Reclassification of *Saccharococcus caldoxylosilyticus* as *Geobacillus caldoxylosilyticus* (Ahmad *et al.* 2000) comb. nov.

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A polyphasic study was performed on five thermophilic strains belonging to the genus *Bacillus*, isolated from soil of different geographical areas. 16S rRNA gene sequence analysis placed these isolates in RNA group 5, with *Saccharococcus caldoxylosilyticus* and [*Bacillus*] *thermoglucosidasius* being the closest phylogenetic neighbours. The type species of *Saccharococcus*, *Saccharococcus thermophilus*, was only moderately related to these two species and the novel isolates. DNA–DNA hybridization studies and comparison of morphological, chemotaxonomic and phenotypic features supported the close relationship between the novel isolates and *Saccharococcus caldoxylosilyticus*. These data justify the reclassification of *Saccharococcus caldoxylosilyticus*. Following the transfer of the validly described *Bacillus* species of group 5 into the genus *Geobacillus*, the reclassification of *Saccharococcus caldoxylosilyticus* as *Geobacillus caldoxylosilyticus* comb. nov. is proposed. This species can be distinguished genomically from *Geobacillus thermoglucosidasius*, *Geobacillus stearothermophilus*, *Geobacillus thermodenitrificans* and *Saccharococcus thermophilus* by a specific PCR–RFLP assay targeting the 16S rDNA.

Keywords: *Geobacillus caldoxylosilyticus* comb. nov., thermophilic bacteria, soil

INTRODUCTION

The first evidence that the genus *Bacillus* was phylogenetically heterogeneous and required extensive taxonomic revision goes back to 1991 (Ash *et al.*, 1991), when the genus was divided into five phylogenetically distinct RNA groups. Three thermophilic species, *Bacillus stearothermophilus*, *Bacillus kaustophilus* and *Bacillus thermoglucosidasius*, constituted group 5. In subsequent studies (Rainey & Stackebrandt, 1993; Rainey *et al.*, 1994), other valid and invalid thermophilic *Bacillus* species, such as *Bacillus thermo- leovorans*, ‘*Bacillus caldotenax*’, ‘*Bacillus caldovelox*’

and ‘*Bacillus caldolyticus*’, and the spherical *Saccharococcus thermophilus* (Nystrand, 1984) were affiliated to group 5.

On the basis of phylogenetic evidence and supported by the availability of taxonomically salient characteristics, the genus *Bacillus* has been dissected into novel genera (Shida *et al.*, 1996; Heyndrickx *et al.*, 1998; Touzel *et al.*, 2000; Fortina *et al.*, 2001), new species have been described (Combet-Blanc *et al.*, 1995; Pettersson *et al.*, 1996; Heyndrickx *et al.*, 1999) and invalid species have been validated (Sunna *et al.*, 1997; Manachini *et al.*, 2000). Only recently, Ahmad *et al.* (2000) described a new species of *Saccharococcus*, *Saccharococcus caldoxylosilyticus*. However, in contrast to the type species, the novel species exhibited a typical *Bacillus*-type morphology. Still more recent (Nazina *et al.*, 2001) is the proposal of a new genus, *Geobacillus*, for the validated thermophilic *Bacillus* species of genetic group 5.

Abbreviations: ARDRA, amplified rDNA restriction analysis; RAPD, randomly amplified polymorphic DNA; RSA, ribosomal spacer analysis.

The EMBL accession numbers for the 16S rDNA sequences of strains T524A (= DSM 12833) and T52A are AJ271738 and AJ271739.

In a previous work (Mora *et al.*, 1998), we deepened some aspects of the taxonomic and genotypic diversity within the thermophilic *Bacillus* group 5 by the study of 85 novel isolates from different soil samples, using DNA-based fingerprinting methods such as amplified rDNA restriction analysis (ARDRA), randomly amplified polymorphic DNA (RAPD) fingerprints and ribosomal spacer analysis (RSA). The results of this study demonstrated the power of typing methods not only to group and identify successfully a major proportion of the thermophilic *Bacillus* isolates, but also to find strains belonging to novel genomovars of described species and novel species not yet described. In some cases, the number of novel strains was too small to provide a reliable description of the intra-species variability, recommended for the characterization of a bacterial species defined by the polyphasic approach to taxonomy (Ursing *et al.*, 1995; Vandamme *et al.*, 1996).

