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***Geobacillus subterraneus* subsp. *aromaticivorans* subsp. nov., a novel thermophilic and alkaliphilic bacterium isolated from a hot spring in Sirnak, Turkey**

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A new thermophilic spore-forming strain Ge1^T was isolated from the Guclukonak hot spring in Sirnak, Turkey. The strain was identified by using a polyphasic taxonomic approach. Strain Ge1^T was Gram-positive, spore-forming, alkaliphilic rod-shaped, motile, occurring in pairs or filamentous. Growth was observed between 30 and 65°C (optimum 60°C) and at pH 5.5–10.0 (optimum pH 9.0). It was capable of utilizing starch, growth was observed at 0–3% NaCl (w/v) and was positive for catalase and urease. The major cellular fatty acids were *iso*-C_{15:0} and *iso*-C_{17:0}, and the predominant lipoquinone found was menaquinone MK7 type. The DNA G+C content of the genomic DNA of strain Ge1^T was 52.0%. Comparative 16S rRNA gene sequence studies showed that the isolate belonged to the genus *Geobacillus*. The DNA-DNA hybridization mean values between the representative strain Ge1^T and the closely related species *G. subterraneus*, *G. thermodenitrificans*, *G. thermocatenulatus*, *G. vulcani* and *G. thermoleovorans* were 69.3%, 57%, 37%, 27% and 26%, respectively. The results of DNA-DNA hybridization, physiological and biochemical tests allowed genotypic and phenotypic differentiation of strain Ge1^T. Based on these results, we propose assigning a novel subspecies of *Geobacillus subterraneus*, to be named as *Geobacillus subterraneus* subsp. *aromaticivorans* subsp. nov. with the type strain Ge1^T (DSM 23066^T = CIP 110341^T).

Key Words—DNA-DNA hybridization analysis; *Geobacillus subterraneus* subsp. *aromaticivorans* subsp. nov.; lipids; taxonomy; thermophile

Introduction

On the basis of physiological characteristics, the results of fatty acid analysis, DNA-DNA hybridization studies and 16S rRNA gene sequence analysis, Nazina et al. (2001) proposed a new genus, *Geobacillus* gen. nov., containing subsurface isolates as two new species, *Geobacillus subterraneus* sp. nov. and *Geoba-*

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain Ge1^T is HE613733.

cillus uzenensis sp. nov. They also proposed the transfer of the validly described species of group 5, *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*, respectively. The *Geobacillus* species are a phenotypically and phylogenetically coherent group of thermophilic bacilli. The 16S rRNA gene sequence heterogeneity within the genus is 92.6% (Nazina et al., 2001). At present, this genus includes the following species: *Geobacillus stearothermophilus* (Nazina et al., 2001), *G. thermocatenulatus* (Golavacheva et al., 1975), *G. thermoleovorans* (Zarilla and Perry, 1987), *G. kaustophilus*, *G. thermoglucosidans* (nom. corrig., formerly 'thermoglucosidasius') (Coorevits et al., 2011; Nazina et al., 2001), *G. thermodenitrificans* (Manachini et al., 2000), *G. subterraneus* and *G. uzenensis* (Nazina et al., 2001), *G. toebii* (Sung et al., 2002), *G. lituanicus* (Kuisiene et al., 2004), *G. vulcani* and *G. gargensis* (Nazina et al., 2004), *G. debilis* (Banat et al., 2004), *G. tepidamans* (Schäffer et al., 2004), *G. caldxylosilyticus* (Ahmad et al., 2000; Fortina et al., 2001) and *G. jurassicus* (Nazina et al., 2005), which have growth temperatures ranging from 35 to 78°C. *G. pallidus* (Banat et al., 2004) has recently been transferred to the genus *Aeribacillus* (Miñana-Galbés et al., 2010). Moreover, a recent study (Dinsdale et al., 2011) described the emendation of *G. thermoleovorans* and *G. thermocatenulatus* with the merger of *G. kaustophilus*, *G. lituanicus*, *G. thermoleovorans* subsp. *strombolienensis* and *G. vulcani* to *G. thermoleovorans*, and the merger of *G. thermocatenulatus* and *G. gargensis* to *G. thermocatenulatus*. In addition, *Bacillus thermantarcticus*, proposed at first with the name of the genus "*Bacillus*" by Nicolaus et al. (1996), was recently transferred to genus *Geobacillus* as *G. thermantarcticus*; moreover, the same authors transferred *G. tepidamans* to *Anoxybacillus* as *A. tepidamans* (Coorevits et al., 2011).

