

Classification of isolates from locations in Austria and Yellowstone National Park as *Geobacillus tepidamans* sp. nov.

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Two moderately thermophilic, Gram-positive, spore-forming bacteria were isolated from different geographical locations and sources; strain GS5-97^T from a beet sugar factory in Leopoldsdorf, Lower Austria, and strain YNP10 from a geothermally heated soil, Yellowstone National Park, USA. The sequences of their 16S rRNA genes were found to be 99·8% identical, and DNA–DNA hybridization experiments revealed that strains GS5-97^T and YNP10 share 89·9 mol% similarity to each other, but only 34·3 and 39·2 mol% similarity, respectively, to *Geobacillus caldxylosilyticus* DSM 12041^T, which is their closest related type strain. A polyphasic analysis showed that these two isolates were more similar to each other than to other characterized geobacilli. Their DNA G + C content was 43·2 and 42·4 mol%, respectively, and they were identical with respect to many phenotypic features (e.g. T_{opt} 55 °C; pH_{opt} 7·0). Both strains clearly displayed best growth when cultured aerobically. They differed slightly in their cellular fatty acid profiles and polar lipid pattern, and genotypically they could also be distinguished based on randomly amplified polymorphic DNA fingerprints and internal transcribed spacer analysis. Freeze-etching experiments revealed oblique surface layer (S-layer) lattices in both strains, and biochemical analyses of the purified S-layer proteins indicated the occurrence of glycosylation. Based on the properties of these organisms relative to those currently documented for the genus *Geobacillus* and for the various sister genera in the *Bacillus* radiation, a novel species is proposed, *Geobacillus tepidamans* sp. nov., with GS5-97^T (= ATCC BAA-942^T = DSM 16325^T) as the type strain. Strain YNP10 has been deposited in the American Type Culture Collection as ATCC BAA-943.

In the course of systematic surveys on the occurrence of prokaryotic glycoproteins (for reviews, see Schäffer & Messner, 2001; Messner & Schäffer, 2003), several isolates originating from extraction plants of Austrian beet sugar factories have been isolated (Hollaus & Klaushofer, 1973; Messner *et al.*, 1984, 1997; Meier-Stauffner *et al.*, 1996). Initial culture work with extraction juice samples of the 1991 sugar campaign resulted in the isolation of five different pure

cultures, designated GS1-97 to GS5-97^T. Whereas strains GS1-97 to GS4-97 could be assigned to the species *Geobacillus stearothermophilus* and *Aneurinibacillus thermoaerophilus* (Schäffer *et al.*, 1999), the taxonomic affiliation of strain GS5-97^T appeared different. Previously, partial 16S rRNA gene sequence analysis showed high similarity (99·8% sequence identity) of strain GS5-97^T to a previously uncharacterized organism, referred to as strain YNP10 (GenBank/EMBL/DDBJ accession no. AF391974), which was isolated from a geothermally heated soil in Yellowstone National Park, USA. The novelty of these strains at the phylogenetic level and their disparate geographical distribution was intriguing and offered an opportunity to initiate examination of a potential case of species cosmopolitan distribution (reviewed by Staley & Gosink, 1999). Initial analysis of these novel organisms showed that the

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of *Geobacillus tepidamans* strain GS5-97^T is AY563003.

Details of media composition, morphological characteristics, chemical analyses and fatty acid compositions are available as supplementary material in IJSEM Online.

highest sequence similarity values matching recognized species were in the range 96–94% and included *Geobacillus caldxylosilyticus*, *Saccharococcus thermophilus*, *Geobacillus thermoglucosidasius*, *Geobacillus thermocatenulatus*, *Geobacillus thermoleovorans* and *G. stearothermophilus* (in decreasing order), and they formed the basis for selection of reference strains for the current study (*G. caldxylosilyticus* DSM 12041^T, *S. thermophilus* ATCC 43125^T, *G. thermoglucosidasius* DSM 2542^T and *Geobacillus thermodenitrificans* DSM 465^T).

