Sinibacillus soli gen. nov., sp. nov., a moderately thermotolerant member of the family *Bacillaceae*

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Two Gram-staining-positive, rod-shaped and endospore-forming bacteria that represent a single species, designated strains GD05^T and GD051, were isolated from a tropical forest soil and a hot spring sediment, respectively. Cells of both strains were facultatively anaerobic, catalase- and oxidase-positive, and could grow optimally at 50 °C, pH 8.0 and with 1 % (w/v) NaCl. Analysis of the 16S rRNA gene sequence revealed that these two isolates belonged to the family *Bacillaceae*, but did not show sequence similarities of more than 95 % to members of other related genera. The G+C content of the genomic DNA was 43.7–44.1 mol%. The major cellular fatty acids were anteiso-$C_{15:0}$, iso-$C_{15:0}$, iso-$C_{16:0}$ and anteiso-$C_{17:0}$. The main polar lipids were diphosphatidylglycerol and phosphatidylglycerol, and the major menaquinone was MK-7. The peptidoglycan type was A1γ (*meso*-diaminopimelic acid direct). On the basis of this polyphasic taxonomic analysis, the novel strains represent a novel species of a new genus in the family *Bacillaceae*, order *Bacillales*, for which the name *Sinibacillus soli* gen. nov., sp. nov. is proposed. The type strain is GD05^T (=CCTCC AB 2013105^T=KCTC 33117^T).

The family *Bacillaceae* in the order *Bacillales* is a large taxonomic group containing more than 40 genera with many different physiological features. Most bacteria of the family *Bacillaceae* are Gram-positive and endospore-forming rods. More than 25 genera of this family include halophilic or halotolerant species isolated from saline or hypersaline lakes, saline soils, seawater, etc., such as the genera *Halobacillus* (Spring *et al.*, 1996), *Oceanobacillus* (Lu *et al.*, 2001), *Ornithinibacillus* (Mayr *et al.*, 2006), *Terribacillus* (An *et al.*, 2007), *Sediminibacillus* (Carrasco *et al.*, 2008), *Streptohalobacillus* (Wang *et al.*, 2011) and *Saliterribacillus* (Amoozegar *et al.*, 2013). However, only few genera of the family *Bacillaceae*, such as *Bacillus* (Zarilla & Perry, 1987) and *Geobacillus* (Nazina *et al.*, 2001), include thermophilic or thermotolerant species. In this study, two new moderately thermotolerant bacteria, designated strains GD05^T and GD051, were isolated and characterized. On the basis of a polyphasic taxonomic characterization, these two isolates were assigned to the family *Bacillaceae*, and represent a novel species in a new genus, *Sinibacillus soli* gen. nov., sp. nov.

Strain GD05^T was isolated from a soil sample collected from a tropical forest in Xuwen County, Guangdong Province, South China. The sample was collected at about 10 cm below soil surface and had pH 6.5, a total organic carbon of 6 % and a total N of 0.5 %. Soil sample (10 g) was added into 100 ml 0.85 % NaCl solution and stirred for 30 min. Then tenfold serial dilutions were made from 10^{-1} up to 10^{-8}, and 100 µl of each dilution was spread onto the surface of trypticase soya agar (TSA; pH 7.2) which contained (l^{-1}) 15.0 g tryptone, 5.0 g soytone, 5.0 g sodium chloride and 15.0 g agar and incubated at 50, 60 or 70 °C. After incubation for 5 days, single colonies were picked and transferred to fresh TSA medium for further purification at their respective culture temperatures. This procedure was repeated until purified colonies were obtained. Strain GD051 was a by-product from an investigation of the microbial community of an electrochemical biofilm in our laboratory. The electrochemical biofilm was formed on the anode of a microbial fuel cell which was inoculated with a hot-spring sediment sample taken from Conghua City, Guangdong, China, and maintained at 60 °C. The isolates were preserved at −80 °C in trypticase soya broth (TSB; TSA without agar) supplemented with 15 % (v/v) glycerol for further study.