In this study, we report the isolation and characterization of thermophilic bacilli from uncultivated soil of four different geographical areas, five of which shared high phenotypic, chemosystematic and genotypic similarities with the type strain of the recently described species *Saccharococcus caldxylosilyticus*.

METHODS

Bacterial strains and isolation procedure. Isolates, their origin and reference organisms used are listed in Table 1. The new isolates were recovered from soil samples from different geographical areas through pasteurization at 90 °C for 10 min and subsequent growth on CESP agar [pH 7.2, containing (l⁻¹): 15 g casitone, 5 g yeast extract, 3 g soytone, 2 g peptone, 0.015 g MgSO₄, 0.007 g FeCl₃, 0.002 g MnCl₂·H₂O] at 65 °C for 24 h.

Strains were routinely maintained at 4 °C after growth at 55 °C for 6–12 h on the same medium. Long-term preservation was achieved by suspending cells in broth cultures of the same medium supplemented with 15% glycerol and storing them at –80 °C.

Morphological characteristics. 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 cultures grown at 55 °C.

Phenotypic analysis. All assays were performed in triplicate and repeated when inconsistent results were obtained. The media and temperature used to determine physiological characteristics were as described above, unless stated otherwise.

The following characteristics were determined by the methods described by Smith *et al.* (1952): anaerobic growth in glucose broth, anaerobic production of gas from nitrate, citrate utilization, starch and casein hydrolysis and indole, acetoin and catalase production. Reduction of nitrate was examined using the method of Lanyi (1987) and the urease test followed the method described by Atlas (1993). Utilization of compounds as sole carbon sources was tested on agar plates containing (l⁻¹) 1 g (NH₄)₂HPO₄, 0.2 g KCl, 0.2 g

Table 1. Strains used in this study

Strain	Source
Novel isolates	
TS24A	Soil, Esna (Egypt)
TS2A	Soil, Lingu (China)
TS3B	Soil, Lingu (China)
TS33B	Soil, Sassari (Italy)
TSu3A	Soil, Denizli (Turkey)
Reference strains	
<i>Saccharococcus caldxylosilyticus</i> DSM 12041 ^T (= ATCC 700356 ^T)	Soil, Melbourne (Australia)*
<i>Saccharococcus thermophilus</i> DSM 4749 ^T (= ATCC 42125 ^T)	
<i>Geobacillus thermoglucosidasius</i> DSM 2542 ^T (= ATCC 42472 ^T)	
<i>Geobacillus thermodenitrificans</i> DSM 465 ^T	
<i>Geobacillus thermocatenulatus</i> DSM 730 ^T	
<i>Geobacillus stearothermophilus</i> 22 ^T	
<i>Geobacillus stearothermophilus</i> T10	
<i>Geobacillus thermoleovorans</i> DSM 5366 ^T	
<i>Geobacillus kaustophilus</i> DSM 7363 ^T	
' <i>Bacillus caldovelox</i> ' DSM 411	
' <i>Bacillus caldotenax</i> ' DSM 406	
' <i>Bacillus caldolyticus</i> ' DSM 405	

* Isolated by Ahmad *et al.* (2000).

MgSO₄, 0.2 g yeast extract, 0.004 g bromocresol purple, pH 7, supplemented with 0.5% (w/v) of each carbon source, sterilized separately. The temperature range for growth was determined by observing growth at different temperatures over the range 40 to 75 °C in a variable-temperature incubator (Politermomatic-Rossi); growth was recorded daily for up to 4 d. For pH studies, the pH of the medium was adjusted from 5 to 10. Media were adjusted to the initial pH indicated with either 1 M NaOH or 1 M HCl. Tolerance to salinity was determined in CESP broth supplemented with 2–10% (w/v) NaCl.

Cellular fatty acid composition. The analysis of fatty acid methyl ethers was performed by GLC as described by Schumann *et al.* (1997).

DNA base composition and DNA homology. DNA was isolated and purified as described previously (Manachini *et al.*, 1985). The 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 the internal standard.