Strain GS5-97^T was enriched from sugar beet extraction juice samples in modified S-VIII medium (SVIII/glc) (media compositions, Table S1, supplementary material in IJSEM Online) at 55 °C (Messner *et al.*, 1984) and subcultured in SVIII/glc broth until pure cultures were obtained (Schäffer *et al.*, 1999). Strain YNP10 was isolated from a high temperature soil heated by an underground source of steam (documented to be geothermal for at least 7 years, unpublished data). Soil temperatures at the surface were 65 °C, increasing to 92 °C at the 12 cm depth. Aseptically taken soil samples (~1–5 g) were transported back to the laboratory in sterile tubes suspended in heated water contained in a ThermosTM bottle. Soil samples were serially diluted in 0.1 M NH₄PO₄ buffer (pH 6.0; 65 °C), and aliquots from each dilution spread onto 0.1% yeast extract agar. Single colonies were repeatedly subcultured until a pure culture was obtained. Strains GS5-97^T and YNP10 were stored at –75 °C in SVIII/glc and 0.1% yeast extract broth, respectively, amended with 25% glycerol.

Cell morphology of both novel strains was investigated by light microscopy and electron microscopy of freeze-etched and ultrathin-sectioned bacterial cells (Messner *et al.*, 1984; Sleytr *et al.*, 1988). Both organisms formed straight rods with average dimensions of about 4 µm in length and 1 µm

in diameter, and frequently the cells occurred in short chains (two to three cells). The cells were peritrichously flagellated and were completely covered by an oblique surface layer (S-layer) lattice (Fig. 1a) (also Table S2, supplementary material in IJSEM Online). They display a typical Gram-positive cell wall profile (Fig. 1b), with relatively thin cell walls as has been frequently observed in thermophilic bacilli (Sleytr & Messner, 1992). Sporulation of both organisms was tested on 0.7% standard Nutrient medium (Oxoid), supplemented with 0.1% MnSO₄. After growth in sporulation medium with shaking (100 r.p.m.) at 55 °C for 2 days, terminal spherical endospores were observed in both isolates (Fig. 1c). SDS-PAGE revealed apparent molecular masses of the constitutive S-layer protomers in the range of 106–166 kDa for strain GS5-97^T and of 140–230 kDa for strain YNP10 (Fig. S1a; supplementary material in IJSEM Online). Staining of the gels for carbohydrates (Segrest & Jackson, 1972) showed that the S-layer proteins of both organisms were glycosylated (Fig. S1b; supplementary material in IJSEM Online). For analysis of the S-layer glycoprotein, both strains were grown in continuous culture (modified SVIII growth medium, Table S1, supplementary material in IJSEM Online) in a Braun Biostat C 15-1 fermenter (B. Braun, Melsungen, Germany), at 55 °C, a constant pH value of 7.0, and with a dilution rate of 0.3 h⁻¹. Oxygen saturation (*p*O₂) was maintained at a value above 30% by manual adjustment of aeration and stirring speed. The process was controlled by monitoring optical density at 600 nm, pH, redox potential (Ag electrode; Mettler-Toledo, Wien, Austria) and *p*O₂. The strain-specific S-layer glycans were determined by HPAEC-PED (Bock *et al.*, 1994; Altman *et al.*, 1995) and structurally investigated by ¹H and ¹³C NMR spectroscopy (Kosma *et al.*, 1995). The S-layer glycoprotein glycan chains of strain GS5-97^T are built of