To establish the phylogenetic position of strains GD05^T and GD051, genomic DNA was extracted using a DNA extraction kit (Aidlab) and the 16S rRNA gene was amplified by PCR with the forward primer 27F and the reverse primer 1492R (Baker *et al.*, 2003). The PCR product was gel-purified using a Gel Extraction kit (D2500-01; Omega Bio-tek), cloned into a plasmid vector using a TA cloning kit (TaKaRa) and then double-checked by sequencing both strands. The sequences of strains GD05^T (1489 bp) and GD051 (1490 bp) displayed 99.5 % similarity over the length of the 16S rRNA gene sequence. A

Abbreviation: *meso*-DAP, *meso*-diaminopimelic acid.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains GD05^T and GD051 are KC404830 and KF188705, respectively.

Three supplementary figures and two supplementary tables are available with the online version of this paper.

similarity search using BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi) revealed that both isolates were members of the family *Bacillaceae*, but they showed low sequence similarities to the type strains of the type species of other members of this family. The most closely related taxa to strains GD05^T and GD051 were *Ornithinibacillus contaminans* (94.9 % sequence similarity), *Ornithinibacillus bavariensis* (94.5 %) and *Virgibacillus kekensis* (93.9 %). Other type strains of species of the genera *Ornithinibacillus* and *Virgibacillus* and all type strains of species of the genus *Oceanobacillus* shared 93.9–93.6 %, 93.7–91.5 % and 93.6–91.6 % 16S rRNA gene sequence similarities with strain GD05^T, respectively. Except for the above genera, the isolates showed no more than 93 % similarity with other members of the family *Bacillaceae*.

Pairwise sequence similarity was calculated using the EzTaxon-e server (http://eztaxon-e.ezbiocloud.net/; Kim *et al.*, 2012). Phylogenetic analysis was carried out using MEGA version 5.0 (Tamura *et al.*, 2011) after multiple alignment of the sequence data with CLUSTAL X (Thompson *et al.*, 1997). For the neighbour-joining and minimum-evolution trees, the substitution model maximum composite likelihood method was chosen, and for the maximum-likelihood tree, the Tamura-Nei model was used. Statistical support for the branches of the phylogenetic trees was determined using bootstrap analysis (based on 1200 resamplings) (Felsenstein, 1985). The phylogenetic analysis revealed that strains GD05^T and GD051 belonged to the family *Bacillaceae* and formed an independent group with a bootstrap value of 70 % in the neighbour-joining tree and minimum-evolution tree (Fig. 1), which also confirmed the phylogenetic position of the two new isolates.

Based on the analysis of 16S rRNA gene sequence similarities and phylogenetic trees, *Ornithinibacillus*, *Virgibacillus* and *Oceanobacillus* were the most closely related genera to the new isolates, so the type strains of the type species of these three genera (*Ornithinibacillus bavariensis* DSM 15681^T, *Virgibacillus pantothenticus* DSM 26^T and *Oceanobacillus iheyensis* DSM 14371^T) were selected as reference strains for comparison in this study. These strains were purchased from the German Collection of Microorganisms and Cell Cultures (DSMZ). To phenotypically characterize the new isolates, standard phenotypic tests were performed. The recommended minimal standards for describing new taxa of aerobic, endospore-forming bacteria were followed (Logan *et al.*, 2009).