With the ultimate goal of studying the microbial community present in the Guclukonak hot spring in Sirnak, located in the southeast of Turkey, some thermophilic bacilli from the mud were isolated.

In this paper, a novel member of the genus *Geobacillus* is reported on the basis of a polyphasic approach. The characteristics of this isolate are com-

pared with its near neighbor and on the basis of DNA-DNA hybridization values and biochemical properties, we propose that it represents a novel subspecies of *Geobacillus subterraneus*.

Materials and Methods

Location of sampling. Bacteria were isolated from the mud of Guclukonak hot spring in Sirnak which is a city in the southeast of Turkey. The temperature and pH of the muddy water were 60°C and 6.9, respectively. The hot spring water contained Ca, Mg, SO₄, Cl and hydrogen sulphide.

Culture medium and growth condition. All samples were immediately incubated in the temperature range 25–70°C in Nutrient Broth (NB medium, Oxoid). Cultures were purified from the samples grown in the solid NB medium, containing 2% agar (Oxoid), at 60°C. After 1 week of incubation a number of cream colonies had developed. They were purified using the repeated serial dilution technique followed by re-streaking on the solid NB medium and the purity of the isolates was examined based on cell shape under a microscope and colony homogeneity on the plates. Several isolates were selected and the taxonomic properties of strain Ge1^T will be presented in this paper. Subculturing was performed on the same medium for 24 h at 60°C and isolates were maintained as glycerol stock at –70°C for further studies.

Geobacillus subterraneus DSM 13552^T, *G. thermodenitrificans* DSM 466^T, *G. thermocatenulatus* DSM 730^T, *G. vulcani* DSM 13174^T, *G. thermoleovorans* DSM 5366^T, *G. stearothermophilus* DSM22^T, *G. uzenensis* DSM13551^T, *G. jurassicus* DSM 15726^T, *G. gargensis* DSM 15378^T, *G. kaustophilus* DSM 7263^T and *G. lituanicus* DSM 15325^T were obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen, Brunswick, Germany (DSMZ) and were grown according to the DSMZ catalogue.

Morphological and physiological tests. The temperature range for growth was determined by incubating the isolate from 25 to 75°C. For the spore formation test, enrichment medium plus 0.001% (w/v) MnCl₂·4H₂O was used. The pH tolerance of strains was tested at 60°C at different pH values by using buffered NB medium (50 mM MES, HEPES, TAPS and CAPSO) over these pH values: 5.0, 5.5, 6.0, 6.5, 7.5, 8.0, 8.5, 9.0, and 10.0. In order to study the utilization of single carbon sources, the isolates were grown statically using

M162 minimal medium (Poli et al., 2006). The organic compounds tested (1%, w/v) were D-glucose, D-lactose, D-maltose, D-fructose, D-galactose, D-xylose, D-mannose, D-trehalose, glycerol, D-cellobiose, sodium acetate and sucrose. In order to test the capability to use hydrocarbons, the NB medium was diluted (15 times) and 1% (v/v) hydrocarbons were added and incubated for 72 h. All growth tests were done at 60°C and the growth was scored positive if the $A_{540\text{nm}}$ was greater than 0.300 after 3 days. Cellular morphology and motility were determined by phase-contrast microscopy (Zeiss) and colony morphology was determined with a Leica M8 stereomicroscope using cultures grown on media NB agar plates for 24 h at the optimal temperature. Gram staining and KOH test were performed as previously reported (Poli et al., 2009).