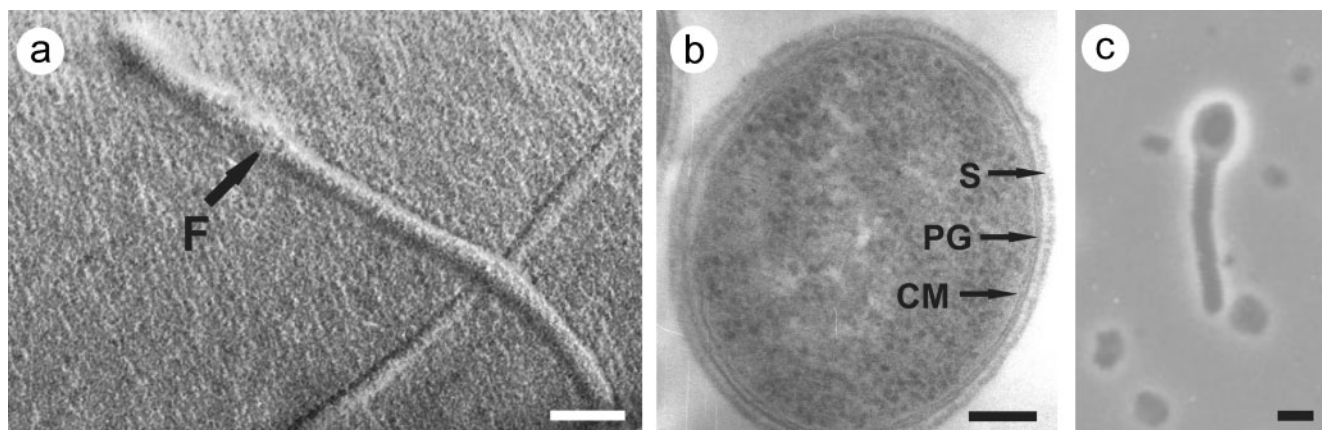


Fig. 1. Ultrastructure and morphology of *Geobacillus tepidamans* sp. nov. (a) Electron micrograph of an intact cell of strain YNP10 after freeze-etching and metal-shadowing, showing the oblique S-layer lattice. F, flagellum. Bar, 100 nm. (b) Electron micrograph of an ultrathin-sectioned cell of strain GS5-97^T. S, S-layer; CM, cytoplasmic membrane; PG, peptidoglycan. Bar, 100 nm. (c) Phase-contrast micrograph of a spore-forming cell of strain YNP10. Bar, 500 nm. Both strains were identical for all features shown.

disaccharide repeats with the structure $[\rightarrow 3)\text{-}\alpha\text{-L}\text{-rhamnopyranose-(1}\rightarrow 2)\text{-}\alpha\text{-D}\text{-fucopyranose-(1}\rightarrow]_n$; a complete structural characterization will be published elsewhere (C. Schäffer & P. Messner, unpublished results). The glycan chain structure of strain YNP10 was identical to that of the previously analysed S-layer glycoprotein of *Aneurinibacillus thermoaerophilus* DSM 10155, consisting of disaccharide repeats with the structure $[\rightarrow 4)\text{-}\alpha\text{-L}\text{-rhamnopyranose-(1}\rightarrow 3)\text{-D-glycero-}\beta\text{-D-manno-heptopyranose-(1}\rightarrow]_n$ (Kosma *et al.*, 1995). Comparison of the glycan structures indicated that the glycosylated S-layer proteins (Sleytr & Messner, 2003) can be used to discriminate the investigated organisms at the strain level.

For all growth experiments, cultures were initiated by inoculating with a standardized number of cells (OD_{595} 0.01), and they were performed in triplicate. After overnight incubation on SVIII/glc agar at 55 °C, both GS5-97^T and YNP10 formed opaque colonies (~3 mm diameter) with lobed margins. Both strains demonstrated robust growth when cultures were adequately aerated (e.g. vigorous shaking of batch cultures or high air-bubbling rates in fermenters) in a variety of complex media such as R2A, SVIII and LB broths. Culture reactions to pH and temperature were assessed in R2A medium (Difco). T_{opt} was determined to be 55 °C for both strains, with a doubling time in R2A broth of 26 and 52 min for GS5-97^T and YNP10, respectively. For pH control across the pH range examined, the R2A medium was amended with acetic acid/acetate, MES, MOPS and Tris buffers. The medium was adjusted to the initial treatment pH with HCl or NaOH, and measured at the conclusion of the experiment to verify that it had not changed. The cardinal temperatures for growth were determined in oil-bath shaker incubators set at predetermined temperatures. Cell doubling times were determined by tracking increases in culture optical density (595 nm). T_{min} and T_{max} were determined to be 39 and 67 °C for both strains. Each strain grew optimally at pH 7, but was capable of growth from pH 6 to 9.