Cell morphology and the presence of flagella were determined by transmission electron microscopy (JEM 1400; JEOL) after cells were grown on TSA for 12 h. In preparation for transmission electron microscopy, bacterial cells were suspended in phosphate buffer solution (140 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4 and 1.8 mM KH_2PO_4, pH 7.4), dried on a nickel-coated mesh, and negatively stained with phosphotungstic acid. The presence of endospores was investigated using a staining solution kit (HB8300; Qingdao Hope-Bio Technology) and observed by light microscopy (BX51; Olympus). The motility of cells was tested by observing the growth spread in semi-solid TSA medium. Gram staining, urease activity, nitrate reduction, hydrolysis of aesculin, starch and casein, production of indole and Voges–Proskauer tests were performed as recommended by Smibert & Krieg (1994). Hydrolysis of Tween 80 was examined as described by Dong & Cai (2001). Catalase activity was determined by observing bubble production in 3 % (v/v) hydrogen peroxide solution and oxidase activity was determined using an oxidase reagent (bioMérieux). Haemolysis was assessed by spot-inoculation on TSA supplemented with 5 % ovine blood (Oxoid) followed by incubation at 37 °C or 50 °C for 1–3 days. Growth was tested at 20–70 °C, and NaCl tolerance was examined in the range 0–10.0 % (w/v) NaCl (in increments of 0.5 %). The pH range (pH 4.0–10.0 at intervals of 0.5 pH units) for growth was determined in TSB buffered with citrate/phosphate buffer or Tris/hydrochloride buffer (Breznak & Costilow, 1994). Anaerobic growth was tested in anaerobic chambers (Sheldon Manufacturing) for 2 weeks. Oxidative or fermentative utilization of glucose was determined on Hugh & Leifson's medium (Leifson, 1963). The use of acetate, lactate, pyruvate, D-glucose, L-arabinose, sucrose, inositol, D-mannitol, D-fructose, D-ribose, rhamnose and raffinose as carbon and energy sources was examined aerobically using a basal medium (0.2 g $MgSO_4.7H_2O$, 5.0 g NaCl, 0.5 g K_2HPO_4, 0.5 g K_2HPO_4, 0.02 g yeast extract and 1.5 % agar in 1000 ml distilled water, at pH 7.2) containing $(NH_4)_2SO_4$ (0.2 %, w/v). The utilization of $(NH_4)_2SO_4$, NH_4Cl, L-alanine, L-arginine, L-asparagine, L-cysteine, L-glycine, L-valine, L-serine, L-histidine and L-proline as nitrogen sources was tested in the above basal medium supplemented with D-glucose (1 %, w/v) under aerobic conditions. Acid production and some other physiological and biochemical properties were examined using the API 20E and API 50CH systems (bioMérieux) at 37 °C. Results were recorded after 4 h, 12 h, 24 h, 48 h and 72 h, and positive reactions were those with positive results recorded at any of these observation times.

Strains GD05^T and GD051 were Gram-staining-positive, motile and facultatively anaerobic. Cells were long rods with peritrichous flagella, which occurred singly or in short chains (Fig. S1, available in the online Supplementary Material). Spherical to ellipsoidal endospores were formed at the terminal position in swollen sporangia (Fig. S2). Both isolates were moderately thermophilic and grew optimally at 50 °C, with the difference that strain GD051 could grow at 63 °C but strain GD05^T could not. NaCl was essential for cell growth, but cells could not grow with more than 6 % NaCl (w/v). The growth temperature and NaCl tolerance characteristics of strains GD05^T and GD051 are distinct from those of all members of the genera *Ornithinibacillus*, *Virgibacillus* and *Oceanobacillus*, which can tolerate at least 10 % (w/v) NaCl but cannot grow at a temperature higher than 55 °C (Table S1). Growth occurred within a narrow pH range (pH 7.0–9.0) and optimally at pH 8.0. Voges–Proskauer reaction, nitrate