DNA–DNA reassociation was determined by the optical renaturation rate method (Kurtzman *et al.*, 1979) with a model Response spectrophotometer equipped with an Ad-

Fig. 1. 16S rDNA sequence-based phylogenetic dendrogram constructed from evolutionary distances, showing the phylogenetic position of the strains tested, next to members of RNA group 5 of the thermophilic bacilli and related taxa. *G.*, *Geobacillus*. Bar, 1% sequence similarity.

vance Kinetics Graphic version 1.3 thermoprogrammer (Gilford system). For all samples tested, the melting temperature (T_m) was calculated in $5 \times$ SSC, the same salt concentration used for the determination of DNA–DNA homology. The temperature of hybridization was 25°C below the previously calculated T_m . The equation of Seidler & Mandel (1971) was used to calculate the extent of DNA–DNA reassociation.

16S rDNA sequence determination and phylogenetic analysis. Genomic DNA extraction, PCR-mediated amplification of the 16S rDNA and sequencing of the PCR products were performed as described previously (Rainey *et al.*, 1994). The sequence reactions were electrophoresed using a model 373A automatic DNA sequencer (Applied Biosystems). The sequences obtained were aligned and compared with those of members of the thermophilic bacilli and a similarity matrix was obtained using the Ribosomal Database Project service (Maidak *et al.*, 1999). Phylogenetic reconstructions were done using the algorithms of De Soete (1983), available in the Ribosomal Database Project, and of Saitou & Nei (1987), which is part of the PHYLIP package (Felsenstein, 1993).

PCR-based techniques. For the PCR, 100 μl of an overnight culture in CESP broth was added to 400 μl $1 \times$ TE buffer (10 mM Tris/HCl, 1 mM disodium EDTA, pH 8) containing 0.45 mg lysozyme ml^{-1} . Following incubation for 30 min at 37°C , SDS and proteinase K were added at final concentrations of 0.6% (w/v) and 7 U ml^{-1} . After incubation for 30 min, the solution was extracted with an equal volume of phenol. The DNA was then precipitated by adding 0.1 vols sodium acetate and 2 vols 95% ethanol. The DNA pellet was air-dried and subsequently dissolved in 50 μl sterilized water (HPLC grade).

ARDRA and RSA were performed as described previously (Mora *et al.*, 1998).

The amplification of a region of 270 bp encompassing part of the variable region V4 and part of V3 and ranging from positions 466 to 732 (*E. coli* sequence) was performed in 25 μl containing 1 μl bacterial genomic DNA solution obtained as above, 10 μl $10 \times$ PCR buffer (Amersham Pharmacia Biotech), 200 μM of each dNTP, 2.5 mM MgCl_2 , 0.5 U *Taq* polymerase (Amersham) and 0.5 μM of each of primers pol16SF (466, 5'-TCGAATAGGCGGTACGG-3') and pol16SR (732, 5'-GCCTTCGCCACTGGTGT-3').

The temperature profile consisted of a primary DNA denaturation step at 94°C for 2 min followed by 30 cycles of 45 s at 94°C , 45 s at 60°C and 45 s at 72°C . Following amplification, 5 μl product was analysed by PAGE (6%, 29:1 acrylamide:bisacrylamide, TBE buffer). The gel was run at 5 V cm^{-1} in the appropriate buffer, stained in a solution containing 0.5 μg ethidium bromide ml^{-1} and photographed under UV light. The 270 bp amplified fragments were then digested for 1 h at 37°C in a 20 μl reaction containing 7 μl PCR product solution, 2 μl incubation buffer and 5 U *Hae*III (Amersham). Restriction digests were subsequently analysed by PAGE as described above.