Unless otherwise stated the strain was characterized using the modified methods of Gordon and Pang (1973). Starch hydrolysis was tested by flooding cultures on solid enrichment medium NB containing 0.2% (w/v) starch with Lugol's iodine. For casein hydrolysis a solid medium plus an equal quantity of skimmed milk was used. For gelatine hydrolysis and sensibility to lysozyme, enrichment medium plus 1.0% (w/v) gelatine and 0.001% (w/v) lysozyme was used, respectively. The consumption of glucose either by oxidation or fermentation and respiration on nitrate and nitrite were tested as described by Poli et al. (2012). Indole production was detected in the medium containing 0.5% (w/v) peptone and 0.3% (w/v) meat extract in 1 L distilled water at pH 9.0 plus 1% (w/v) tryptophan using Kovacs' reagent. For the tyrosine decomposition test, solidified standard growth medium NB plus 0.05% (w/v) L-tyrosine was used. Hydrolysis of hippurate was tested in standard growth medium NB plus 1% (w/v) sodium hippurate. Urease was determined as described by Poli et al. (2012). Lipase and phosphatase activity were determined according to Lanyi (1987). Oxidase activity was determined by assessing the oxidation of tetramethyl-*p*-phenylenediamine and catalase activity was determined by assessing bubble production in a 3% (v/v) hydrogen peroxide solution. For nitrate and nitrite reduction, liquid growth NB medium plus 0.1% (w/v) KNO_3 and 0.001% (w/v) NaNO_2 were used, respectively. The methyl red and Voges-Proskauer tests were performed according to Clark and Lubs (1915) and Barrit (1936), respectively. Sensitivity of the strain to the antibiotics was tested by using the enrichment-solid NB medium and sensi-discs (6 mm, Oxoid)

(Poli et al., 2012).

Lipid and fatty acid compositions. Polar lipids were obtained from 3.0 g of freeze-dried cells grown in aerobic conditions on NB medium at 60°C and harvested at the stationary growth phase. Polar lipids were extracted with $\text{CHCl}_3/\text{MeOH}/\text{H}_2\text{O}$ (65 : 25 : 4, by vol.). The lipid extract was analyzed by thin layer chromatography (TLC) on silica gel (0.25 mm, F254, Merck) eluted with $\text{CHCl}_3/\text{MeOH}/\text{H}_2\text{O}$ (65 : 25 : 4, by vol.). All polar lipids were detected by spraying the plates with 0.1% (w/v) $\text{Ce}(\text{SO}_4)_2$ in 2 N H_2SO_4 followed by heating at 100°C for 5 min. Phospholipids and aminolipids were detected on the plates upon spraying with the Dittmer-Lester and the ninhydrin reagents, respectively, and glycolipids were visualized with α -naphthol (Nicolaus et al., 2001). Polar lipids were also identified by ^1H -NMR spectra. Quinones were analyzed by LC/MS on a reverse-phase RP-18 Lichrospher (250 × 4 mm) column eluted with *n*-hexane/ethylacetate (99 : 1, v/v) with a flow rate of 1.0 ml/min and identified by ESI/MS and ^1H NMR spectra (Nicolaus et al., 2001). Fatty acid methyl esters (FAMES) were obtained from complex lipids by acid methanolysis and analyzed using a Hewlett-Packard 5890A gas chromatograph fitted with a FID detector, as previously reported (Nicolaus et al., 2001).

NMR spectra, recorded at the NMR Service of Institute of Biomolecular Chemistry of CNR (Pozzuoli, Italy), were acquired on a Bruker DPX-300 operating at 300 MHz, using a dual probe.

16S rDNA sequencing, phylogenetic analysis and DNA-DNA hybridization studies. Genomic DNA extraction, PCR mediated amplification of the 16S rDNA and purification of the PCR products was carried out as described previously (Rainey et al., 1996). Purified PCR products were sequenced using the CEQ™-DTCS-Quick Start Kit (Beckmann Coulter) as directed in the manufacturer's protocol at the Deutsche Sammlung von Mikroorganismen und Zellkulturen, Brunschweig, Germany (DSMZ).

