Novibacillus thermophilus gen. nov., sp. nov., a Gram-staining-negative and moderately thermophilic member of the family Thermoactinomycetaceae

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Two Gram-staining-negative, facultatively anaerobic bacterial strains, SG-1T and SG-2, were isolated from a saline soil sample and a compost sample, respectively. The cells were non-motile rods that occurred singly or in chains, and endospores were not observed under tested growth conditions. Optimum growth occurred at 50 °C, pH 7.5–8.0 and with 5–7 % (w/v) NaCl. The DNA G+C content was 49.5–50.5 mol%. The strains contained MK-7 as the predominant menaquinone and iso-C15 : 0and anteiso-C15 : 0as the major fatty acids. The polar lipids consisted mainly of diphosphatidylglycerol and phosphatidylglycerol. The cell-wall peptidoglycan type was A1c (meso-DAP direct). Phylogenetic analyses revealed that the new isolates belonged to the family Thermoactinomycetaceae, exhibiting low 16S rRNA gene sequence similarity (90.8–91.3 %) to the nearest type strain, Mechercharimyces asporophorigenes YM11-542T, and formed a well-supported lineage that was clearly distinguished from all currently described genera in this family. Based on our polyphasic taxonomic characterization, we propose that strains SG-1T and SG-2 represent a novel genus and species within the family Thermoactinomycetaceae, for which we propose the name Novibacillus thermophilus gen. nov., sp. nov. The type strain of Novibacillus thermophilus is SG-1T (=KCTC 33118T=CGMCC 1.12771T).

Within the phylum Firmicutes, as the type order of the class Bacilli, the order Bacillales contains a morphologically diverse assemblage of unicellular bacteria that includes aerobic, aerotolerant, facultatively anaerobic and strictly anaerobic strains, growing under psychrophilic, mesophilic or thermophilic conditions. Members of this order are generally Gram-staining-positive and endospore-forming. As an exception, the species Bacillus horti was detected to be Gram-staining-negative, possessing a thicker peptidoglycan layer than Gram-negative bacteria but a thinner one than ordinary Gram-staining-positive bacteria (Yumoto et al., 1998). Endospore formation has not been observed under tested growth conditions for some species, such as Bacillus foraminis (Tiago et al., 2006), Bacillus thermoamylovorans (Combet-Blanc et al., 1995) and Aquisalibacillus elongatus (Márquez et al., 2008).

The family Thermoactinomycetaceae in the order Bacillales was proposed by Matsuo et al. (2006), consisting of the genera Laceyella, Thermoflavimicrobium, Thermoactinomyces, Seinonella, Planifilum and Mechercharimyces. At the time of writing (April 2015), with seven new genera, Desmospora, Kroppenstedtia, Lihuaxuella, Marininema, Melghirimyces, Polycladomyces and Shimazuella, the family comprised 13 recognized genera. These bacteria are characterized by the formation of a single, non-stalked spore on the aerial or substrate hyphae, or consecutive spores on straight or branched spore-phores (Tsubouchi et al., 2013). Most species of the family are thermophilic, although several members of the genera Seinonella, Mechercharimyces and Shimazuella are mesophilic. In this study, we report two moderately thermotolerant and Gram-staining-negative strains, SG-1T and SG-2, which were isolated from a saline soil sample and a compost sample, respectively. Based on a polyphasic taxonomic analysis, the
two strains are proposed to represent a novel genus and species within the family *Thermoactinomyctaceae*.

