40 h; and (d) giant cells after 66 h. Bar, 5 µm.

growth, reduction of nitrate and production of indole. They were able to utilize aesculin and urea. In addition to these characteristics, we studied other physiological properties, not yet investigated in *Bacillus thermosphaericus* species, that would improve its characterization. In particular, all isolates and the type strain were able to grow in presence of 3 % NaCl and, with the exception of strain TU1A, up to 5 % NaCl. With the exception of strain TU1A, the isolates and the type strain were able to grow in media prepared at pH 9.0. Moreover, all tested strains failed to hydrolyse casein and to produce acetylmethylcarbinol. Some of these characteristics distinguished this group of strains from the other thermophilic *Bacillus* species, such as round spore formation, Gram-negative type cell wall and the inability to grow anaerobically or to use sugars as a source of carbon and energy.

Chemotaxonomic analyses

The amino acid composition of the peptidoglycan of the three strains matches the data given for *Bacillus thermosphaericus* DSM 10633^T (Andersson *et al.*,

1995). The cross-linkage of the peptidoglycan was of the L-Lys ← D-Asn type (variation A4α) (A11.31); this peptidoglycan type is uncommon within the documented thermophilic bacilli. The phylogenetically nearest neighbour of *Bacillus thermosphaericus*, *Bacillus thermocloaceae*, possesses a directly cross-linked peptidoglycan with *meso*-diaminopimelic acid as the diagnostic amino acid (Demharter & Hensel, 1989).

Polar lipids, not included in the original description of *Bacillus thermosphaericus* DSM 10633^T, were also similar, consisting of phosphatidylglycerol, diphosphatidylglycerol, phospholipids and glycolipids of unknown composition.

Analysis of the fatty acids (Table 1) revealed that all strains of the *Bacillus thermosphaericus* cluster were characterized by high levels of iso-C_{16:0}, when grown on solid fatty acid medium (trypticase soy agar; TSA) at 50 °C. Indeed, under these conditions, the strains tested showed predominant amounts of iso-C_{16:0} (58–61 %), while iso-C_{15:0}, iso-C_{17:0} and C_{16:0} fatty acids occurred in smaller amounts (13, 11 and 6 % respectively). Other thermophilic bacilli, such as some

Table 1 Percentage cellular fatty acid composition of the strains tested

The abbreviations for fatty acids are illustrated by the following examples: C_{16:0}, hexadecanoic acid; i-C_{15:0}, 13-methyl tetradecanoic acid; ai-C_{17:0}, 14-methyl hexadecanoic acid.

Strain	C _{14:0}	C _{15:0}	C _{16:0}	C _{18:0}	i-C _{15:0}	i-C _{16:0}	i-C _{17:0}	ai-C _{15:0}	ai-C _{17:0}
<i>Bacillus thermosphaericus</i> DSM 10633 ^{T*} ‡		3.0	6.0		13.0	61.0	11.0		1.0
<i>Bacillus thermosphaericus</i> DSM 10633 ^{T*}		2.8	5.8		13.8	58.8	13.0		1.0
TH9A ^{T*}		3.5	6.0		15.8	53.6	12.2		1.0
<i>Bacillus thermosphaericus</i> DSM 10633 ^{T†}	1.1	1.0	11.6	1.0	38.6	5.1	35.0	1.2	3.0
TH29A [†]			11.4		40.3	7.7	29.5		7.2
TU1A [†]	1.0		23.3	1.1	13.7	11.6	35.7	1.2	10.7
TH9A ^{T†}			12.3		11.9	19.4	42.9		13.5

* Strains grown in TSA medium.

† Strains grown in CASO medium.

‡ According to Andersson *et al.* (1995).