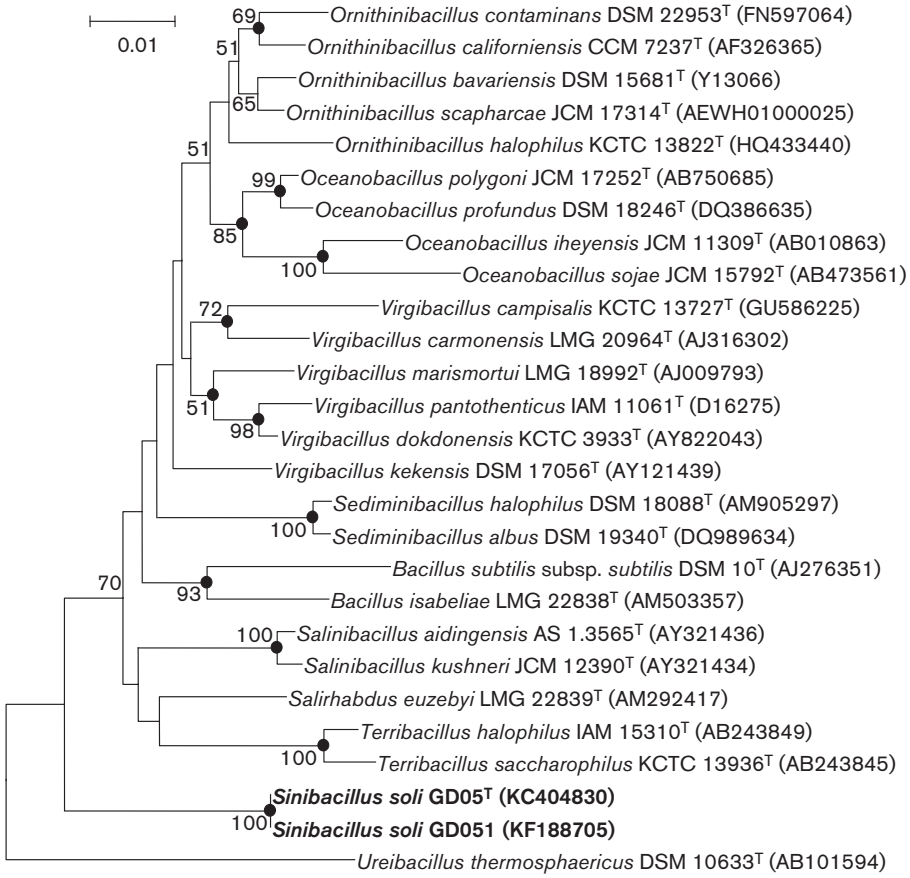

Fig. 1. Phylogenetic tree reconstructed using the neighbour-joining method based on 16S rRNA gene sequences, showing the position of strains GD05^T and GD051 and closely related species of genera within the family *Bacillaceae*. Bootstrap values, generated from 1200 resamplings, at or above 50 % are indicated at branching points. Filled circles indicate branches found in phylogenetic consensus trees generated with the minimum-evolution and maximum-likelihood methods. *Ureibacillus thermosphaericus* DSM 10633^T was used as an outgroup. Bar, 0.01 nucleotide substitutions per nucleotide position.

reduction, production of H_2S and indole, and casein hydrolysis were negative, and oxidase, catalase and hydrolysis of gelatin were positive. Detailed phenotypic features are included in the species description and Tables 1, S1 and S2.

Menaquinones were extracted with methanol using freeze-dried cells according to Collins *et al.* (1977) and analysed by HPLC (model 1260; Agilent) as described by Groth *et al.* (1997). Strain GD05^T contained a quinone system that consisted of the major component MK-7 (97 %) and the minor component MK-8 (3 %), and strain GD051 contained a similar quinone system of MK-7 (95 %) and MK-8 (5 %). The G+C content of the genomic DNA was determined by HPLC according to the method of Mesbah *et al.* (1989). The G+C contents of the genomic DNA of strains GD05^T and GD051 were 43.7 and 44.1 mol%, respectively, which were higher than those of the reference strains (Table 1). In addition, the DNA G+C contents of the type strains of species of the genera *Ornithinibacillus*, *Virgibacillus* and *Oceanobacillus* were reported to be in the range 36–41 mol%, 36–43 mol% and 35.8–40.2 mol%, respectively (Table S1). Therefore, the DNA G+C contents of the two novel isolates are higher than those of all members of closely related genera.

Polar lipids were extracted, separated by two-dimensional TLC and identified according to the method of Minnikin *et al.* (1984) by spraying individual plates with appropriate detection reagents: molybdophosphate for total lipids, molybdenum blue for phospholipids, ninhydrin reagent for amino-containing lipids and α-naphthol reagent for glycolipids. As shown in Fig. S3, the polar lipid pattern of strain GD05^T consisted of diphosphatidylglycerol (DPG), phosphatidylglycerol (PG) and three unidentified phospholipids; for strain GD051, DPG and PG were the predominant and moderate polar lipids, respectively, and the trace components were an unidentified aminophospholipid and an unidentified phosphoglycolipid. Polar lipid profiles containing DPG as the predominant polar lipid and PG as a moderate component were in accordance with those reported for the genera *Ornithinibacillus*,

Table 1. Differential characteristics of strains GD05^T and GD051 and members of related genera in the family *Bacillaceae*

Strains: 1, GD05^T; 2, GD051; 3, *Ornithinibacillus bavariensis* DSM 15681^T; 4, *Virgibacillus pantothenticus* DSM 26^T; 5, *Oceanobacillus iheyensis* DSM 14371^T. Data were taken from this study, except for isolation source, cell size, DNA G+C content and peptidoglycan type of *Ornithinibacillus bavariensis* DSM 15681^T (Mayr *et al.*, 2006), *V. pantothenticus* DSM 26^T (Heyndrickx *et al.*, 1998; Heyrman *et al.*, 2003) and *Oceanobacillus iheyensis* DSM 14371^T (Lu *et al.*, 2001). +, Positive; −, negative. For spore shape: E, ellipsoidal; S, spherical; for spore position: ST, subterminal; T, terminal.