Strain SG-1<sup>T</sup> was isolated from a soil sample obtained in Xipo village, Xuwen County (20.34° N 110.17° E), which is located at the southern tip of Guangdong Province, China, and where the fields are often flooded by seawater. The soil sample had a pH of 8.2 and an organic matter content of 1.34 %. For strain isolation, 10 g soil sample was added to 100 ml liquid 2216E medium (HB0132-1; Qingdao Hope Bio-Technology Co.) containing (l<sup>-1</sup>) 5.0 g peptone, 1.0 g yeast extract, 0.1 g ferric citrate, 19.45 g NaCl, 5.98 g MgCl<sub>2</sub>, 3.24 g Na<sub>2</sub>SO<sub>4</sub>, 1.8 g CaCl<sub>2</sub>, 0.55 g KCl, 0.16 g Na<sub>2</sub>CO<sub>3</sub>, 0.08 g KBr, 0.034 g SrCl<sub>2</sub>, 0.022 g H<sub>2</sub>BO<sub>3</sub>, 0.004 g Na<sub>2</sub>SiO<sub>3</sub>, 0.0024 g NaF, 0.0016 g NaNO<sub>3</sub>, 0.008 g Na<sub>2</sub>HPO<sub>4</sub>; pH 7.6) and incubated at 50 °C for 2 weeks. Tenfold serial dilution was then done from 10<sup>-1</sup> to 10<sup>-5</sup>, and 100 μl of each dilution was spread onto the surface of fresh 2216E agar and incubated at 50 °C until single colonies were present. Strain SG-1<sup>T</sup> was subsequently obtained in pure culture by repeatedly streaking onto 2216E agar plates. The sample for isolation of strain SG-2 was obtained from a composting demonstration plant in Dongguan city, Guangdong Province, China (23.04° N 113.75° E). The compost windrow consisting of sewage sludge and crop straw was 2 m high and 30 m long. Samples were collected at about 1 m below the top surface of the compost pile after composting for 13 days, when the internal temperature exceeded 55 °C. Strain SG-2 was obtained during an investigation of thermophilic micro-organisms in the compost samples using LB agar ([l<sup>-1</sup>] 1.0 g NaCl, 20 g agar (Dong & Cai 2001) at 50 °C. The new isolates were cryopreserved at −80 °C in 15 % (v/v) glycerol. Experiments on the new isolates were carried out at 50 °C, pH 7.5 and with 1.0 % NaCl (w/v) unless indicated otherwise.

Taxonomic characteristics of the new isolates were studied following the standards recommended by Logan <i>et al.</i> (2009). Based on phylogenetic analysis of 16S rRNA gene sequences, the closely related type strains *Mechercharimyces asporophigenes* DSM 44955<sup>T</sup>, *Mechercharimyces mesophilus* DSM 44894<sup>T</sup>, *Lihuxella thermophila* DSM 46701<sup>T</sup>, *Microaerobacter geothermalis* DSM 22679<sup>T</sup> and *Caldalkalibacillus uzonensis* DSM 17740<sup>T</sup> were selected as reference strains. These reference strains were obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ, Braunschweig, Germany) and were cultured in the media and under the conditions recommended by the DSMZ.

Cell morphology was determined by using a transmission electron microscope (JEM 1400; JEOL) and a scanning electron microscope (JSM-6330F; JEOL) with cells grown on 2216E agar at 50 °C for 12 h. Colonies were observed on 2216E agar and LB agar after 2 days of growth. The motility of cells was tested by observing the spread of growth in semi-solid medium with 0.2 % agar added. Growth was also examined on MacConkey agar. Growth was tested at 20–70 °C (at intervals of 5 °C) in LB medium. The pH range for growth was determined at pH 4.0–11.0 (at intervals of 0.5 pH units) using the following buffer systems: pH 4.0–5.0, 0.1 M citric acid/0.1 M sodium citrate; pH 5.5–8.5, 0.1 M KH<sub>2</sub>PO<sub>4</sub>/0.1 M NaOH; pH 9.0–10.0, 0.1 M NaHCO<sub>3</sub>/0.1 M Na<sub>2</sub>CO<sub>3</sub>; pH 10.5–11.0, 0.05 M Na<sub>2</sub>HPO<sub>4</sub>/0.1 M NaOH (Zhang <i>et al.</i>, 2009). Tolerance of NaCl was examined at 0–15.0 % (w/v) NaCl (in increments of 0.5 % NaCl) in a mineral salts medium [MSM, containing 0.2 g MgSO<sub>4</sub>7H<sub>2</sub>O, 0.5 g Na<sub>2</sub>PO<sub>4</sub>12H<sub>2</sub>O, 0.5 g K<sub>2</sub>HPO<sub>4</sub>, 0.1 g CaCl<sub>2</sub>2H<sub>2</sub>O, 0.02 g yeast extract and 15 g agar in 1000 ml distilled water, pH 7.5] supplemented with (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> (0.2 %, w/v) and glucose (1 %, w/v) as nitrogen and carbon sources, respectively. 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 on blood agar plates (HBPM004; Qingdao Hope Bio-Technology Co.) by incubation at 50 °C for 1–3 days. Urease activity, hydrolysis of aesculin, starch and casein, production of indole and the Voges–Proskauer test were assessed as recommended by Smibert & Krieg (1994). Citrate utilization, activities of β-galactosidase, arginine dihydrolase, lysozyme, decarboxylase, ornithine decarboxylase and tryptophan deaminase, H<sub>2</sub>S production and hydrolysis of gelatin, l-tyrosine and Tweens 80 and 20 were examined by the classical methods described by Dong & Cai (2001). Enzyme activities were also determined by using the API ZYM strip (bioMérieux) according to the manufacturer’s instruction. Acid production from carbohydrates was examined in tubes containing (l<sup>-1</sup>) 1.0 g (NH<sub>4</sub>)<sub>2</sub>HPO<sub>4</sub>, 0.2 g KCl, 0.2 g MgSO<sub>4</sub>, 15.0 g NaCl, 0.2 g yeast extract, 6.0 g agar, 10.0 g substrate and 15 ml 0.04 % bromocresol purple; positive results were indicated by the yellow colour of the medium.