Table 2 Major menaquinones of strains tested

Strain	Menaquinones
<i>Bacillus thermosphaericus</i> DSM 10633 ^T	MK-7
TH29A	MK-7, MK-8 (91:2)
TU1A	MK-9, MK-8, MK-10, MK-7 (51:37:6:3)
TH9A ^T	MK-9, MK-8, MK-10, MK-7, MK-11 (58:22:14:1:1)

representatives of rRNA group 5 *sensu* Ash *et al.* (1991) and the new genus *Thermobacillus* (Touzel *et al.*, 2000) contain iso-C_{16:0} as the predominant component, but at lower levels, up to 37 and 48 %, respectively. After growth in a different medium (liquid CASO medium, supplemented with yeast extract and glucose) the quantitative composition of fatty acids was different from that previously obtained (Table 1), revealing that the fatty acid composition depends largely not only on the incubation temperature but also on the growth medium used. However, this different growth medium permitted us to distinguish strains DSM 10633^T and TH29A from strains TH9A^T and TU1A. In this case the first pair of strains was characterized by the predominance of iso-C_{15:0} and iso-C_{17:0}, accounting for about 40 and 33 %, respectively, while the second pair possessed significantly smaller amounts of iso-C_{15:0} (12 %). Some differences in the quantitative composition of the other fatty acids occurred between the two pairs of strains, but were less significant.

Significant chemical differences between the strains tested was detected in the composition of isoprenoid quinones (Table 2). While *Bacillus thermosphaericus* DSM 10633^T and isolate TH29A possessed almost exclusively menaquinone type MK-7 (which accounted for more than 90 % of the total), strains TH9A^T and TU1A showed major amounts of MK-9 (58 and 51 % respectively) and MK-8 (22 and 37 %), with MK-7 as a minor component (1 and 6 %).

Fig. 2. 16S rDNA sequence-based phylogenetic dendrogram constructed from evolutionary distances, showing the phylogenetic position of strains tested, next to members of the genus *Bacillus* and related taxa. Bar, 5 % sequence similarity.

Phylogenetic analysis

A continuous stretch of 1305 nt of 16S rDNA was determined, ranging from position 18 (5') to 1361 (3') (*Escherichia coli* numbering; Brosius *et al.*, 1978).

Table 3 G + C content and levels of DNA–DNA reassociation between *Bacillus thermosphaericus* DSM 10633^T and new soil isolates

Percentage reassociation values are means of three determinations; the maximum difference noted between determinations was 7%. Values in parentheses indicate that, by definition, the reassociation value was 100%.

Strain	G + C (mol %)	1	2	3	4
1. <i>Bacillus thermosphaericus</i> DSM 10633 ^T	35.7	(100)			
2. TH9A ^T	39.6	4	(100)		
3. TU1A	41.5	6	98	(100)	
4. TH29A	39.2	93	25	27	(100)

Fig. 3. Diversity of normalized ribotype patterns found within isolates related to strains of *Bacillus thermosphaericus*.

The highest binary 16S rDNA similarity values were found with *Bacillus thermosphaericus* DSM 10633^T (99.8–98.2% similarity), while those determined with all other reference strains were significantly lower (91.5–86.2%; data not shown). The 16S rDNA relationships between the strains and the reference organisms of *Bacillus* and affiliated taxa, depicted in Fig. 2, shows that *Bacillus thermosphaericus* strains fall within the radiation of the genus *Bacillus* and represent a distinct lineage within this group, with *Bacillus thermocloaceae* DSM 5250^T being the neighbouring species of *Bacillus thermosphaericus* DSM 10633^T (Andersson *et al.*, 1995). Moreover, it is interesting to note that the strains tested can once again be separated into two groups. One group included *Bacillus thermosphaericus* DSM 10633^T and isolate TH29A with a 16S rDNA similarity value of 99.8%. The other group included the isolates TH9A^T and TU1A, highly similar (99.7%) to each other, but sharing only 98.2% similarity with the former group.