The G+C mol% content was evaluated by modifying the procedure originally described by Gonzalez and Saiz-Jimenez (2002) as reported in Poli et al. (2009). DNA sample purification and DNA-DNA percent homology evaluation by filter hybridization were performed as previously described (Poli et al., 2009). The 16S rRNA gene sequence of Ge1^T strain was submitted to EMBL Nucleotide Sequence Database and has been assigned accession number HE613733.

Sequences of related taxa were obtained from GenBank/EMBL/DDBJ databases. The values for pairwise 16S rRNA gene sequence similarity among the closest species were determined using the EzTaxon server (<http://www.Eztaxon.org>) (Chun et al., 2007). A phylogenetic tree was constructed by using the software package MEGA version 5 (Tamura et al., 2011) after multiple alignment of the data by CLUSTAL_X (Thompson et al., 1997). Distances (distance options according to the Kimura two-parameter model; Kimura, 1980) and clustering were based on the neighbor-joining method. Tree topology was re-examined by the bootstrap method of resampling (Felsenstein, 1985) using 500 bootstraps.

Random amplified polymorphic DNA-PCR (RAPD-PCR), used to produce fingerprint patterns of Ge1^T and *Geobacillus subterraneus* DSM 13552^T, *Geobacillus thermodenitrificans* DSM 466^T, *Geobacillus thermocatenulatus* DSM 730^T and *Geobacillus vulcani* DSM 13174^T, was performed as previously described (Roni-mus et al., 1997).

Results and Discussion

Phylogenetic analysis and DNA-DNA hybridizations

This is the first study that has been carried out on the isolation of thermophilic bacteria in the Guclukonak hot spring (Sirnak-Turkey). The total 16S rRNA gene sequence of the strain Ge1^T (EMBL nucleotide sequence accession number is HE613733) falls within the radiation of the genus *Geobacillus* and showed high similarity to *Geobacillus subterraneus* DSM 13552^T (99.6%) and to *Geobacillus thermodenitrificans* DSM 466^T (99.2%). The bacterium shared a similarity of 98.4% to *G. thermocatenulatus* DSM 730^T, 98.8% to *G. vulcani* DSM 13174^T, 98.7% to *G. thermoleovorans* DSM 5366^T, 98.7% to *G. kaustophilus* DSM 7263^T, 98.4% to *G. stearothermophilus* DSM 22^T, 98.3% to *G. jurassicus* DSM 15726^T, 98.2% to *G. uzenensis* DSM 13551^T, 98.1% to *G. gargensis* DSM 15378^T and 98.0% to *G. lituanicus* DSM 15325^T. A phylogenetic tree was constructed using the neighbor-joining method showing the position of the novel isolate with respect to other related species of genus *Geobacillus* (Fig.1). It

Fig. 1. Phylogenetic tree based on neighbor joining of 16S rRNA gene sequences, showing the position of the novel isolate with respect to other related species.

Bootstrap values (expressed as percentages of 500 replications) >50% are shown at branch points.

Fig. 2. RAPD-PCR fingerprint analysis.

Primers OPR2 (lanes 1–6) and GTG5 (lanes 7–12) were used for the RAPD-PCR fingerprint analysis of Ge1^T and its closest relatives. Lines: 1) Ge1^T DSM 23066^T; 2) *Geobacillus subterraneus* DSM 13552^T; 3) *Geobacillus thermodenitrificans* DSM 466^T; 4) *Geobacillus thermocatenulatus* DSM 730^T; 5) *Geobacillus vulcani* DSM 13174^T; 6) OPR2-Negative Control; Lines: 7) Ge1^T DSM 23066^T; 8) *Geobacillus subterraneus* DSM 13552^T; 9) *Geobacillus thermodenitrificans* DSM 466^T; 10) *Geobacillus thermocatenulatus* DSM 730^T; 11) *Geobacillus vulcani* DSM 13174^T; 12) GTG5-Negative Control; L: ladder.

has been largely accepted that if the level of 16S rRNA similarity is greater than 97% other additional phenotypic and genotypic characteristics should be used for taxonomic purposes.