Phenotypic characterizations of the investigated strains were based on the methods of Gordon *et al.* (1973), unless indicated otherwise (Table 1). All assays were performed in duplicate. Tolerance of NaCl was determined in nutrient broth amended with varying concentrations of NaCl. Growth was inhibited in the presence of NaCl at concentrations of 3 and 2 % for GS5-97^T and YNP10, respectively. Further tests included catalase, Voges-Proskauer reaction, starch and casein hydrolysis, citrate utilization, nitrate reduction and acid production and growth with various carbohydrates (glucose, galactose, mannose, trehalose, rhamnose, fructose, sucrose, maltose, lactose and xylose) (Table 1). Anaerobic growth tests were conducted in crimp-sealed serum bottles purged with filter-sterilized N₂, amended with 0.2 % sodium thioglycolate and 0.1 % sodium formaldehyde/sulfoxylate as oxygen scavengers, and resazurin was included (0.0001 %, w/v) as an oxygen indicator. Anaerobic growth tests were negative for

fermentation (LB amended 1 %, w/v, with glucose, galactose, mannose, trehalose, rhamnose, fructose, sucrose, maltose, lactose and xylose), as well as for anaerobic respiration (5 mM each of NaAsO₄, KNO₃, FeCl₃, Na₂SO₃ and K₂SO₄ as electron acceptors), although growth occurred in all aerobic controls for both types of experiments. Urease activity was determined as described elsewhere (Lanyi, 1987). Hydrolysis of DNA was assessed using DNase Test agar (Difco) flooded with a 0.02 % methyl green solution after suitable growth had occurred (methyl green was not included in the agar medium as it proved inhibitory to growth).

For 16S rRNA gene sequencing, DNA was purified using extraction methodology adapted from Mazel *et al.* (1986). The near full-length 16S rRNA gene was amplified using purified DNA as template, along with the *Bacteria*-specific primer 8F and the universal primer 1492R (Amann *et al.*, 1995). In addition, internal primers, which included 1070F (Ferris *et al.*, 1996), 338F, 338R, 522F, 522R and 785F (Amann *et al.*, 1995; Jackson *et al.*, 2001), were used. For phylogenetic analysis, an initial BLAST (Altschul *et al.*, 1997) search of public databases approximated the phylogenetic affiliation of GS5-97^T and YNP10 and identified closely related sequences, which were downloaded and aligned using CLUSTAL W (Thompson *et al.*, 1994). Phylogenetic trees were constructed using the UPGMA distance analysis treeing program in the PAUP* (<http://paup.csit.fsu.edu>) software package, including *Alicyclobacillus acidocaldarius* and *Aneurinibacillus thermoaerophilus* as outgroups. Phylogenetic analysis of the PCR-amplified 16S rRNA genes included 1374 nucleotides (this study).

The chemotaxonomic analyses included cellular fatty acid analysis and characterization of polar lipids of GS5-97^T, YNP10 and related reference strains. For determination of the cellular fatty acid composition as fatty acid methyl esters (FAMES), 48 h cultures of either strain, grown in trypticase soy broth at 55 °C, were used. Fatty acids were extracted, methylated with methanolic HCl and analysed by gas chromatography. The FAME analysis was performed by Microbial ID (Newark, DE, USA). It revealed a predominance of iso-C_{15:0}, C_{16:0} and iso-C_{17:0}, summing to 74.4 and 80.8 % of the totals (Table S3, supplementary material in IJSEM Online). Also present in each strain are C_{14:0}, iso-C_{16:0} and anteiso-C_{17:0}. Overall, the fatty acid profiles of GS5-97^T and YNP10 more closely resembled each other and were clearly distinguishable from other *Geobacillus* species. Interestingly, strains GS5-97^T and YNP10 lack significant amounts of iso-C_{16:0}, which in addition to iso-C_{15:0} and iso-C_{17:0}, has been viewed as characteristic of the geobacilli (Nazina *et al.*, 2001). Nevertheless, these results still support the classification of GS5-97^T and YNP10 as a novel species within the genus *Geobacillus*.

For polar lipid analysis, solvent extraction of lyophilized biomass was performed according to Bligh & Dyer (1959), and extracts were analysed by one-dimensional TLC

Table 1. Differential characteristics of *Geobacillus tepidamans* sp. nov. strains GS5-97^T and YNP10 and related thermophiles from the genus *Geobacillus*

Taxa: 1, *G. tepidamans* strain GS5-97^T; 2, *G. tepidamans* strain YNP10; 3, *G. caldoxylosilyticus*; 4, *S. thermophilus*; 5, *G. thermoglucosidasius*; 6, *G. thermodenitrificans*; 7, *G. subterraneus*; 8, *G. stearothermophilus*; 9, *G. thermocatenulatus*; 10, *G. thermoleovorans*; 11, *G. kaustophilus*; 12, *G. uzensis*; 13, *G. toebii*. Characteristics are scored as: +, positive; -, negative; W, weak, V, variable within the group; ND, not determined.