DNA–DNA homology studies and G + C content

The levels of DNA–DNA homology and the G + C contents of the strains are shown in Table 3. It can be seen that the strains tested belonged to two different homology groups, the first comprising strain DSM 10633^T and isolate TH29A, and the second the new isolates TH9A^T and TU1A. The DNA G + C content of *Bacillus thermosphaericus* DSM 10633^T, determined by the thermal denaturation method, agrees with that obtained by HPLC (Andersson *et al.*, 1995). Isolate TH29A showed a G + C value about 3.5 mol % higher, but a difference in thermal stability greater than 2% is

now considered a more realistic standard to delimit bacterial species (Ursing *et al.*, 1995). This less strict definition seems to be particularly sound for the two strains in question, which were closely related either phylogenetically or genetically and phenotypically. The DNA G + C content of the other homology group ranged between 39.6 and 41.5 mol %.

All strains tested were negative for the presence of extrachromosomal elements.

Ribotyping analyses

The riboprint patterns obtained for the strains studied (Fig. 3) differ from those of all other *Bacillus* strains (Qualicon Database; data not shown) and also permit the differentiation of the two homology groups. Moreover, while strains TH9A^T and TU1A showed a similar ribopattern that can be considered characteristic of this group, *Bacillus thermosphaericus* DSM 10633^T and isolate TH29A displayed a certain degree of genomic diversity, denoting a genomic uniqueness of these strains that can be used in future identification at the strain level.

DISCUSSION

In this paper we describe the characterization of a group of thermophilic spore-forming bacteria, including strains TH29A, TH9A^T and TU1A, isolated from uncultivated soil from three different geographical areas. The isolates, based on their phenotypic, chemosystematic and phylogenetic properties, appeared to belong to *Bacillus thermosphaericus*. This latter species, previously described by Andersson *et al.* (1995),

Table 4 Salient features of some genera of aerobic endospore-forming bacteria

Abbreviations: DAP, *meso*-diaminopimelic acid; S, spherical; O, oval; O-S, oval to spherical spores; i-C iso methyl-branched; ai-C, anteiso methyl-branched; v, character varies according to species; ND, no data available.

Character	<i>Ureibacillus</i>	<i>Bacillus</i> *	<i>Virgibacillus</i> †	<i>Brevibacillus</i> ‡	<i>Aneurinibacillus</i> ‡	<i>Alicyclobacillus</i> §
No. of species	2	> 60	2	10	2	3
Murein	Lys-D-Asn	v¶	DAP	DAP	DAP	ND
Spore shape	S	O-S	O-S	O	O	O
Gram reaction	—	+ /v	+	+	+	+
Anaerobic growth	—	v	+	v	—	—
Optimum growth:						
pH	7.0–8.0	7.0–9.5	7.0	7.0	7.0	3.0
Temp. (°C)	50–55	15–55	37	30–48	37	48–65
Major cellular fatty acids	i-C _{16:0} , i-C _{15:0} , i-C _{17:0}	v	i-C _{15:0} , ai-C _{15:0}	ai-C _{15:0} , i-C _{15:0}	i-C _{15:0} , C _{16:0} , i-C _{16:0}	ω-Alicyclic acids
Level of intragenus 16S rRNA gene sequence similarity (%)	> 98	ND	> 99	> 93.2	98.6	> 92.7
G+C content (mol%)	36–41.5	32–69	37	46–57	42–43	52–60

* Data from Claus & Berkeley (1986).

† Data from Heyndrickx *et al.* (1998).

‡ Data from Shida *et al.* (1996).

§ Data from Wisotzkey *et al.* (1992).

¶ Predominant type is DAP.

represents a particular thermophilic *Bacillus* species, for which we believe there are sufficient justifications for the proposal of a new taxon.