RESULTS

16S rDNA sequence and phylogenetic analysis

Almost complete 16S rDNA sequences (1444 nt) were determined for isolates TS24A and TS2A. In both sequences, an ambiguity ($Y = C$ or T) was determined in the variable region V4 at position 633 of the *E. coli* sequence (Winker & Woese, 1991). Comparison of these 16S rDNA sequences (binary similarity of 99.9%) with those of several thermophilic bacilli and the two *Saccharococcus* species revealed a high phylogenetic similarity of strains TS24A and TS2A to *S. caldoxylosilyticus* DSM 12041^T (99.6%). A bootstrap value of 95% supported the clustering of these three organisms found by the two distance-matrix algorithms applied. The dendrogram obtained using the algorithm of De Soete (1983) is depicted in Fig. 1. The closest phylogenetic neighbour of these strains was *Geobacillus* (formerly *Bacillus*) *thermoglucosidasius*, followed by *S. thermophilus*. Lower similarities were found to *Geobacillus thermodenitrificans*, *Geobacillus stearothermophilus* and *Geobacillus thermoleovorans*.

Morphology and biochemical properties

Cells of all strains isolated from soil samples were motile, Gram-positive, catalase-positive and rod-shaped, varying in length from 4 to 6 μm and 0.5–1 μm in diameter, normally present as single cells or in short chains. Oval spores were located terminally within a

Fig. 2. Phase-contrast micrograph of *Geobacillus caldoxylosilyticus* strain TS24A grown at 55 °C on CESP agar. Bar, 5 μm.

swollen sporangium (Fig. 2). Colonies exhibited a regular, flat morphology with entire margins and were off-white to beige. All the isolates grew optimally between 50 and 65 °C. No growth was observed below 42 °C and some strains showed weak growth at 45 °C. All strains showed a maximum temperature for growth of 70 °C and did not grow at pH 5 or 11; strains TSu3A and TS33B were able to grow at pH 9. No growth was detected in the presence of 3% NaCl. The isolates reduced nitrate to nitrite and strains TS2A, TS24A and TS33B also showed the ability to reduce

nitrite to gas. The strains used a wide variety of carbon sources when grown aerobically. The following compounds were assimilated within 24–36 h: glucose, maltose, trehalose, lactose, galactose and cellobiose. Fructose, sucrose, arabinose, ribose and xylose were assimilated within 48–56 h, whereas utilization of rhamnose was variable within the species. The new isolates could be also characterized phenotypically on the basis of their ability to use citrate as a sole carbon source. Starch and casein were hydrolysed.

Anaerobic production of gas from nitrate and anaerobic growth in glucose broth were weak. All strains were negative for indole and urease production and for the Voges–Proskauer test (acetylmethylcarbinol production).

Comparison of phenotypic data for the soil isolates with those of some related reference strains (Table 2) showed a pattern similar to that of *S. caldoxylosilyticus* DSM 12041^T, while those of the other reference strains were distinctly different. The only slight differences in the reactions of strain DSM 12041^T and the soil isolates were the ability of the former strain to grow better under anaerobic conditions and to utilize citrate poorly only. Other characteristics that were not investigated originally, such as nitrate reduction and casein and urea hydrolysis, were similar to those obtained for the soil isolates. Comparison with the phylogenetically nearest species, *G. thermoglucosidasius* DSM 2542^T, indicated that the new isolates

Table 2. Phenotypic characteristics of soil isolates and some related thermophilic bacilli

Taxa are identified as: 1, *S. caldoxylosilyticus*; 2, *S. thermophilus*; 3, *G. thermoglucosidasius*; 4, *G. stearothermophilus*; 5, *G. thermodenitrificans*. Characters are scored as: +, positive; –, negative; w, weak reaction; ND, not determined (data obtained in our laboratory are shown in parentheses); v, variable within the group. Data were obtained from this study or from Ahmad *et al.* (2000) (*S. caldoxylosilyticus*), Nystrand (1984) (*S. thermophilus*), Suzuki *et al.* (1983) (*G. thermoglucosidasius*), Logan & Berkeley (1984) and Claus & Berkeley (1986) (*G. stearothermophilus*) and Manachini *et al.* (2000) (*G. thermodenitrificans*). All isolates and reference strains were positive for the Gram reaction, catalase and utilization of glucose, fructose, maltose, trehalose, mannose and sucrose. They were all negative for indole production and the Voges–Proskauer test.

