Paenibacillus guangzhouensis sp. nov., an Fe(III)-
and humus-reducing bacterium from a forest soil

Jibing Li,1,2 Qin Lu,2 Ting Liu,3 Shungui Zhou,2 Guiqin Yang2
and Yong Zhao1

1College of Food Science and Technology, Shanghai Ocean University, Shanghai 201306,
PR China
2Guangdong Institute of Eco-Environmental and Soil Sciences, Guangzhou 510650, PR China
3College of Orient Science and Technology, Hunan Agricultural University, Changsha 410128,
PR China

A Gram-reaction-variable, rod-shaped, motile, facultatively aerobic and endospore-forming
bacterium, designated strain GSS02T, was isolated from a forest soil. Strain GSS02T was
capable of reducing humic substances and Fe(III) oxides. Strain GSS02T grew optimally at 35 °C,
at pH 7.8 and in the presence of 1 % NaCl. The predominant menaquinone was MK-7. The major
cellular fatty acids were anteiso-C15:0 and iso-C16:0 and the polar lipid profile contained mainly
phosphatidylethanolamine, diphasphatidylglycerol and phosphatidylglycerol, with moderate
amounts of two unknown aminophospholipids and a minor amount of one unknown lipid. The DNA
G+C content was 53.4 mol%. Comparative 16S rRNA gene sequence analysis showed that
strain GSS02T was related most closely to Paenibacillus terrigena JCM 21741T (98.1 %
similarity). Mean DNA–DNA relatedness between strain GSS02T and P. terrigena JCM 21741T
was 58.8 ± 0.5 %. The phylogenetic, chemotaxonomic and phenotypic results clearly demon-
strated that strain GSS02T belongs to the genus Paenibacillus and represents a novel species,
for which the name Paenibacillus guangzhouensis sp. nov. is proposed. The type strain is
GSS02T (=KCTC 33171T=CCTCC AB 2013236T).

The genus Paenibacillus, which was first proposed by Ash et al. (1993/1994), belongs to the family Paenibacillaceae.
At the time of writing, 154 species and four subspecies of the genus Paenibacillus have been described (http://www.
bacterio.net/index.html). In general, bacteria of the genus

Paenibacillus are aerobic or facultatively anaerobic, rod-shaped and endospore-forming (Ash et al., 1993/1994),
with anteiso-C15:0 as the major fatty acid and a DNA G+C content of 40–54 mol% (Ash et al., 1993/1994; Shida et al.,
1997a, b). Since the genus was first described, members of the genus Paenibacillus have been isolated from various
ecological habitats, including air (Rivas et al., 2005), a cold spring (Tang et al., 2011), a glacier (Kishore et al., 2010),
alkaline soil (Yoon et al., 2005), a fermented substance (Oh et al., 2008), a wetland (Baik et al., 2011), a molybdenum
mine (Benardini et al., 2011), sediments (Moon et al., 2011; Park et al., 2011), and the rhizosphere soils of herb and
woody plants (Zhang et al., 2013). In this study, a strain of humic substance- and Fe(III)-
reducing bacterium was isolated from a forest soil and characterized based on
evidence from phylogenetic, chemotaxonomic and phenotypic properties, and its Fe(III)-
and humus-reducing abilities were demonstrated.

Strain GSS02T was isolated from a forest soil sample taken from Huolu Mountain in Guangzhou, Guangdong Province,
China (GPS coordinates 23°18′N 113°39′E). For isolation, 2 g of the forest soil sample was inoculated in a sterile
bottle containing 20 ml sterilized mineral salts medium [MSM, containing (per litre) 0.6 g NaH2PO4, 0.25 g NH4Cl,
0.1 g KCl, 0.2 g yeast extract, 10.0 ml vitamin stock solution and 10.0 ml mineral stock solution (Zachara et al., 1998; pH 7.2) supplemented with 1 mM anthraquinone-2,6-disulfonate (AQDS; Sigma) and 5 mM glucose as electron acceptor and electron donor, respectively. The bottle was then purged with O2-free N2/CO2 (80:20, v/v) for 15 min and sealed with a butyl-rubber stopper and an aluminium cap. After incubation at 30 °C for 5 days, the colour of the medium changed to bright orange and the enriched population was serially diluted and spread on Luria–Bertani agar (LA; Difco). The plates were incubated at 30 °C for 4 days. In total, 20 colonies were isolated, purified and identified. Only one isolate was considered to represent a novel species. The colony was designated as strain GSS02T and was routinely confirmed with the heat resistance method (Tiago et al., 2001). Endospore morphology was observed using a light microscope (OLYMPUS BX51, Japan) and was further examined electron microscope (JEM-1400; Jeol) after incubation for 2 days on LA plates. Anaerobic growth was determined at 30 °C for 1 week. The Gram reaction, motility, oxidase activity, and hydrolysis of Tween 80, Tween 20, casein and starch were tested as described by Dong & Cai (2009). Additional biochemical features were determined with the use of API 20E, API 50CH and API 1D32G kits (bioMérieux) according to the manufacturer’s instructions. All of the experiments were performed more than twice. Cells of strain GSS02T were Gram-reaction-variable, endospore-forming, motile, oxidase-positive and catalase-positive. Differential phenotypic, physiological and biochemical characteristics of strain GSS02T compared with the reference strains are listed in Table 1 and Table S1 (available in the online Supplementary Material).