Strains SG-1<sup>T</sup> and SG-2 were facultatively anaerobic, non-flagellated, non-motile, rod-shaped bacteria that occurred singly or in long chains (Fig. S1, available in the online Supplementary Material). The strains formed white, small, rounded colonies on 2216E agar and produced dark-yellowish-pigmented colonies on LB agar. Growth occurred at 30–65 °C (optimally at 50 °C) and at pH 6.5–10.5 (optimally at pH 7.5–8.0). Cells could grow without NaCl and with up to 10 % (w/v) NaCl, and optimum growth occurred with 5–7 % (w/v) NaCl. Using the API ZYM system, tests for alkaline phosphatase, acid phosphatase and N-acetyl-β-glucosaminidase were positive, while tests for C<sub>4</sub> esterase, C<sub>8</sub> lipase, leucine arylamidase, naphthol-AS-BI-phospho-4-nitroaniline, C<sub>14</sub> lipase, valine arylamidase, cystine arylamidase, trypsin, z-chymotrypsin, z-galactosidase, β-glucuronidase, z-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase, z-mannosidase and β-fucosidase were negative. Detailed phenotypic features are included in the species description and in Tables 1 and S1.

The Gram reaction was determined by a conventional Gram-staining method (Dong & Cai, 2001). Ultrathin sections for observing the cell wall were made using the...
Cells at each growth stage was checked by subculturing for 48 h at 50°C inoculated onto 2216E and LB solid media and incubated 20 min. Aliquots (0.3 ml) of the heated cultures were obtained at the exponential (4 h), late-exponential (8 h) and 10 days) phases and were heated at 80°C in a water bath. No growth was observed after heat treatment of cultures of growth under the various conditions examined. In addition, the cells were sensitive to heat treatment, and no growth was observed after heat treatment of cultures at 80°C for 20 min, supporting the above conclusion.

Utilization of various substrates as sole carbon and energy sources under aerobic conditions was examined in a basal medium (0.2 g MgSO₄·7H₂O, 15.0 g NaCl, 0.5 g NaH₂PO₄·H₂O, 0.5 g K₂HPO₄, 0.1 g CaCl₂·2H₂O, 0.02 g yeast extract and 15 g agar in 1000 ml distilled water, pH 7.5) supplemented with (NH₄)₂SO₄ (0.2%, w/v) as the nitrogen source. Utilization of NH₄Cl, L-serine, L-tyrosine, L-arginine, L-cysteine, L-proline, L-leucine, L-lysine and L-threonine as sole nitrogen sources was examined in the same basal medium supplemented with D-glucose (1%, w/v) as the carbon source. Anaerobic growth was assessed in sterilized mineral salts medium (1 g L⁻¹; 0.6 g Na₂HPO₄·2H₂O, 0.25 g NH₄Cl, 0.1 g KCl, 10.0 ml vitamin stock solution and 10.0 ml mineral stock solution, pH 7.5) [the vitamin and mineral stock solutions were the same as those described by Zachara et al. (1998)] in anaerobic chambers (Sheldon Manufacturing) for 10 days with different electron donors (sodium citrate, sodium formate, sodium lactate, sodium acetate, sodium pyruvate, adipic acid, malic acid, proline, sucrose, glucose or xylose) and acceptors (anthraquinone) method described by Khelifi et al. (2010). The cell-wall types of the two isolates were further confirmed by detecting lipopolysaccharide (LPS); this analysis was performed at the China National Analytical Center, Guangzhou. Cells stained Gram-negative (Fig. S2), and electron micrographs revealed a Gram-negative type of cell wall (Fig. S3). Analysis of LPS confirmed that the new isolates have a true Gram-negative cell wall (data not shown).