Characteristic	1	2	3	4	5
Isolation source	Forest soil	Hot-spring sediment	Pasteurized milk	Soil	Deep-sea sediment
Cell size (μm)	0.3–0.4 × 1.4–2.9	0.3–0.5 × 1.4–3.2	0.4 × 2.0–6.0	0.5–0.7 × 2.0–5.0	0.6–0.8 × 2.5–3.5
Chains of cells	+	+	+	+	−
Endospore shape	S/E	S	E	S/E	E
Endospore position	T	T	T	T/ST	T/ST
Anaerobic growth	+	+	−	+	−
Optimal growth temperature (°C)	50	50	40	37	30
Optimal growth salinity (%)	1	1–1.5	3–4	3.5	3
Optimal pH for growth	8.0	8.0	8.5–9	7.0	8.0–8.5
Growth at/with:					
25 °C	−	−	+	+	+
55 °C	+	+	−	−	−
63 °C	−	+	−	−	−
0 % (w/v) NaCl	−	−	+	+	+
7–10 % (w/v) NaCl	−	−	+	+	+
pH 6.5	−	−	−	+	+
pH 10.0	−	−	+	−	+
Citrate utilization	−	−	−	+	−
H_2S production	−	−	−	+	−
Haemolysis	+	+	+	−	−
Hydrolysis of:					
Tween 80	+	−	−	−	−
Casein	−	−	−	+	+
DNA G+C content (mol%)	43.7	44.1	36.4	38.3	35.8
Peptidoglycan type	A1γ (*meso*-DAP direct)	A1γ (*meso*-DAP direct)	A4β (L-Orn←D-Asp)	A1γ (*meso*-DAP direct)	*meso*-DAP direct

Virgibacillus and *Oceanobacillus* (Heyrman *et al.*, 2003; Mayr *et al.*, 2006).

The peptidoglycan type of strain GD05^T was analysed by the Identification Service of the German Collection of Microorganisms and Cell Cultures (DSMZ, Braunschweig, Germany) and that of strain GD051 was isolated and studied by TLC on cellulose plates using published protocols (Schumann, 2011). For the total hydrolysate (4 M HCl, 100 °C, 16 h) determined by GC-MS, peptidoglycan of strain GD05^T consisted of the amino acids alanine (Ala), glutamic acid (Glu) and *meso*-diaminopimelic acid (*meso*-DAP) in a molar ratio of 1.0 : 1.0 : 0.9 besides traces of glycine (Gly); the amino acids Ala, Glu and *meso*-DAP were present in the peptidoglycan of strain GD051 in a molar ratio of 1.1 : 1.0 : 0.8, and the trace amino acids were Gly, serine (Ser) and aspartic acid (Asp). In addition to these amino acids, the partial hydrolysate (4 M HCl, 100 °C, 0.75 h) contained the peptides L-Ala–D-Glu and *meso*-DAP–D-Ala. From these data it was concluded that the two isolates showed the peptidoglycan type A1γ *meso*-DAP-direct (Schleifer & Kandler, 1972; type A31 according to http://www.dsmz.de/catalogues/catalogue-microorganisms/specific-catalogues/peptidoglycans.html). This peptidoglycan type was found in the majority of endospore-forming rod-shaped bacteria, including members of the genera *Virgibacillus* (Heyrman *et al.*, 2003) and *Oceanobacillus* (Mayr *et al.*, 2006). This peptidoglycan type could also clearly distinguish strains GD05^T and GD051 from the species of the most closely related genus, *Ornithinibacillus*, which have a peptidoglycan type of A4β (L-Orn–D-Asp) (Mayr *et al.*, 2006).