Cell and colony morphology were studied using a transmission electron microscope (JEM-1400; Jeol) after incubation for 2 days on LA plates. Anaerobic growth was tested by incubating cultures on LA plates in Bactron anaerobic chambers (Sheldon Manufacturing). Salt tolerance was tested in Luria–Bertani broth (LB; Difco) with the addition of 0–10 % (w/v) NaCl with increments of 1 %. The temperature range for growth was tested at 4, 10, 15, 20, 25, 30, 37, 40 and 50 °C in LB, and pH range for growth was tested at pH 4.0–11.0 (with intervals of 0.5 pH unit) by using the buffer systems according to Zhang et al. (2009). Growth on MacConkey agar was determined at 30 °C for 1 week. The Gram reaction, motility, oxidase activity, catalase activity, and hydrolysis of Tween 80, Tween 20, casein and starch were tested as described by Dong & Cai (2001). Endospore morphology was observed using a light microscope (OLYMPUS BX51, Japan) and was further confirmed with the heat resistance method (Tiago et al., 2006; Logan et al., 2009). Additional biochemical features were determined with the use of API 20E, API 50CH and API 1D32G kits (bioMérieux) according to the manufacturer’s instructions. All of the experiments were performed more than twice. Cells of strain GSS02T were Gram-reaction-variable, endospore-forming, motile, oxidase-positive and catalase-positive. Differential phenotypic, physiological and biochemical characteristics of strain GSS02T compared with the reference strains are listed in Table 1 and Table S1 (available in the online Supplementary Material).

The 16S rRNA gene sequence was PCR-amplified using two bacterial universal primers (27F and 1492R; Baker et al., 2003). The PCR products were gel-purified using a D2500-01 Gel Extraction kit (Omega Bio-tek) and double-checked by sequencing both strands by Sangon (Shanghai, China). The sequence was then submitted to GenBank (Shanghai, China) for similarity search using the EzTaxon-e server (http://www.ezbiocloud.net/eztaxon; Kim et al., 2012). Phylogenetic analysis was performed using MEGA version 4.0 (Tamura et al., 2007) after multiple alignments with CLUSTAL X (Thompson et al., 1997). Distances were calculated using distance options according to the maximum-composite-likelihood model (Tamura et al., 2007). Clustering was performed with the neighbour-joining method (Saitou & Nei, 1987) and the minimum-evolution method (Rzhetskaya & Nei, 1993). Statistical support for the branches of the phylogenetic trees was determined using bootstrap analysis based on 1200 resamplings (Felsenstein, 1985).

Table 1. Differential phenotypic, physiological and biochemical characteristics between strain GSS02T and its closest related neighbours in the genus Paenibacillus

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram reaction</td>
<td>Variable</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Anaerobic growth</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Catalase</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Arginine dihydrolase</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Voges–Proskauer test</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Nitrate reduction</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Hydrolysis of:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gelatin</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Starch</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Tween 20</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Tween 80</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Growth at:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 °C</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>40 °C</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Indole production</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>DNA G + C content (mol%)</td>
<td>53.4</td>
<td>48.1</td>
<td>49.9</td>
<td>43–46</td>
</tr>
</tbody>
</table>

Table 1 and Table S1 (available in the online Supplementary Material).
trees based on 16S rRNA gene sequences, strain GSS02T represents a novel species of the genus *Paenibacillus*.