RAPD-PCR was used to produce fingerprint patterns of the isolate Ge1^T and the related strains. RAPD-PCR fingerprint profiles of strain Ge1^T were clearly different from those produced by its closest relatives within the range 50–5,000 bp (Fig. 2). Strain Ge1^T produced fingerprints (lanes 1 and 7) very different with respect to *Geobacillus subterraneus* (lanes 2 and 8) and to the other species of *Geobacillus* genus employed, using both OPR2 and GTG5 primers. In particular, strain Ge1^T and *G. subterraneus* differed at the level of bands 100 and almost 1,000 bp utilizing OPR-2 primer and at the level of band 2,000 bp using GTG5 primer.

The DNA-DNA reassociation analysis between strain Ge1^T and the closest type strain of *G. subterraneus* were performed in triplicate (71%, 69% and 68%). The average was 69.3%. The DNA-DNA reassociation values found between strain Ge1^T and the type strains of *G. thermodenitrificans*, *G. thermocatenulatus*, *G. vulcani*, *G. thermoleovorans*, *G. kaustophilus*, *G. stearothermophilus*, *G. jurassicus*, *G. uzenensis*, *G. gargasensis* and *G. lituanicus* were 57%, 37%, 27%, 26%, 26%, 25%, 23%, 22%, 20% and 16%, respectively. The value of 69.3% for the DNA-DNA reassociation analysis between strain Ge1^T and the closest strain *G. subterraneus* was borderline with the recommended threshold value of 70%, which is accepted as the definition of a

genospecies (Wayne et al., 1987). Therefore, additional studies, such as lipid and fatty acid analysis, carbon source utilization, and other biochemical features, were required to establish strain Ge1^T as a new subspecies of *Geobacillus subterraneus*.

Morphological and biochemical analysis

Cells of isolate Ge1^T were Gram-positive, aerobe, motile rods. Cells formed cream circular colonies with a diameter of 1.5–2.0 mm and smooth margins on solid medium NB at 60°C. The temperature growth range was from 35 to 65°C with an optimum growth at 60°C. The pH growth range was from 5.5 to 10.0 with an optimum at pH 9.0. The isolate was grown on NB pH 9.0 at 60°C unless otherwise stated and subculturing was performed on the same medium. The isolate was maintained as glycerol stock at –70°C for further studies.

Growth was observed up to 1% NaCl (w/v). The isolate Ge1^T utilized, as carbon sources, D-galactose, D-lactose, D-maltose, D-trehalose, D-fructose, D-mannose, D-xylose, sucrose, glycerol, sodium acetate, cellobiose and weakly glucose. The isolate Ge1^T was positive for nitrate and nitrite reduction, lipase, phosphatase, urease, oxidase, starch and gelatine hydrolysis, while it was negative for indole production, hippurate and casein-hydrolysis (Table 1). Ge1^T was sensitive to lysozyme and it was negative for methyl red and Voges-Proskauer tests. It was positive for ONPG activity, for oxidation of D-glucose and respiration on nitrate. The isolate was resistant to Nystatin 100 µg and Novobiocin 5 µg, while it was sensitive to Ampicillin 10 µg, Bacitracin 10 U, Chloramphenicol 30 µg, Fusidic acid 10 µg, Gentamycin 10 µg, Kanamycin 5 µg, Lincomycin 15 µg, Neomycin 10 µg, Penicillin 2 U, Streptomycin 10 µg, Tetracycline 30 µg and Tilcomycin 15 µg. Comparative analysis conducted between Ge1^T and *Geobacillus subterraneus* DSM 13552^T showed that they use different hydrocarbons (1%, v/v): strain Ge1^T was not able to utilize toluene, while *Geobacillus subterraneus* DSM 13552^T was not able to utilize xylene on phenanthroline as the sole carbon and energy source. Both strains utilized *n*-decane and squalene. In Table 1 are shown the differences in terms of phenotypic and biochemical characteristics between strain Ge1^T and its closest relatives. In particular, strain Ge1^T differed from the closest related species, *G. subterraneus*, in terms of pH values, NaCl range, methyl red test, utilization of D-lactose, glycerol, cellobiose and

Table 1. Differential characteristics of strain Ge1^T and its closest phylogenetic relatives.