Characteristic	1	2	3*	4*	5*	6*	7†	8†	9†	10†	11†	12†	13‡
Catalase activity	W	+	+	+	+	+	ND	V	ND	ND	ND	ND	+
Growth in the presence of:													
Lysozyme (0.001 %, w/v)	-	-	-	-	+	-	ND	ND	ND	ND	ND	ND	ND
NaCl (3 %, w/v)	-	-	-	-	-	+	+	V	-	+	ND	+	+
NaCl (5 %, w/v)	-	-	-	-	-	-	+	V	-	-	ND	-	-
Citrate	-	-	W	-	-	-	-	-	V	+	ND	-	-
Indole	-	-	-	-	-	-	-	ND	ND	ND	ND	ND	ND
Urease	-	-	-	+	+	-	ND	-	ND	ND	ND	-	ND
Nitrate reduction to nitrite	+	+	+	+	+	+	+	V	-	+	ND	+	+
Gas from nitrite	-	-	+	-	-	+	ND	-	ND	ND	ND	ND	+
Hydrolysis of:													
Starch	W	W	+	-	+	W	+	+	+	-	V	+	-
Casein	-	-	+	-	+	W	-	V	+	ND	+	-	+
Tween 80	W	W	+	-	-	+	ND	ND	ND	ND	ND	ND	ND
Gelatin	-	-	-	-	-	-	-	+	-	-	ND	+	-
DNA	+	+	+	-	+	+	ND	ND	ND	ND	ND	ND	ND
Acid production from:													
Glucose	+	+	+	+	+	+	ND	ND	ND	ND	ND	+	+
Galactose	+	+	+	-	-	+	+	V	-	+	+	+	-
Mannose	+	+	+	+	+	+	+	ND	+	+	ND	+	ND
Trehalose	+	+	+	+	+	+	ND	ND	ND	ND	ND	+	ND
Rhamnose	-	-	+	+	+	-	-	-	+	-	-	-	-
Fructose	+	+	+	+	+	+	ND	ND	ND	ND	ND	ND	ND
Sucrose	+	+	+	+	+	+	ND	ND	ND	ND	ND	ND	ND
Maltose	+	+	+	+	+	+	ND	ND	+	ND	ND	+	ND
Lactose	W	W	+	-	-	+	-	V	ND	-	-	-	-
Xylose	+	+	+	-	+	+	-	-	+	-	V	-	-

*Fortina *et al.* (2001).

†Nazina *et al.* (2001).

‡Sung *et al.* (2002).

(Minnikin *et al.*, 1984). Phosphate and amino groups were detected on the plates upon spraying with the molybdenum blue and the ninhydrin reagent, respectively, and glycolipids were visualized with anisaldehyde (Meier-Stauffer *et al.*, 1996; Messner *et al.*, 1997). A comparison of the polar lipid patterns by one-dimensional TLC again revealed high levels of similarity between GS5-97^T and YNP10, but significant differences to the reference strains, particularly after staining of the lipids with ninhydrin (Fig. S2, supplementary material in IJSEM Online). All investigated organisms showed one prominent spot representing an unidentified amino group-containing polar lipid species with an R_F value of 0.41. Furthermore, a second, less prominent, lipid species with an R_F value of 0.48 was unique to GS5-97^T and YNP10. In addition to the prominent-staining lipid species, several weak spots were visible in all

organisms, mainly ranging from R_F values of 0.15 to 0.35. The patterns of these weak spots were different in both novel strains and therefore allowed discrimination at the strain level. The general conclusion from these particular experiments was again that strains GS5-97^T and YNP10 are more similar to each other than to other taxonomically recognized organisms and should therefore belong to the same, novel species.