The presence of endospores was investigated using 2216E agar, LB agar and another medium containing (1 g L⁻¹) 5.0 g Bacto peptone (Difco), 3.0 g meat extract (Difco), 5 mg MnSO₄·H₂O (Merck) and 16 g agar (Difco). Plates were inoculated with 0.3 ml aliquots of an overnight liquid culture and then incubated at 50°C for up to 15 days to determine the presence of endospores using staining solution kit HB8300 (containing carbol fuchsin and methylene blue staining reagents; Qingdao Hope-Bio Technology Co.,) and observed by light microscopy (Olympus BX51). Heat resistance of the cells was determined using cultures in 2216E and LB media. Aliquots (5 ml) of the cultures were obtained at the exponential (4 h), late-exponential (10 h), stationary (20 h) and late-stationary (48 h, 72 h and 10 days) phases and were heated at 80°C for 20 min. Aliquots (0.3 ml) of the heated cultures were inoculated onto 2216E and LB solid media and incubated for 48 h at 50°C (Tiago et al., 2006). The viability of cells at each growth stage was checked by subculturing them on the same media before heating. The result showed that endospores were not observed at any stage of growth under the various conditions examined. In addition, the cells were sensitive to heat treatment, and no growth was observed after heat treatment of cultures at 80°C for 20 min, supporting the above conclusion.

### Table 1. Differential characteristics of strain SG-1T and type strains of related taxa

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
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<td>Mycelia formation</td>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
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<tr>
<td>Sporulation</td>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Cell width (µm)</td>
<td>0.4–0.5</td>
<td>0.4–0.5</td>
<td>0.5–0.7</td>
<td>0.5–0.9</td>
<td>0.4–0.5</td>
<td>0.7–0.8</td>
</tr>
<tr>
<td>Cell length (µm)</td>
<td>0.7–2.3</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>1.9–9.0</td>
<td>5.5–12</td>
</tr>
<tr>
<td>Chains of cells</td>
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<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Motility</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Gram-staining</td>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
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<td>Catalase</td>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
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<td>Nitrate reduction</td>
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<td>+</td>
<td>−</td>
<td>−</td>
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<td>Aerobic growth</td>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
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<tr>
<td>Anaerobic growth</td>
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<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Temperature range (°C)</td>
<td>30–65</td>
<td>20–40</td>
<td>15–35</td>
<td>30–65</td>
<td>35–70</td>
<td>45–65</td>
</tr>
<tr>
<td>Optimum temperature (°C)</td>
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<td>50</td>
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<td>50</td>
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<td>NaCl range (% w/v)</td>
<td>0–10</td>
<td>0–10</td>
<td>0–4</td>
<td>0–1.0</td>
<td>0–7.0</td>
<td>0–6.0</td>
</tr>
<tr>
<td>Optimum NaCl (% w/v)</td>
<td>5.0–7.0</td>
<td>3–4</td>
<td>2.0</td>
<td>0.5</td>
<td>2.0–2.5</td>
<td>1.5</td>
</tr>
<tr>
<td>pH range</td>
<td>6.5–10.5</td>
<td>5–10</td>
<td>5.5–10</td>
<td>6.0–8.0</td>
<td>6.0–7.5</td>
<td>6.5–10</td>
</tr>
<tr>
<td>Optimum pH</td>
<td>7.5–8.0</td>
<td>7.0</td>
<td>7.0–8.0</td>
<td>7.0</td>
<td>7.0</td>
<td>8.0–8.5</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>50.5</td>
<td>45.2⁺⁺⁺</td>
<td>45.1⁺⁺⁺</td>
<td>55.6ᵇ</td>
<td>41.8⁺⁺⁺</td>
<td>45.0ᵈ</td>
</tr>
<tr>
<td>Major isoprenoid quinone</td>
<td>MK-7</td>
<td>MK-9ᵃ</td>
<td>MK-9ᵃ</td>
<td>MK-7ᵇ</td>
<td>MK-7ᶜ</td>
<td>MK-7ᵈ</td>
</tr>
</tbody>
</table>