Phenotypically, the three new thermophilic isolates and *Bacillus thermosphaericus* DSM 10633^T can be easily distinguished from other taxa on the basis of round spore formation, negative Gram reaction, urease activity and an inability to grow anaerobically or to use sugars as a source of carbon and energy. Moreover, within the thermophilic bacilli, only the *Bacillus thermosphaericus* group has peptidoglycan type A4α (L-Lys ← D-Asn) and a G+C content lower than the other thermophilic bacilli. Moreover, this cluster displays iso-C_{16:0} as the main fatty acid, a feature unusual for the genus *Bacillus* with respect to the high content (about 60%). These phenotypic and chemotaxonomic differences justify the separation of the strains tested from the other thermophilic bacilli at the genus level.

This conclusion is in agreement with the phylogenetic position of the strains. The 16S rDNA sequence data confirm the isolated position of *Bacillus thermosphaericus* described by Andersson *et al.* (1995) and show that *Bacillus thermocloaceae* is the closest phylogenetic neighbour of *Bacillus thermosphaericus*. However, *Bacillus thermocloaceae* differs in several phenotypic and chemotaxonomic properties (among which are peptidoglycan type and fatty acid composition)

(Demharter & Hensel, 1989). Our results also show that the three new thermophilic isolates are phylogenetically related to the reference strain. Sequence comparison reveals intragroup similarity values of more than 98.2%. In contrast, the levels of similarity between members of this group and members of previously described genera are consistently less than 91.5%. These intra- and intergroup similarity values indicate that the strains tested are cohesive and distinct from previously described genera.

These accumulated data support the proposal that *Bacillus thermosphaericus* DSM 10633^T and strains TH29A, TH9A^T and TU1A represent the core of a new genus for which the name *Ureibacillus* gen. nov. is proposed. This new genus is also characterized by the presence of the polar lipids phosphatidylglycerol, diphosphatidylglycerol, phospholipids and glycolipids of unknown composition.

Further characterization of these strains indicates that, within the new genus, two different species can be differentiated. The high level of DNA relatedness (93%) between strains DSM 10633^T and TH29A underlines that these organisms belong to the same species. This finding is also supported by a high level of 16S rDNA sequence similarity (99.8%). The other two isolates, TH9A^T and TU1A, belong to a different species, sharing only between 4 and 27% similarity with members of the former species. It is interesting to

Table 5 Characteristics for distinguishing between *Ureibacillus* species and the phylogenetically related *Bacillus thermocloaceae* species

For abbreviations, see Table 4 legend.

Characteristic	<i>Ureibacillus thermosphaericus</i>	<i>Ureibacillus terrenus</i>	<i>Bacillus thermocloaceae</i> *
Gram reaction	—	—	+
Spore shape	S	S	O
Murein	Lys-D-Asn	Lys-D-Asn	DAP
Growth at:			
pH 7.0	+	+	—
pH 9.0	+	v	+
37 °C	+	—	+
65 °C	—	+	+
5% NaCl	+	v	±
Hydrolysis of urea	+	+	ND
Major cellular fatty acids	i-C _{16:0} , i-C _{15:0} , i-C _{17:0}	i-C _{16:0} , i-C _{15:0} , i-C _{17:0}	i-C _{15:0} , i-C _{17:0}
Predominant menaquinone	MK-7	MK-9, MK-8	MK-7, MK-8
G + C content (mol %)	35.7–39.2	39.6–41.5	42–44
Habitat of isolation	Urban air	Soil	Sewage sludge

* Data from Demharter & Hensel (1989).

note that strains TH9A^T and TU1A (showing a high similarity, 99.7%) exhibit a lower level of 16S rDNA similarity (98.2%) with the other strains, according to the presence of two different homology groups.

These two different homology groups can also be distinguished on the basis of their isoprenoid quinone composition. While strains DSM 10633^T and TH29A possess exclusively menaquinones of type MK-7 (accounting for more than 90% of the total), strains TH9A^T and TU1A possess major amounts of MK-9 and MK-8, with MK-7 as a minor component.