For spectroscopic DNA-DNA hybridization experiments, DNA from the novel strains was isolated using a French pressure cell (Thermos Spectronic) and purified by chromatography on hydroxyapatite according to Cashion *et al.* (1977). DNA-DNA hybridization experiments of the two novel isolates, as well as with *Geobacillus caldoxylosilyticus* DSM 12041^T, were carried out as described by De Ley *et al.*

(1970), with modifications according to Huss *et al.* (1983) and Escara & Hutton (1980), using a model 2600 spectrophotometer, equipped with a Gilford model 2527-R thermoprogrammer and plotter. Renaturation rates were computed with the TRANSFER.BAS program (Jahnke, 1992). Data were interpreted on the basis of Wayne *et al.* (1987). DNA–DNA hybridization was performed by the DSMZ (Deutsche Sammlung für Mikroorganismen und Zellkulturen, Braunschweig, Germany). The level of DNA relatedness between strains GS5-97^T and YNP10 was 89.9%, indicating that both strains belong to the same species. In contrast, DNA relatedness of GS5-97^T and YNP10 to *G. caldxylosilyticus* DSM 12041^T, the closest neighbour in the initial partial 16S rRNA gene sequence analysis, was only 34.4 and 39.6%, respectively. These data indicate that both strains do not belong to the species *G. caldxylosilyticus* and therefore justify the establishment of a novel species within the genus *Geobacillus*.

For determination of the DNA G+C content, genomic DNA was purified from disrupted cells (Cashion *et al.*, 1977) and subsequently hydrolysed with P1 nuclease. Nucleotides were dephosphorylated with bovine alkaline phosphatase, and the resulting deoxyribonucleosides were analysed by HPLC as described previously (Mesbah *et al.*, 1989). The G+C content analysis was performed by the DSMZ. The G+C content of the DNA of strain GS5-97^T was 43.2 mol% and that of strain YNP10 was 42.4 mol%. Both values are close to that originally reported for *G. caldxylosilyticus* DSM 12041^T (44.2 mol%; Ahmad *et al.*, 2000), which showed the highest similarity values in the 16S rRNA gene sequence comparisons. These data indicate that GS5-97^T and YNP10 group well within the radiation of strains belonging to the genus *Geobacillus*.

The notion of a novel species designation was also supported by random amplified polymorphic DNA (RAPD) analysis. For RAPD analysis, DNA from the novel strains and the selected reference strains was prepared using 6 ml of cells from the exponential phase of growth (Mazurier *et al.*, 1992). The analysis was performed using the Ready-To-Go RAPDTM analysis kit (Amersham Biosciences) and 25 pmol, each, of primers 1 (5'-GGTGC GGAA-3'), 2 (5'-GTTTCGCTCC-3'), 3 (5'-GTAGACCCGT-3'), 4 (5'-AAGAGCCGT-3'), 5 (5'-AACGCGCAAC-3') and 6 (5'-CCCCTCAGCA-3'), according to the manufacturer's instructions (Messner *et al.*, 1997). DNA from *Escherichia coli* strains BL21 (λ DE3) and C1a was used as positive controls. The results of the RAPD analysis (not shown) concur with those of the polar lipid and internal transcribed spacer (ITS) analysis (see below) and therefore are not shown here.

ITS analysis by PCR amplification of the 16S–23S ITS region followed the procedure described by Mora *et al.* (1998). Briefly, 45 ng of genomic DNA was added to a PCR mixture containing 12.5 μ l Promega PCR master mix, 10.5 μ l sterile DEPC-treated water and the forward ITSF (5'-GTCGTAACAAGGTAGCCGTA-3') and reverse ITS

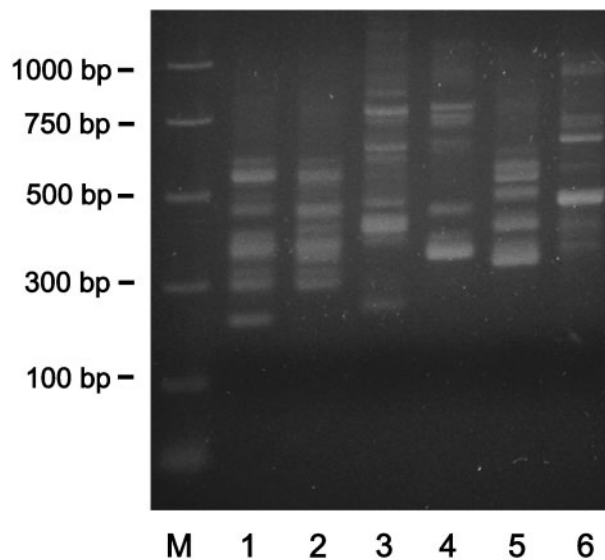


Fig. 2. Internal transcribed spacer analysis of *Geobacillus tepidamans* sp. nov. Lanes: molecular mass standards; 1, GS5-97^T; 2, YNP10; 3, *G. caldxylosilyticus* DSM 12041^T; 4, *S. thermophilus* ATCC 43125^T; 5, *G. thermoglucosidasius* DSM 2542^T; 6, *G. thermodenitrificans* DSM 465^T.