*Data obtained from: a, Matsuo et al. (2006); b, Yu et al. (2012); c, Khelifi et al. (2010); d, Zhao et al. (2008).
2,6-disulfonate (AQDS), fumaric acid or sodium nitrate]. For all the above investigations, cell growth was evaluated by measuring the OD_{600} and the occurrence of cell growth was indicated by OD_{600}>0.2.

Similar results were obtained for strains SG-1^T and SG-2. Under aerobic conditions, both isolates used D-glucose, D-mannitol, D-ribose, L-serine, L-proline, glycogen, trisodium citrate, sodium acetate, 4-hydroxybenzoic acid and 3-hydroxybenzoic acid as carbon sources and NH_4Cl, L-serine, L-aspartic acid, L-glutamic acid, L-proline, L-alanine and l-lysine as nitrogen sources. Under anaerobic conditions, with AQDS, fumarate or nitrate as the sole electron acceptor, both isolates grew with lactate, acetate, succrose and D-glucose as the sole electron donor. However, differences also existed between them: strain SG-2 used inositol as a carbon source but strain SG-1^T did not, and strain SG-1^T used L-arginine and L-threonine as nitrogen sources but strain SG-2 did not. The detailed results are shown in the species description.

Genomic DNA of strains SG-1^T and SG-2 was extracted using a DNA Extraction kit (Aidlab). The 16S rRNA gene was amplified by PCR using universal primers 27F and 1492R (Baker et al., 2003). The PCR product was gel-purified using 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. 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 16S rRNA gene sequences of strains SG-1^T (1481 bp) and SG-2 (1482 bp) displayed 99.6 % similarity. Strain SG-1^T shared the highest sequence similarity with *Mechercharimyces asporophigenens* YM11-542^T (90.8 %), *Lihuaxuella thermophila* YIM77831^T (90.7 %) and *Mechercharimyces mesophilus* YM3-251^T (90.6 %), and strain SG-2 shared the highest sequence similarity with *Mechercharimyces asporophigenens* YM11-542^T (91.0 %), *Mechercharimyces mesophilus* YM3-251^T (90.8 %) and *Lihuaxuella thermophila* YIM77831^T (90.7 %). All these strains belong to the family *Thermoactinomycetaceae* in the order *Bacillales*. Among type strains that shared more than 90 % 16S rRNA gene sequence similarity with the two isolates, *Microaerobacter geothermalis* Nad S1^T (90.4 % similarity) was the only one that was not a member of the family *Thermoactinomycetaceae*. In the phylogenetic analysis, the most closely related type strains were in the family *Thermoactinomycetaceae* (*Mechercharimyces asporophigenens* YM11-542^T and *Mechercharimyces mesophilus* YM3-251^T), and *Microaerobacter geothermalis* Nad S1^T and *Caldalkalibacillus uzonensis* JW/WZ-YB58^T were the most closely related type strains that did not belong to the family *Thermoactinomycetaceae*. Trees reconstructed using the neighbour-joining, maximum-likelihood and minimum-evolution methods clearly placed the new isolates in a distinct phylogenetic lineage within the order *Bacillales*, with bootstrap values of 87, 91 and 86 %, respectively (Fig. 1). In brief, strains SG-1^T and SG-2 were most closely related to the family *Thermoactinomycetaceae* and formed a distinct cluster within the order *Bacillales*.