Phenotypic trait	Soil isolates	1	2	3	4	5
Cell morphology	Rods	Rods	Cocci/irregular clusters	Rods	Rods	Rods
Spore formation	+	+	–	+	+	+
Growth in the presence of 3% NaCl	–	–	ND	–	v	+
Starch hydrolysis	+	+	–	+	+	w
Casein hydrolysis	+	ND (+)	ND	+	v	+
Citrate (Simmons')	+	w	–	–	–	–
Anaerobic growth	w	+	–	–	–	+
Reduction of:						
NO ₃ [–] to NO ₂ [–]	+	ND (+)	v	+	v	+
NO ₂ [–] to gas	v	ND	–	–	–	+
Urease	–	ND (–)	–	+	–	–
Utilization of:						
Rhamnose	v	+	+	+	–	–
Lactose	+	+	–	–	v	+
Galactose	+	+	+	–	v	+
Xylose	+	+	+	+	–	+
Arabinose	+	+	+	–	–	+

Table 3. Cellular fatty acid compositions of soil isolates and some related thermophilic bacilli

Values are percentages of total fatty acids. —, Not detected. Taxa are identified as: 1, strain TS24A; 2, strain TS2A; 3, *S. caldoxylosilyticus* DSM 12041^T; 4, *S. thermophilus* DSM 4749^T; 5, *G. thermoglucosidasius* DSM 2542^T; 6, *G. thermodenitrificans* DSM 465^T; 7, *G. stearothermophilus* DSM 22^T. 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.

Fatty acid	1	2	3	4	5	6	7
C _{14:0}	0.5	1.1	—	1.7	1.0	—	0.6
i-C _{15:0}	51.3	45.8	56.7	58.5	22.8	40.0	34.8
ai-C _{15:0}	1.6	1.7	1.1	2.1	1.9	1.3	4.4
C _{15:0}	1.3	2.2	1.7	—	2.8	1.4	—
i-C _{16:0}	4.3	6.1	6.8	1.1	15.1	7.2	5.9
C _{16:0}	2.3	7.5	2.2	13.5	17.1	5.8	14.5
i-C _{17:0}	30.3	28.9	26.7	20.3	26.3	33.8	23.5
ai-C _{17:0}	3.7	5.2	2.5	2.8	10.1	6.1	15.3
C _{17:0}	—	—	—	—	1.7	1.2	—
i-C _{17:1}	1.2	1.6	2.3	—	—	1.9	—
C _{18:0}	—	—	—	—	0.9	—	—

could be distinguished easily phenotypically on the basis of urease production and the utilization of citrate, lactose, galactose and arabinose.

Cellular fatty acid composition

The cellular fatty acid profiles of strains TS24A and TS2A were characterized by the prevalence of i-C_{15:0} (45.8–48.9%) and i-C_{17:0} (28.9–29.1%), with minor amounts of i-C_{16:0}, C_{16:0} and ai-C_{17:0} (Table 3). *S.*

caldoxylosilyticus DSM 12041^T showed a similar profile. On the basis of fatty acid composition, the isolates could not be differentiated from *G. thermodenitrificans* DSM 465^T or *G. stearothermophilus* DSM 22^T, but they were easily distinguishable from their closest phylogenetic neighbour, *G. thermoglucosidasius*. The main differences were the smaller amount of i-C_{15:0} (22.8%) and the larger amounts of i-C_{16:0} (15.1%), C_{16:0} (17.1%) and ai-C_{17:0} (10.1%) in *G. thermoglucosidasius* DSM 2542^T. *S. thermophilus* DSM 4749^T showed an intermediate position between the soil isolates and *S. caldoxylosilyticus* and *G. thermoglucosidasius*, with the i-C_{15:0} and ai-C_{17:0} contents similar to those of our isolates and a large amount of C_{16:0}, as in *G. thermoglucosidasius*. Another feature that distinguished *S. thermophilus* from the other reference strains was the low content of i-C_{16:0}.

DNA base compositions and DNA homology

The levels of DNA–DNA homology and the G + C contents of the strains are shown in Table 4. Using DNA of strain TS24A as a reference, all the soil isolates belonged to the same homology group, with high levels of DNA relatedness, ranging from 84 to 99%. Low similarities only were found to the phylogenetically related *G. thermoglucosidasius* DSM 2542^T, *G. thermodenitrificans* DSM 465^T and *G. stearothermophilus* DSM 22^T. *S. thermophilus* and *S. caldoxylosilyticus* revealed a reassociation of 35%, which is significantly lower than the value reported by Ahmad *et al.* (2000) (67%). These data showed that the new isolates, together with *S. caldoxylosilyticus*, represent a novel, genomically homogeneous taxon, well differentiated from the other three related species.