Characteristics	1	2	3	4	5	6
Temperature range (°C)	35–65	45–65	45–70	42–69	35–78	37–72
Optimum temperature (°C)	60	55–60	55–60	55–60	55–65	60
pH range	5.5–10.0	6.2–7.6	6.0–8.0	6.5–8.5	6.2–7.8	5.5–9.0
Optimum pH	9.0	6.8–7.0	6.8–7.0	6.8–7.0	6.8–7.0	6.0
Motility	+	+	+	+	–	+
NaCl (% w/v)	0–1	0–3	0–5	0–5	0–4	0–3
Sensitivity to lysozyme (0.001%)	+	+	+	+	+	–
Catalase	–	–	+	+	v	–
Oxidase	+	+	+	+	v	–
O/F (D-Glucose)	O	O	O/F	O	O/F	O
MR-VP	–/–	+/–	–/–	+/–	+/–	+/–
Hydrolysis of						
Gelatine	+	–	–	–	–	+
Starch	+	+	–	–	+	+
Casein	–	–	–	+	–	+
Urea	+	–	–	–	–	–
Hippurate	–	+	+	+	+	–
ONPG	+	–	+	+	+	+
Tyrosine degradation	+/–	–	+	++	–	–
Utilization of						
D-Glucose	w	+	+	+	+	+
D-Galactose	+	+	+	+	–	+
D-Fructose	+	+	–	+	–	+
D-Lactose	+	–	+	–	+	+
Sucrose	+	+	–	+	–	+
D-Mannose	+	+	+	+	–	+
Glycerol	+	–	–	+	+	+
D-Xylose	+	+	–	+	+	+
Sodium acetate	+	–	–	+	+	+
D-Cellobiose	+	–	+	+	+	+
DNA G+C content (mol%)	52.0	52.3	48.2–52.3	45–46	55.2	53.0

Strains: 1, *Geobacillus subterraneus* subsp. *aromaticivorans*, strain Ge1^T; 2, *G. subterraneus* 34^T (DSM 13552^T); 3, *G. thermophilus* (DSM 466^T); 4, *G. thermocatenulatus* (DSM 730^T); 5, *G. thermoleovorans* (DSM 5366^T); 6, *G. vulcani* (DSM 13174^T).

+, Positive; –, negative; w, weak response; v, variable; O, Oxidation; F, Fermentation; MR, Methyl Red test; VP, Voges-Proskauer test; Data for temperature range and optimum, NaCl range, pH range and optimum and G+C content for taxa 2 from Nazina et al. (2001); for taxa 3 from Manachini et al. (2000); for taxa 4 from Golovacheva et al. (1975); for taxa 5 from Zarilla and Perry (1987) and from Nazina et al. (2001); for taxa 6 from Caccamo et al. (2000) and Nazina et al. (2004); all other data are from this study. All of the species tested were positive for nitrate and nitrite reduction, and respiration on nitrate, utilization of D-trehalose and D-maltose and negative for indole production.

sodium acetate, hydrolysis of gelatine, urea, hippurate, ONPG activity and tyrosine degradation.