On the basis of DNA–DNA homology values and a distinctive phenotypic trait (menaquinone type), we propose to place two species within the genus *Ureibacillus*: *Ureibacillus thermosphaericus* (formerly *Bacillus thermosphaericus*), including the type strain DSM 10633^T, isolate TH29A (DSM 12655 = LMG 19472) and all strains previously described as *Bacillus thermosphaericus* that share > 70% DNA–DNA homology with the type strain; and *Ureibacillus terrenus* sp. nov., including the type strain TH9A^T (= DSM 12654^T = LMG 19470^T) and strain TU1A (DSM 12656 = LMG 19471).

The description of the new genus and its species follow and a comparison of some of the salient features of other genera and species of aerobic spore-forming bacteria are shown in Tables 4 and 5.

Description of *Ureibacillus* gen. nov.

Ureibacillus (Ur.e.i.ba.cil'lus. L. n. *urea* urea; L. dim. n. *bacillus* from *Bacillus*, a genus of aerobic endospore-forming bacteria; *Ureibacillus* a ureolytic aerobic bacillus).

Cells are motile, Gram-negative rods (0.5–0.7 × 1–6 µm), single or in chains. They bear spherical endospores which lie in terminal or subterminal positions in swollen sporangia. Members of the genus are aerobic, thermophilic bacteria. The cross-linkage of peptidoglycan is of the L-Lys–D-Asn type (variation A4α). The polar lipids are phosphatidylglycerol, diphosphatidylglycerol, phospholipids and glycolipids of unknown composition. The major cellular fatty acid is iso-C_{16:0}. The G + C content ranges from 35.7 to 41.5 mol%. The levels of 16S rRNA gene sequence similarity are more than 98% for the members of this genus. The type species is *Ureibacillus thermosphaericus*.

Description of *Ureibacillus thermosphaericus* (Andersson et al. 1995) comb. nov.

The description is identical to that given by Andersson et al. (1995) and to the description of the genus given above. Colonies are circular, entire, flat, transparent and swarming. Growth occurs at temperatures ranging from 37 to 60 °C, in the presence of 5% NaCl and at pH 9.0. Utilizes aesculin and urea. Negative for anaerobic growth, Voges–Proskauer reaction, indole production, nitrate reduction, starch, casein and gelatin hydrolysis, acid production from arabinose, ribose, xylose, glucose, maltose, mannose, rhamnose, trehalose, sucrose and citrate. The major quinone is menaquinone 7. The G + C content is 35.7 mol% for the type strain and 39.2 for strain TH29A. The type strain is DSM 10633^T, isolated from urban air and municipal landfill sites.

Description of *Ureibacillus terrenus* sp. nov.

Ureibacillus terrenus (ter.re'nus. L. adj. *terrenus* from earth, referring to the habitat of the organism).

The main description is identical to the description of the genus and to that given for *Ureibacillus thermosphaericus*. Can be differentiated from *Ureibacillus thermosphaericus* by its isoprenoid quinone composition. The main menaquinone types are MK-9 and MK-8, with MK-7 as minor component. Other properties distinguishing *Ureibacillus terrenus* are growth temperature, ranging from 42 to 65 °C, and the ability to grow at pH 9.0 and in the presence of 5% NaCl which is variable within the species. The G + C content is 39.6 mol% for the type strain and 41.5 for strain TU1A. The habitat is soil. The type strain is DSM 12654^T (= LMG 19470^T).