For cellular fatty acid analysis, strains GD05^T and GD051 and the reference strains were grown in TSB at 37 °C. At the exponential growth phase, cells were collected and fatty acids in whole cells were saponified, methylated and extracted according to the standard protocol of MIDI (Sherlock Microbial Identification System, version 6.0B). The fatty acids were analysed with GC (model 6850; Agilent) and identified using the TSBA6.0 database of the

Microbial Identification System (Sasser, 1990). The fatty acid profiles of the two isolates and the reference strains are presented in Table 2. The predominant fatty acid of strains GD05^T and GD051 (anteiso-$C_{15:0}$, 30.0 and 33.4 %, respectively) was distinct from that of strains *Ornithinibacillus bavariensis* DSM 15681^T (iso-$C_{15:0}$, 32.5 %) and *Oceanobacillus iheyensis* DSM 14371^T (iso-$C_{15:0}$, 31.7 %). Although similar to strains GD05^T and GD051 in that the predominant fatty acid was anteiso-$C_{15:0}$, *V. pantothenticus* DSM 26^T had much larger proportions of anteiso-$C_{15:0}$ (49.7 %) and anteiso-$C_{17:0}$ (27.5 %). The fatty acid profile of *Oceanobacillus iheyensis* in this study was consistent with that described by Kim *et al.* (2007), but was different from that reported by Hirota *et al.* (2013) who detected anteiso-$C_{15:0}$ as the predominant fatty acid, which suggested that the fatty acid profile of *Oceanobacillus iheyensis* varies with culture conditions. In this study, the fatty acids of the new isolates and *Oceanobacillus iheyensis* were determined using cells cultured under the same conditions, so the comparison was credible.

Phylogenetic analysis of the 16S rRNA gene sequences showed that strains GD05^T and GD051 represent a novel branch within the Gram-staining-positive, endospore-forming bacteria in the family *Bacillaceae*. As shown in Table S1, the two isolates can be distinguished from related genera of the family *Bacillaceae* by some morphological, chemotaxonomic, biochemical and physiological properties. Being capable of growing under anaerobic conditions can separate the new isolates from members of the genera *Ornithinibacillus*, *Terribacillus*, *Salirhabdus* and *Salinibacillus*. Cells forming chains can separate them from members of the genera *Oceanobacillus*, *Salirhabdus* and *Salinibacillus*. The NaCl concentration for growth (range and optimum) and temperature for growth (range and optimum) of the new isolates were distinct from those of all other genera in Table S1. These two isolates can be differentiated from members of the phylogenetically closest genus, *Ornithinibacillus*, by the peptidoglycan type of the cell wall. Finally, the DNA G+C contents of both new isolates were higher than those of members of the other genera in Table S1 except for *Terribacillus* and *Sediminibacillus*. In conclusion, the data suggest that both strains represent a novel species of a new genus within the family *Bacillaceae*, for which the name *Sinibacillus soli* gen. nov., sp. nov. is proposed.

Table 2. Cellular fatty acid profiles of strains GD05^T and GD051 and the type species of the related genera *Ornithinibacillus*, *Virgibacillus* and *Oceanobacillus*

Strains: 1, GD05^T; 2, GD051; 3, *Ornithinibacillus bavariensis* DSM 15681^T; 4, *Virgibacillus pantothenticus* DSM 26^T; 5, *Oceanobacillus iheyensis* DSM 14371^T. Data were taken from this study. Values are percentages of the total fatty acids; –, fatty acids representing <1.0 % of the total.

Fatty acid	1	2	3	4	5
$C_{14:0}$	–	–	1.1	–	1.3
iso-$C_{14:0}$	7.5	2.9	1.3	2.4	1.0
iso-$C_{15:0}$	15.3	11.9	**32.5**	4.1	**31.7**†
anteiso-$C_{15:0}$	**30.0**	**33.4**	7.6	**49.7**	19.8
$C_{16:0}$	1.9	3.9	6.9	2.9	6.6
iso-$C_{16:0}$	13.3	9.8	7.5	7.9	3.4
$C_{16:1}\omega7c$ alcohol	3.6	–	–	–	–
iso-$C_{17:0}$	7.4	6.0	14.4	1.3	9.8
anteiso-$C_{17:0}$	11.5	18.1	14.4	**27.5**	12.2
$C_{18:1}\omega9c$	1.5	3.0	1.8	1.6	2.1
$C_{18:0}$	1.2	–	1.3	1.2	4.0
Summed feature 3*	1.7	3.0	1.4	1.4	–
Summed feature 4*	1.8	–	–	–	–
Summed feature 8*	–	–	1.1	1.0	3.2

*Summed feature 3 comprises $C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$; summed feature 4 comprises iso-$C_{17:1}$ I/anteiso-$C_{17:1}$ B; summed feature 8 comprises $C_{18:1}\omega7c$ and/or $C_{18:1}\omega6c$.