For chemotaxonomic analysis, strains were grown in LB medium at 50 °C and pH 7.5 to the exponential growth phase (when the OD_{600} reached approximately 70 % of the maximum optical density). Cells were collected by centrifugation at 12 000 r.p.m. at 4 °C, and freeze-dried using a vacuum freeze-drying apparatus (FD-1A-50; Beijing Bio-cool). Menaquinones were extracted with methanol using freeze-dried cells according to Collins et al. (1977) and analysed by HPLC (Agilent 1260) as described by Groth et al. (1997). Polar lipids were extracted from the freeze-dried cells, separated by two-dimensional TLC and identified according to Minnikin et al. (1984) by spraying individual plates with appropriate detection reagents: molybdatophosphate for total lipids, molybdenum blue for phospholipids, ninhydrin reagent for amino-containing lipids and α-naphthol reagent for glycolipids. The fatty acids in whole cells were saponified, methylated and extracted according to the standard MIDI protocol (Sherlock Microbial Identification System, version 6.0B). For fatty acid analysis, strains were grown in a complex medium (containing 1 % peptone, 0.5 % yeast extract and 5 % NaCl; pH 7.5) at 50 °C to the exponential growth phase. Freeze-dried cells were prepared as described above. The fatty acids were analysed by GC (Agilent Technologies 6850) and identified using the TSBA6.0 database of the Microbial Identification System (Sasser, 1990). The G+C content of the genomic DNA was determined by HPLC according to the method of Mesbah et al. (1989).

Strains SG-1^T and SG-2 contained a quinone system that consisted of the major compound MK-7 (87.3 and 92.1 %, respectively) and the minor compound MK-8 (12.7 and 7.9 %, respectively), which was completely different from that of members of the closest genus, *Mechercharimyces* (with MK-9 as the predominant quinone). The dominant fatty acid component was iso-C_{15 : 0} (47.1–48.7 %; Table S2), which has been found in only a few Gram-staining-negative bacteria (Zhang et al., 2003), such as *Gemmatimonas aurantia*. The polar lipid pattern of strain SG-1^T consisted of major components diphostathidylglycerol, phosphatidylglycerol and phosphatidylethanolamine and, as minor components, three unidentified glycolipids, two unidentified phospholipids and one unknown aminophospholipid (Fig. S4). For strain SG-2, the major polar lipids were diphostathidylglycerol,
phosphatidylglycerol and one unidentified phospholipid, and the minor components were one unidentified phospholipid, one unidentified glycolipid and one unknown aminophospholipid (Fig. S5). The absence of phosphatidylethanolamine in strain SG-2 indicated that this strain differed from strain SG-1T. Both isolates contained diphosphatidylglycerol and phosphatidylglycerol as major polar lipids, which was consistent with the reference taxa Microaerobacter geothermalis (Khelifi et al., 2010) and Lihuaxuella thermophila (Yu et al., 2012). The DNA G+C content of strains SG-1T and SG-2 was 50.5 and 49.5 mol%, respectively, which was lower than that of Lihuaxuella thermophila and higher than those of the other reference strains (Table 1).

For peptidoglycan analysis, cells were grown in LB medium at 50°C and pH 7.5 to the exponential growth phase. After centrifugation, the wet biomass was suspended in 2-propanol/water (1:1, v/v) and then sent to the DSMZ at ambient temperature. The peptidoglycan of strain SG-1T was isolated and studied by the Identification Service of the DSMZ using published protocols (Schumann, 2011). For analysis of the peptidoglycan type, the total hydrolysate (4 M HCl, 100°C, 16 h) contained the amino acids alanine, glutamic acid and meso-diaminopimelic acid (meso-DAP); 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 strain SG-1T showed the peptidoglycan type A1c meso-DAP-direct (Schleifer & Kandler, 1972; type A31 according to http://www.dsmz.de/catalogues/catalogue-microorganisms/groupsof-organisms-and-their-applications/peptidoglycans.html). This peptidoglycan type is found in the majority of Gram-negative bacteria and many
endospore-forming, rod-shaped bacteria (Mayr et al., 2006). During this analysis, only a very small amount of peptidoglycan could be obtained after repeated extraction, which was in accordance with the observation of a Gram-negative type of cell wall.

Based on phylogenetic analysis of 16S rRNA gene sequences, strains SG-1T and SG-2 were most closely related to the family Thermoactinomycetaceae and formed a distinct cluster within the order Bacillales. They exhibited many different characteristics in comparison with the most closely related type strains: they were the only strains that had a Gram-negative cell wall and grew under both aerobic and anaerobic conditions; it is unusual for a Gram-negative bacterium to have iso-C\textsubscript{15} : \textsubscript{0} as the dominant fatty acid and MK-7 as the major respiratory quinone. The new isolates can be distinguished from reference strains of the family Thermoactinomycetaceae by the absence of mycelium formation and sporulation. Other differences between strain SG-1T and the phylogenetically closest genus Mechercharinomyces of the family Thermoactinomycetaceae include growth temperature (range and optimum), the absence of fatty acid C\textsubscript{12} : \textsubscript{10} as the dominant fatty acid and MK-7 as the major respiratory quinone. The new isolates can be distinguished from reference strains of the family Thermoactinomycetaceae for which the name Novibacillus thermophilus gen. nov., sp. nov. is proposed.