REFERENCES

- Allerberger, F. & Fritschel, S. J. (1999). Use of automated ribotyping of Austrian *Listeria monocytogenes* isolates to support epidemiological typing. *J Micro Methods* **35**, 237–244.
- Andersson, M., Laukkanen, M., Nurmiaho-Lassila, E.-L., Rainey, F. A., Niemela, 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, J. 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.
- Atlas, R. M. (1993). In *Handbook of Microbiological Media*, p. 967. Edited by L. C. Park. London: CRC Press.
- 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.
- Bruce, J. (1996). Automated system rapidly identifies and characterizes microorganisms in food. *Food Technol January*, 77–81.
- 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.
- Collins, M. D. & Jones, D. (1980). Lipids in the classification and identification of coryneform bacteria containing peptidoglycans based on 2,4-diaminobutyric acid. *J Appl Bacteriol* **48**, 459–470.
- Combet-Blanc, Y., Ollivier, B., Streicher, C., Patel, B. K. C., Dwivedi, P. P., Pot, B., Prensier, G. & Garcia, J.-L. (1995). *Bacillus thermoamylovorans* sp. nov., a moderately thermophilic and amyolytic bacterium. *Int J Syst Bacteriol* **45**, 9–16.
- Demharther, W. & Hensel, R. (1989). *Bacillus thermocloaceae* sp. nov., a new thermophilic species from sewage sludge. *Syst Appl Microbiol* **11**, 272–276.
- DeSoete, G. (1983). A least square algorithm for fitting additive trees to proximity data. *Psychometrika* **48**, 621–626.
- Felsenstein, J. (1993). PHYLIP (Phylogeny Inference Package) version 3.5.1. Seattle: Department of Genetics, University of Washington.
- Groth, I., Schumann, P., Weiss, N., Martin, K. & Rainey, F. A. (1996). *Agrococcus jenensis* gen. nov., sp. nov., a new genus of actinomycetes with diaminobutyric acid in the cell wall. *Int J Syst Bacteriol* **46**, 234–239.
- Groth, I., Schumann, P., Rainey, F. A., Schuetze, B. & Augsten, K. (1997). *Bogoriella caseylytica* gen. nov., sp. nov., a new alkaliphilic actinomycete from a Soda Lake in Africa. *Int J Syst Bacteriol* **47**, 788–794.
- Heyndrickx, M., Lebbe, L., Kersters, K., De Vos, P., Forsyth, G. & Logan, N. A. (1998). *Virgibacillus*: a new genus to accommodate *Bacillus pantothenicus* (Proom & Knight 1950). Emended description of *Virgibacillus pantothenicus*. *Int J Syst Bacteriol* **48**, 99–106.
- Jukes, T. H. & Cantor, C. R. (1969). Evolution of protein molecules. In *Mammalian Protein Metabolism*, pp. 21–132. Edited by H. N. Munro. New York: Academic Press.
- Kurtzman, C. P., Johnson, C. J. & Smiley, M. J. (1979). Determination of conspecificity of *Candida utilis* and *Hansenula jadinii* through DNA reassociation. *Mycologia* **71**, 844–847.
- Lanyi, B. (1987). Classical and rapid identification: methods for medically important bacteria. *Methods Microbiol* **19**, 1–67.
- MacKenzie, S. L. (1987). Gas chromatographic analysis of amino acids as the *N*-heptafluorobutyryl isobutyl esters. *J Assoc Off Anal Chem* **70**, 151–160.
- Manachini, P. L., Fortina, M. G., Parini, C. & Craveri, R. (1985). *Bacillus thermoruber* sp. nov., nom. rev., a red-pigmented thermophilic bacterium. *Int J Syst Bacteriol* **35**, 493–496.
- Marmur, J. & Doty, P. (1962). Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. *J Mol Biol* **5**, 109–118.
- Meier-Stauffer, K., Busse, H.-J., Rainey, F. A., Burghardt, J., Scheberl, A., Hollaus, F., Kuen, B., Makristathis, A., Sleytr, U. B. & Messner, P. (1996). Description of *Bacillus thermoaerophilus* sp. nov., to include sugar beet isolates and *Bacillus brevis* ATCC 12990. *Int J Syst Bacteriol* **46**, 532–541.
- Minnikin, D. E., Collins, M. D. & Goodfellow, M. (1979). Fatty acid and polar lipid composition in the classification of *Cellulomonas*, *Oerskovia* and related taxa. *J Appl Bacteriol* **47**, 87–95.
- Mora, D., Fortina, M. G., Nicastro, G., Parini, C. & Manachini, P. L. (1998). Genotypic characterization of thermophilic bacilli: a study on new isolates and several reference strains. *Res Microbiol* **149**, 711–722.
- Owen, R. J. & Hill, L. R. (1979). The estimation of base composition, base pairing and genome sizes of bacterial deoxyribonucleic acid. In *Identification Methods for Microbiologists*, pp. 277–296. Edited by F. A. Skinner & D. W. Lovelock. London: Academic Press.
- Pettersson, B., Lembke, F., Hammer, P., Stackebrandt, E. & Priest, F. G. (1996). *Bacillus sporothermodurans*, a new species producing highly heat-resistant endospores. *Int J Syst Bacteriol* **46**, 759–764.
- 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**, 205–212.
- Salle, A. Y. (1961). *Laboratory Manual on Fundamental Principles of Bacteriology*. New York: McGraw-Hill.
- Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989). *Molecular Cloning: a Laboratory Manual*, 2nd edn. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.
- Schleifer, K. H. (1985). Analysis of the chemical composition and primary structure of murein. *Methods Microbiol* **18**, 123–156.