The DNA G + C content of the novel isolates ranged from 44.0 to 50.2 mol %.

Table 4. DNA base composition and DNA relatedness among thermophilic soil isolates and phylogenetically closely related thermophilic bacteria

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 content (mol %)	DNA–DNA hybridization (%) with:					
		1	6	7	8	9	10
1. TS24A	47.4	(100)					
2. TS2A	44.0	99					
3. TS3B	46.1	86					
4. TS33B	44.0	84					
5. TSu3A	50.2	91					
6. <i>S. caldoxylosilyticus</i> DSM 12041 ^T	45.8	89	(100)				
7. <i>S. thermophilus</i> DSM 4749 ^T	47.6	51	35	(100)			
8. <i>G. thermoglucosidasius</i> DSM 2542 ^T	45.9	2	39	23	(100)		
9. <i>G. thermodenitrificans</i> DSM 465 ^T	50.3	7			28	(100)	
10. <i>G. stearothermophilus</i> DSM 22 ^T	52.0	2				43	(100)

Fig. 3. ARDRA patterns obtained from soil isolates and some related thermophilic species. Lanes M, 100 bp ladder DNA molecular mass marker (Amersham-Pharmacia).

ARDRA

*Hae*III analysis showed that all soil isolates (isolate TS33B not shown) and *S. caldoxylosilyticus* showed a common pattern, which was composed of restriction fragments of about 125, 235, 270, 300 and 450 bp (Fig. 3). With the exception of strain TSu3A, all of the soil isolates and *S. caldoxylosilyticus* were also characterized by an additional fragment of 570 bp. In these strains, the number of nucleotides calculated for the *Hae*III fragments was greater than the size of the 16S rRNA (about 1540 bp). The reason for the larger sum could be the presence of multiple rDNA alleles within a single genome. This can be verified by the ambiguity at position 633 detected by sequencing of the 16S rDNA (Y = C or T; see above). Indeed, the presence of C at position 633 in one allele generates an additional *Hae*III site of size 570 bp that increased the number of restriction fragments produced by the enzymic restriction.

G. thermoglucosidasius DSM 2542^T showed the most similar pattern to the soil isolates, sharing the 125, 235, 450 and 570 bp fragments. This is in accord with the 16S rDNA analysis.

PCR assay and restriction analysis

Two PCR primers, pol16SF and pol16SR (see Methods), were selected upstream and downstream of position 633 in order to generate a PCR fragment of 270 bp in which the unique *Hae*III site should result

Fig. 4. Polyacrylamide gels showing the *Hae*III restriction profiles of a 270 bp fragment encompassing position 633 (*E. coli* sequence) of the 16S rDNA of the soil isolates and some phylogenetically related thermophilic bacilli. Lanes M, 50 bp ladder DNA molecular mass marker (Amersham-Pharmacia).

from the presence of C at position 633. The subsequent restriction analysis of the 270 bp amplified fragments confirmed the presence of two main 16S rDNA alleles (presence of both C and T residues at position 633) in strains TS24A, TS2A, TS3B and TS33B (Fig. 4) and *S. caldoxylosilyticus*, since both the expected digested products of 170 and 100 bp and the non-digested 270 bp fragment were present (Fig. 4). Strain TSu3A showed only two smaller fragments, confirming the ARDRA pattern (Fig. 3), and thus corroborating the hypothesis of the presence of only one type of 16S rDNA as the main chromosomal allele, containing the

Fig. 5. RSA patterns obtained from soil isolates and some related thermophilic species. Lanes M, 100 bp ladder DNA molecular mass marker (Amersham-Pharmacia).

C residue at position 633. The 270 bp amplified fragments from the other thermophilic *Geobacillus* species were not digested or generated different restriction profiles, as was the case for *Geobacillus thermocatenulatus* DSM 730^T (Fig. 4b).