Description of Novibacillus \textit{gen. nov.}


Cells are Gram-staining-negative, facultatively anaerobic, non-flagellated and non-motile rods that occur singly or in chains. Endospores are not observed under the tested growth conditions. Colonies are small and round. Moderately thermophilic. The cell-wall peptidoglycan type is determined as A\textsubscript{17} (\textit{meso-DAP} direct). Cellular fatty acids consist mainly of iso- and anteiso-branched acids, with iso-C\textsubscript{15} : \textsubscript{0} predominating. The major respiratory quinone is MK-7. Polar lipids consist mainly of diphosphatidylglycerol and phosphatidylglycerol. Phylogenetically, the genus belongs to the family Thermoactinomycetaceae within the order Bacillales. The type species is Novibacillus thermophilus.

Description of Novibacillus thermophilus \textit{sp. nov.}

Novibacillus thermophilus (\textit{ther.mo’phi.lus}. Gr. n. \textit{therm} heat; Gr. adj. \textit{philos} friend to, loving; N.L. masc. adj. \textit{thermophilus} heat-loving, thermophilic).

In addition to the characteristics given in the genus description, exhibits the following properties. Cells are straight to slightly curved rods, 0.4–0.5 \textmu m wide and 0.7–2.3 \textmu m long. Colonies are white on 2216E agar and dark-yellowish on LB agar. Cells cannot grow on MacConkey agar. After incubation for 7 days, growth occurs at 30–65 °C (optimally at 50 °C), pH 6.5–10.5 (optimally at pH 7.5–8.0) and with 0–10% NaCl (optimally with 5.0–7.0% NaCl); no growth is observed at 25 or 70 °C, at pH 6.0 or 11.0 or with 10.5% NaCl. Haemolysis is observed. Casein, gelatin and aesculin are hydrolysed, but l-tyrosine and TWEENs 80 and 20 are not. Hydrolysis of starch is strain dependent. Cells test positive for oxidase, catalase, nitrate reduction and \beta-galactosidase, but negative for the Voges-Proskauer reaction, citrate utilization, urease, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, tryptophan deaminase and production of H\textsubscript{2}S and indole. Acid is produced from D-ribose, L-sorbos and aesculin ferric citrate, and is not produced from glycerol, D-glucose, L-arabinose, L-rhamnose, D-sorbitol, salicin, maltose, sucrose, L-fucose, D-galactose, D-mannose, D-fructose or xylitol. Acid production from D-mannitol is variable. Cells can utilize NH\textsubscript{4}Cl, L-serine, L-aspartic acid, L-glutamic acid, L-proline, L-alanine and L-lysine as nitrogen sources, but not L-tyrosine, L-glycine, L-cysteine or L-leucine. Utilization of L-arginine and L-threonine as nitrogen sources is variable. The following substrates are utilized as carbon sources or with L-arginine or L-threonine as nitrogen sources. With AQDS as electron acceptor, the type strain can use formate and adipic acid as an electron donor.

The type strain, SG-1T (\textit{=KCTC 33118T=CGMCC 1.12771T}), was isolated from a saline soil sample collected from Xuwen County, southern China. The type strain cannot hydrolyse starch and cannot grow with inositol as a carbon source or with L-arginine or L-threonine as a nitrogen source. With AQDS as electron acceptor, the type strain can use formate and adipic acid as an electron donor; with fumarate as electron acceptor, the type strain can use malic acid and xylose as electron donors and cannot use pyruvate as an electron donor. Acid is not produced from D-mannitol by the type strain. The DNA G+C content of the type strain is 50.5 mol%. Another strain of this species, SG-2 (\textit{=MCCC 1K00497}), was isolated from a composting demonstration plant in Dongguan city,
Guangdong Province, China. The genomic DNA G+C content of strain SG-2 is 49.5 mol%.

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References