- Schleifer, K. H. & Kandler, O. (1972). Peptidoglycan types of bacterial cell walls and their taxonomic implications. *Bacteriol Rev* **36**, 407–477.
- Schumann, P., Prauser, H., Rainey, F. A., Stackebrandt, E. & Hirsch, P. (1997). *Friedmaniella antarctica* gen. nov., sp. nov., an L-diaminopimelic acid-containing actinomycete from Antarctic sandstone. *Int J Syst Bacteriol* **47**, 278–283.
- Seidler, R. J. & Mandel, M. (1971). Quantitative aspects of deoxyribonucleic acid renaturation: base composition, state of chromosome replication and polynucleotide homologies. *J Bacteriol* **106**, 608–614.
- Shida, O., Takagi, H., Kadowari, K. & Komagata, K. (1996). Proposal for two new genera, *Brevibacillus* gen. nov. and *Aneurinibacillus* gen. nov. *Int J Syst Bacteriol* **46**, 939–946.
- Smith, N. R., Gordon, R. E. & Clark, F. E. (1952). *Aerobic Spore-forming Bacteria*. Agriculture Monograph No. 16. Washington, DC: US Department of Agriculture.
- Sneath, P. H. A. & Sokal, R. R. (1973). *Numerical Taxonomy: the Principles and Practice of Numerical Classification*. San Francisco: Freeman.
- Touzel, J. P., O'Donohue, M., Debeire, P., Samain, E. & Breton, C. (2000). *Thermobacillus xylanilyticus* gen. nov., sp. nov., a new aerobic thermophilic xylan-degrading bacterium isolated from farm soil. *Int J Syst Bacteriol* **50**, 315–320.
- Ursing, J. B., Rossellò-Mora, R. A., García-Valdés, E. & Lalucat, J. (1995). Taxonomic note: a pragmatic approach to the nomenclature of phenotypically similar genomic groups. *Int J Syst Bacteriol* **45**, 604.
- Wainø, M., Tindall, B. J., Schumann, P. & Ingvorsen, K. (1999). *Gracilibacillus* gen. nov., with description of *Gracilibacillus halotolerans* gen. nov., sp. nov.; transfer of *Bacillus dipsosauri* to *Gracilibacillus dipsosauri* comb. nov., and *Bacillus salexigens* to the genus *Salibacillus* gen. nov., as *Salibacillus salexigens* comb. nov. *Int J Syst Bacteriol* **49**, 821–831.
- Wisotzkey, J. D., Jurtshuk, P. J. R., Fox, G. E., Deinhard, G. & Poralla, K. (1992). Comparative sequence analyses on the 16S rRNA (rDNA) of *Bacillus acidocaldarius*, *Bacillus acidotherstris*, and *Bacillus cycloheptanicus* and proposal for creation of a new genus, *Alicyclobacillus* gen. nov. *Int J Syst Bacteriol* **42**, 263–269.