(5'-CAAGGCATCCACCGT-3') primers at 10 μ M each. The PCR product was electrophoresed in a 2% 3:1TM Agarose gel (Ameresco) in 1 \times TBE at 70 V for 4 h. Gels were stained with ethidium bromide and visualized under UV light. Analysis of the ITS region between the 16S rRNA and 23S rRNA genes for strains GS5-97^T and YNP10 and four reference strains is shown in Fig. 2. ITS profiles for strains GS5-97^T and YNP10 were found to be completely reproducible using the PCR conditions described and were nearly identical with the exception of an additional band of approximately 250 bp present in the profile of strain GS5-97^T. The ITS fingerprints of the four reference strains agreed with previous work by Mora *et al.* (1998) and Fortina *et al.* (2001) and were clearly distinguishable from the novel isolates.

In all features examined, the novel isolates GS5-97^T and YNP10 were consistently significantly more similar to each other than to any reference strain representing any other recognized taxa. Morphological similarity included shape, size, flagella distribution, spore location and S-layer features (Fig. 1). Essentially identical phenotypic characteristics included optimum growth temperature and pH, apparent obligate aerophily, enzyme activity, carbohydrate utilization and acid production (Table 1). Fatty acid profiles were also very similar (Table S3, supplementary material in IJSEM Online). Finally, at the genetic level, YNP10 and GS5-97^T were nearly identical in 16S rRNA gene sequence and shared very similar ITS band profiles (Fig. 2). UPGMA distance-based phylogenetic analysis placed both strains together, but separate from other *Geobacillus* species and