RSA

Amplification of the internal transcribed spacer between the 16S and 23S rDNA is shown in Fig. 5. The soil isolates could be distinguished from the reference strains by the presence of three common main amplification fragments, of about 240, 400 and 510 bp. Amplification products ranging from approximately 270 to 370 bp permitted discrimination of some of the isolates (TS33B, TSu3A). As observed previously (Mora *et al.*, 1998), *G. stearothermophilus* DSM 22^T, *G. thermodenitrificans* DSM 465^T and *G. thermoglucosidasius* DSM 2542^T showed distinctive amplification profiles that were typical of each strain analysed.

DISCUSSION

Initial studies on five thermophilic strains isolated from uncultivated soils of four different geographical areas showed these new isolates to represent a novel, genetically homogeneous taxon within the genus *Bacillus*. Based upon phylogenetic, morphological and biochemical evidence, this new taxon is clearly a member of RNA group 5 (Ash *et al.*, 1991), the closest relative being [*B.*] *thermoglucosidasius*. Whilst this study was underway, a novel species of *Saccharococcus*, *S. caldoxylosilyticus*, was described (Ahmad *et al.*, 2000). In the phylogenetic analysis of the original description of this species (Ahmad *et al.*, 2000), which is spore-forming and rod-shaped but not spherical, the species [*B.*] *thermoglucosidasius* was not included, and

the new species consequently grouped adjacent to *S. thermophilus*. This omission most likely led to the misclassification of this thermophilic and xylose-degrading species.

The inclusion of the five novel isolates not only led to a correction of the phylogenetic position of *S. caldoxylosilyticus* but also revealed that these new isolates had to be considered members of this species, with which they shared high DNA–DNA relatedness, phenotypic and morphological properties and molecular similarities, as revealed by ARDRA analysis using *Hae*III and a specific PCR–RFLP assay targeted to the 16S rDNA. These properties also distinguished *S. caldoxylosilyticus* and the soil isolates from the other thermophilic species of RNA group 5.

Recently, on the basis of phylogenetic and fatty acid analyses, the validly described *Bacillus* species of group 5 have been transferred to a new genus, *Geobacillus*. On the basis of the evidence mentioned above, which is based on properties used conventionally to affiliate species to a genus, such as phylogenetic position and morphology, we therefore propose to reclassify *Saccharococcus caldoxylosilyticus* as a member of genus *Geobacillus*, the generic affiliation of its closest phylogenetic neighbour, *G. thermoglucosidasius*. The *International Code of Nomenclature of Bacteria* (Lapage *et al.*, 1992) requires that, in a new combination, the type strain of the reclassified species is maintained as the type strain.

Description of *Geobacillus caldoxylosilyticus* (Ahmad *et al.* 2000) comb. nov.

Geobacillus caldoxylosilyticus (cal.do.xy.lo.si.ly'ti.cus. L. adj. *caldus* hot; N.L. neut. n. *xylosum* xylose; N.L. adj. *lyticus* dissolving, degrading; *caldoxylosilyticus* hot and xylose-degrading).

The description includes elements of the original description of Ahmad *et al.* (2000) and properties determined in this study. Gram-positive, catalase-positive rods, varying in length from 4 to 6 µm and 0.5–1 µm in diameter, normally present as single cells or in short chains. Oval spores are located terminally within a swollen sporangium. The optimal growth temperature ranges between 50 and 65 °C with a minimum at 42 °C and a maximum at 70 °C. Nitrate reduction is positive. Anaerobic production of gas from nitrate. Acid production from glucose, fructose, sucrose, maltose, trehalose, lactose, galactose, cellobiose, arabinose, ribose and xylose. Starch and casein are hydrolysed. Strains vary in their utilization of rhamnose and citrate. All strains are negative for indole and urease production, the Voges–Proskauer test (acetylmethylcarbinol production) and anaerobic growth. The main cellular fatty acids are i-C_{15:0} and i-C_{17:0}. Phylogenetically, a member of RNA group 5 of *Geobacillus*. The DNA G + C content ranges from 44.0 to 50.2 mol %. Habitat, soil.

The type strain is DSM 12041^T (= ATCC 700356^T).

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