†The predominant fatty acid was iso-$C_{15:0}$ in this study and in that by Kim *et al.* (2007), but was anteiso-$C_{15:0}$ in the study by Hirota *et al.* (2013).

Description of *Sinibacillus* gen. nov.

Sinibacillus (Si.ni.ba.cil′lus. N.L. fem. pl. n. *Sinae* China; L. dim. n. *bacillus* a small rod; N.L. masc. n. *sinibacillus* a rod-shaped microbe isolated from China).

Cells are Gram-staining-positive, motile and endospore-forming rods that occur singly or in short chains. Colonies are small, circular and convex with regular margins. Moderately thermophilic. Phylogenetically related to the genera *Ornithinibacillus*, *Virgibacillus*, *Oceanobacillus* and other genera of the family *Bacillaceae*. The cell-wall peptidoglycan type is A1γ (*meso*-DAP direct). Cellular fatty acids consist mainly of iso- and anteiso-branched acids, with anteiso-$C_{15:0}$ predominating and iso-$C_{15:0}$, iso-$C_{16:0}$ and anteiso-$C_{17:0}$ in moderate amounts The major respiratory quinone is MK-7. Polar lipids mainly consist of diphosphatidylglycerol and phosphatidylglycerol. DNA G+C contents are 43.7–44.1 mol%. The type species is *Sinibacillus soli*.

Description of *Sinibacillus soli* sp. nov.

Sinibacillus soli (so′li. L. neut. gen. n. *soli* of soil, the source of the type strain).

In addition to the characteristics given in the genus description, *Sinibacillus soli* exhibits the following properties. Cells are straight to slightly curved rods, 0.3–0.5 μm wide and 1.4–3.2 μm long. Motile by peritrichous flagella. Spherical to ellipsoidal endospores are produced at a terminal position in swollen sporangia. Colonies are circular, convex, entire, smooth, cream and grow to about 2 mm in diameter on TSA after 48 h of incubation at 50 °C. Anaerobic growth occurs. Oxidase and catalase are positive. Able to grow at 30–63 °C (optimally at 50 °C), pH 7.0–9.0 (optimally at pH 8.0) and with 0.5–6 % (w/v) NaCl concentrations (optimally with 1–1.5 %, w/v, NaCl). Results of the oxidation-fermentation test show oxidation.

Haemolysis is observed after incubation for 2 days at 50 °C on blood agar. Voges–Proskauer reaction and production of H_2S and indole are negative. Nitrate is not reduced. Citrate is not utilized. Urease, β-galactosidase, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase and tryptophan deaminase are negative. Gelatin and aesculin are hydrolysed but starch and casein are not. Hydrolysis of Tween 80 is strain-dependent. Cells can utilize pyruvate, D-glucose, L-arabinose, D-ribose, inositol and D-fructose as sole carbon and energy sources, but not acetate, lactate, sucrose, D-mannitol or raffinose. $(NH_4)_2SO_4$ and NH_4Cl can be used as sole nitrogen sources, but L-alanine, L-arginine, L-asparagine, L-cysteine, L-glycine, L-serine, L-proline, L-valine and L-histidine cannot. In the API 50 CHB gallery, acid is produced from D-ribose, D-xylose, L-xylose, D-sorbitol, *N*-acetylglucosamine, aesculin ferric citrate, inulin, D-tagatose and potassium 5-ketogluconate; acid production from the following carbohydrates is variable: D-arabinose, L-arabinose, D-galactose, D-fructose, L-sorbose and inositol; and acid is not produced from other carbohydrates. The major cellular fatty acids are anteiso-$C_{15:0}$, iso-$C_{15:0}$, iso-$C_{16:0}$ and anteiso-$C_{17:0}$.

The type strain, GD05^T (=CCTCC AB 2013105^T=KCTC 33117^T), was isolated from a tropical forest soil in Guangdong Province, South China. The type strain cannot grow at 63 °C. The type strain can hydrolyse Tween 80 and produce acid from D-arabinose, L-arabinose, D-galactose, D-fructose, L-sorbose and inositol. The DNA G+C content of the type strain is 43.7 mol%.

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