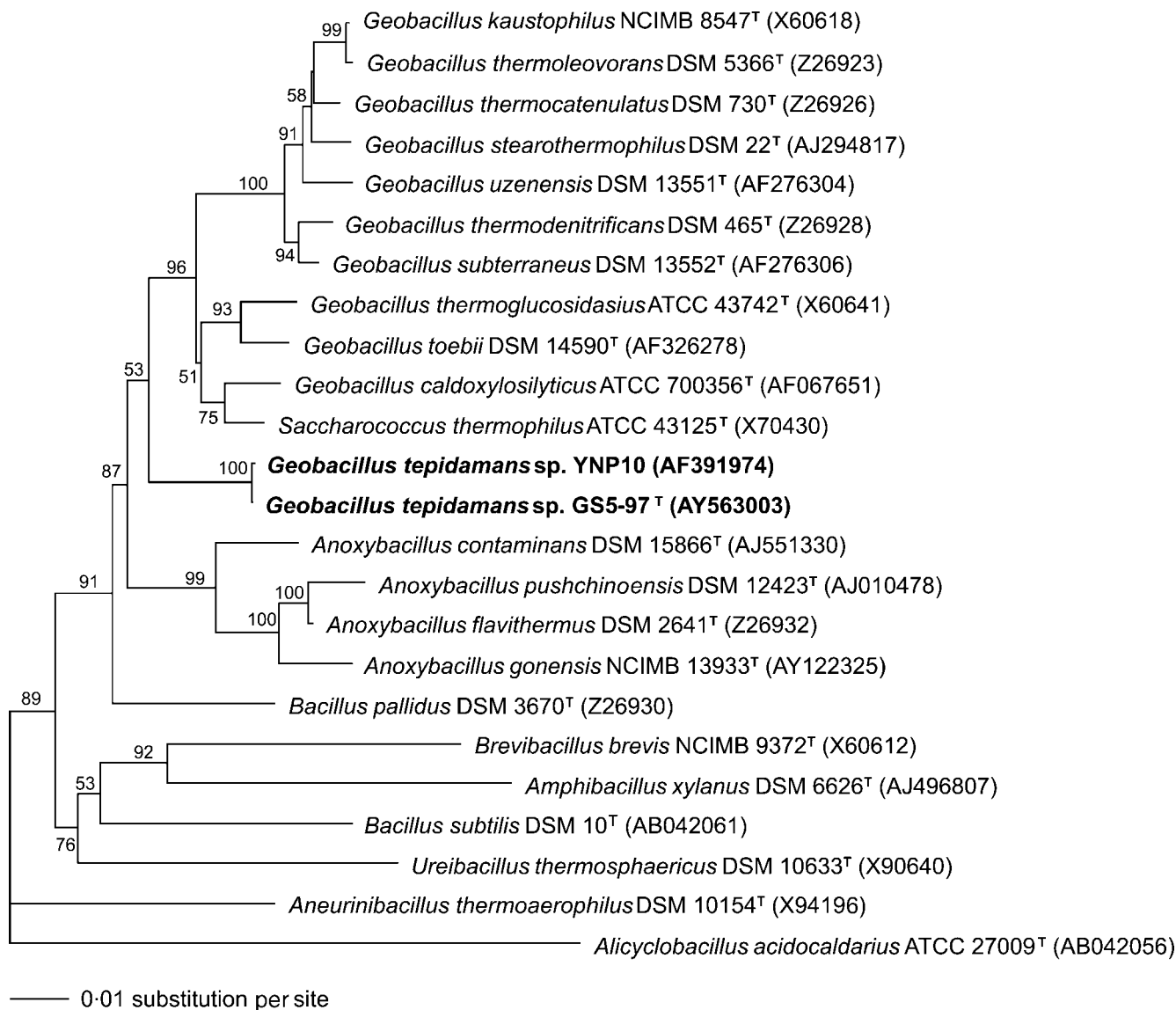


Fig. 3. Dendrogram showing a UPGMA distance tree suggesting the phylogenetic relationship of *Geobacillus tepidamans* sp. nov. strains GS5-97^T and YNP10 relative to characterized type strains of *Geobacillus* and other species within the *Bacillus* radiation. Bootstrap values (%) from 1000 pseudoreplicates are as shown. Bar, 0.01 substitution per site.

other bacilli (Fig. 3). Placement of GS5-97^T and YNP10 was separate from representatives of other species within the *Bacillus* radiation, including the phylogenetically related (but phenotypically distinct) *Anoxybacillus* species. The results of the phenotypic, chemotaxonomic and phylogenetic analyses together with the clear oxygen preference of the novel isolates GS5-97^T and YNP10 support their affiliation with the genus *Geobacillus* and separate them from recognized *Anoxybacillus* species, which are either strict anaerobes (Pikuta *et al.*, 2000) or only microaerotolerant (Belduz *et al.*, 2003; Pikuta *et al.*, 2003; De Clerck *et al.*, 2004). We draw attention to the deep branching location of these novel organisms relative to the balance of the *Geobacillus* clade. It is conceivable that these novel

isolates represent a new species within an as yet undefined genus in the *Bacillus* radiation, although we believe this significant addition to the *Bacillus* phylogeny should await the valid description of additional closely related isolates.

Description of *Geobacillus tepidamans* sp. nov.

Geobacillus tepidamans [te.pid.a'mans. L. adj. *tepidus* (luke) warm; L. part adj. *amans* loving; N.L. part adj. *tepidamans* loving warm (conditions)].

Straight rods, 3.9–4.7 × 0.9–1.2 μm in size, single cells, sometimes in short chains, Gram-positive, motile. Moderately thermophilic and form oval terminal endospores. Require oxygen as an electron acceptor. Covered with an

oblique S-layer lattice, composed of identical S-layer glycoprotein protomers. Grow at 39–67 °C, with an optimum at 55 °C; the pH range for optimal growth is 6–9. For additional strain-specific features, see Table 1. Negative for the Voges–Proskauer reaction (pH 6.5–7) and acid production from basal medium. The major cellular fatty acids are iso-C_{15:0} and iso-C_{17:0}. The G+C content is 43.2 mol% for strain GS5-97^T and 42.4 mol% for strain YNP10. Strain YNP10 has been deposited in the American Type Culture Collection as ATCC BAA-943.

The type strain is GS5-97^T (=ATCC BAA-942^T=DSM 16325^T).

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