Lipid and fatty acid composition

The total lipid content in Ge1^T ranged between 8% and 10% of the total dry weight of cells grown at 60°C under standard conditions and harvested at the stationary phase of growth. A comparison of the polar lipid patterns by one-dimensional TLC (Fig. 3) revealed differences between Ge1^T and its closest relative, *Geobacillus subterraneus* DSM 13552^T. The main phospholipids detected in strain Ge1^T were 1,2 diacyl-

glycero-3-phosphorylethanolamine (PEA), 1,3-bis(*sn*-3-phosphatidyl)-*sn*-glycerol (known as cardiolipin; CL), phosphatidylglycerol (PG) and an unknown aminophosphoglycolipid (UK2). Moreover, the presence of phosphatidylserine (PS) was also detected but in minor amounts with respect to *G. subterraneus*. In addition, the presence of a unknown phospholipid UK1 (not present in *G. subterraneus*), was also recorded. FAME composition, determined on cells grown under standard conditions, was characterised by the abundance of branched acyl chains. The most abundant cellular fatty acid in strain Ge1^T was *iso*-C15:0 (37.4%),

Fig. 3. Thin Layer Chromatography (TLC) of polar lipids of strain Ge1^T and *Geobacillus subterraneus* DSM 13552^T eluted with CHCl₃/MeOH/H₂O (65 : 25 : 4, by vol.).

Total polar lipids were detected by spraying the plates with ninhydrine (A) or with 0.1% (w/v) Ce(SO₄)₂ in 2 N H₂SO₄ (B) followed by heating at 100°C for 5 min. Phospholipids were detected on the plates upon spraying with the Dittmer-Lester reagent (C) followed by heating at 100°C for 5 min.

other components were *iso*-C17:0 (29.6%), *anteiso*-C17:0 (10.8%), *anteiso*-C15:0 (9.5%), *anteiso*-C16:0 (7.0%), C16:0 (3.8%), C18:0 (0.5%), C15:0 (0.3%), and *iso*-C16:0 (0.3%), and traces of C17:0, *iso*-C18:0 and *anteiso*-C18:0 (less than 0.2%) were also present. This composition was similar to and corresponded well with those of members of the genus since the predominance of branched fatty acids is characteristic of *Geobacillus* species (Dinsdale et al., 2011; Nazina et al., 2001). On the other hand, the FAME composition of *Geobacillus subterraneus*, grown under the same conditions as strain Ge1^T, resulted in a major relative percentage of *iso*-C17:0 (36.6%), followed by *iso*-C15:0 (20.4%), *anteiso*-C17:0 (17.8%), *iso*-C16:0 (10.6%), C16:0 (8.8%), *anteiso*-C15:0 (2.5%), and *anteiso*-C16:0 (1.0%), and traces of C15:0 and C17:0 were also recorded. Chromatographic analysis of quinones revealed the presence of one abundant UV-absorbing band in strain Ge1^T. The ¹H-NMR spectrum showed the presence of menaquinone (MK) type. The LC/MS analysis gave a molecular peak corresponding to MK7 as major compound (more than 90% of quinones).

The borderline DNA-DNA hybridisation value between strain Ge1^T and *Geobacillus subterraneus* DSM

13552^T (the average value 69.3%) did not allow us to differentiate Ge1^T from all the other species of this genus into a separate new species (Wayne et al., 1987), but since lipid analyses, fatty acid profile, biochemical and physiological properties support the diversity from *Geobacillus subterraneus*, we propose strain Ge1^T as a novel subspecies of *Geobacillus subterraneus*, for which the name *Geobacillus subterraneus* subsp. *aromaticivorans* is proposed.

Description of *Geobacillus subterraneus* subsp. *subterraneus* (Nazina et al. 2001) subsp. nov.

Geobacillus subterraneus subsp. *subterraneus* (Nazina et al., 2001) subsp. nov. is automatically created with the same authors as those of *Geobacillus subterraneus* subsp. *aromaticivorans* according to the former rule of nomenclature (Garrity et al., 2011).

Geobacillus subterraneus subsp. *subterraneus* (subterraneanus. L. adj. *subterraneus* subterranean, below the Earth's surface). The description of *Geobacillus subterraneus* subsp. *subterraneus*. is identical to the description given by Nazina et al., 2001.

DNA-DNA hybridization between the type strain and the type strain of *G. subterraneus* subsp. *aromaticivorans* is 69.3%.

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.

Emendation of *Geobacillus subterraneus* (Nazina et al., 2001)

In addition to the description given above for *Geobacillus subterraneus* subsp. *subterraneus* (Nazina et al., 2001) subsp. nov., the following features are also found in the present paper: hydrolysis of hippurate and utilization of D-fructose, D-mannose and sucrose occur; squalene is degraded while xylene and phenanthroline are not. ONPG activity is negative. The following polar lipids are present: 1,2 diacylglycerol-3-phosphorylethanolamine (PEA), 1,3-bis(sn-3-phosphatidyl)-sn-glycerol (known as cardiolipin; CL), phosphatidylglycerol (PG), phosphatidylserine (PS) and an unknown aminophosphoglycolipid.

Description of *Geobacillus subterraneus* subsp. *aromaticivorans* subsp. nov.

G. subterraneus subsp. *aromaticivorans* (a.ro.ma.ti.ci.vo'rans. L. adj. aromaticus, aromatic, fragrant; L. part. adj. vorans, devouring; N.L. part. adj. aromaticivorans, devouring aromatic (compounds); i.e. able to degrade xylene and phenanthroline).

Cells are Gram-positive, sporulating rods, occurring in pairs or filamentous. Motile. Colonies on medium NB agar are circular, cream, and smooth. It is thermophilic and aerobic, exhibiting an optimum growth temperature of 60°C, but is able to grow between 30 and 65°C and at pH 9.0. The isolate Ge1^T utilized, as carbon sources, D-galactose, D-lactose and D-maltose, and weakly D-glucose, D-fructose, D-mannose, D-xylose, D-cellobiose, sodium acetate, sucrose, and glycerol. The isolate was resistant to Nystatin 100 µg and Novobiocin 5 µg, while it was sensitive to Ampicillin 10 µg, Bacitracin 10 U, Chloramphenicol 30 µg, Fusidic acid 10 µg, Gentamycin 10 µg, Kanamycin 5 µg, Lincomycin 15 µg, Neomycin 10 µg, Penicillin 2 U, Streptomycin 10 µg, Tetracycline 30 µg and Tilcomycin 15 µg. The isolate Ge1^T was lipase, ONPG, phosphatase, urease, oxidase, gelatine and starch hydrolysis positive while it was negative for catalase, indole, casein and hippurate hydrolysis. Isolate Ge1^T was not sensitive to lysozyme. Strain Ge1^T is able to utilize xylene, squalene, *n*-decane and phenanthroline among

tested hydrocarbons. The main phospholipids present in Ge1^T are 1,2 diacylglycerol-3-phosphorylethanolamine (PEA), 1,3-bis(sn-3-phosphatidyl)-sn-glycerol (known as cardiolipin; CL), phosphatidylglycerol (PG), an unknown phospholipid (UK1) and an unknown aminophosphoglycolipid (UK2). A minor amount of phosphatidylserine (PS) is also detected. MK7 is the most abundant menaquinone. The most abundant cell fatty acid in strain Ge1^T is *iso*-C15:0 (37.4%); other components are *iso*-C17:0 (29.6%), *anteiso*-C17:0 (10.8%), *anteiso*-C15:0 (9.5%), *anteiso*-C16:0 (7.0%), C16:0 (3.8%), C18:0 (0.5%), C15:0 (0.3%), *iso*-C16:0 (0.3%), and trace of C17:0, *iso*-C18:0 and *anteiso*-C18:0 (less than 0.2%). Ge1^T was isolated from the mud of Guclukonak hot spring in Sirmak (Turkey). Type strain Ge1^T has been deposited in the Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany (DSMZ 23066^T) and in the Institute Pasteur, France (CIP 110341^T). The DNA G+C content of the type strain is 52.0 mol%. DNA-DNA hybridization between the type strain and the type strain of *G. subterraneus* subsp. *subterraneus* is 69.3%. The GenBank/EMBL/DBJ accession numbers for the 16S rRNA gene sequence of strain Ge1^T is HE613733.

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