For chemotaxonomic analyses, strain GSS02T and the reference strains were cultivated in LB at 30°C for 24 h, and then harvested by centrifugation, washed with distilled water and freeze-dried. Fatty acids of strain GSS02T and the reference strains were obtained by saponification, methylation and extraction according to the protocols given by the MIDI System (Sherlock Microbial Identification System, version 6.0B), analysed by GC (model 6850; Agilent Technologies) and identified using the Microbial Identification System (Sasser, 1990), which is based on the TSBA6.0 database. Isoprenoid quinones were extracted and purified as described by Collins (1977) and analysed by HPLC (Tamaoka, 1986). Cell walls from strain GSS02T were prepared and analysis of the diagnostic diamino acid was carried out using the methods described by Schleifer (1985). Polar lipids were determined by TLC as described by Minnikin et al. (1977).

The predominant cellular fatty acids (>10%) of strain GSS02T were anteiso-C15:0 and iso-C16:0, which was similar to *P. terrigena* JCM 21741T. The fatty acid compositions of strain GSS02T and the reference species are shown in Table 2. The isoprenoid quinone of the respiratory chain in strain GSS02T was MK-7 (Fig. S3), which was in agreement with the description of the genus *Paenibacillus*. Strain GSS02T contained meso-diaminopimelic acid as the diagnostic diamino acid in the cell-wall peptidoglycan. The polar lipid profile of

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**Fig. 1.** Neighbour-joining tree based on 16S rRNA gene sequences showing the phylogenetic position of strain GSS02T and representatives of some other related taxa. Bootstrap values (expressed as percentages of 1200 replications) ≥50% are shown at branch points. Bar, 0.01 substitutions per nucleotide position.
strain GSS02^T mainly contained phosphatidylethanolamine, diphasphatidylglycerol and phosphatidylglycerol, with moderate amounts of two unknown aminophospholipids and a minor amount of one unknown lipid (Fig. S4).

Chromosomal DNA of strain GSS02^T was extracted using a commercial genomic DNA extraction kit (Aidlab Biotechnologies). The DNA G+C content was analysed with the HPLC method (Mesbah et al., 1989). DNA–DNA hybridization experiments were conducted using the photobiotin-labeled DNA probe microplate method according to Ezaki et al. (1989). Hybridization values are given as the mean (±SD) of at least two hybridization experiments (reciprocal and non-reciprocal values).

The DNA G+C content of strain GSS02^T was 53.4 mol%, which falls within the range of members of the genus Paenibacillus (40–54 mol%). The DNA–DNA hybridization value between strain GSS02^T and P. terrigena JCM 21741^T was 58.8 ± 0.5 %, which is well below the 70 % threshold accepted for species delineation (Wayne et al., 1987), indicating that strain GSS02^T represents a separate genomic species. DNA–DNA hybridization was not conducted between strain GSS02^T and P. harenae DSM 16969^T or P. polymyxa JCM 2507^T, because the 16S rRNA gene sequence similarities between them were below the threshold value of 97 % (Stackebrandt & Goebel, 1994). In conclusion, based on the phenotypic, physiologial, chemotaxonomic and phylogenetic properties, strain GSS02^T represents a novel species of the genus Paenibacillus, for which the name Paenibacillus guangzhouensis sp. nov. is proposed.

The humic substance- and Fe(III)-reducing ability of strain GSS02^T was tested with different electron donors including glucose, acetate, lactate and propionic acid using MSM in 25.2 ml serum bottles. In the reaction systems, bacterial cells were at 5×10^6 ml^-1, and the humic substance analogue AQDS at 1 mmol l^-1. Ferrihydrite (FH), prepared according to Li et al. (2009), was at 10 mmol l^-1 and electron donor at 10 mmol l^-1. Controls without electron donor or without cells were also set up. The reduction experiments were conducted in triplicate, and the same standard anaerobic techniques and anaerobic incubation conditions as those used in enrichment and isolation were also used. Reduction products, Fe(II) in Fe(III) reduction and anthrahydroquinone-2,6-disulfonate (AHQDS) in AQDS reduction, were quantified according to published protocols (Hong et al., 2007; Li et al., 2009; Wang et al., 2009).

As shown in Figs 2 and 3, the concentrations of Fe(II) and AHQDS in the inoculated vials with glucose, acetate, lactate or propionic acid as electron donor were greater than those in the controls, suggesting that strain GSS02^T could reduce AQDS and FH in the presence of these organic compounds under anaerobic conditions. The concentrations of Fe(II) and AHQDS increased with time and were in the order acetate < lactate < propionic acid < glucose. Therefore, it can be concluded that strain GSS02^T was capable of reducing both humic substances and FH under anaerobic conditions.

**Table 2.** Cellular fatty acid profiles of strain GSS02^T and reference strains

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
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<tbody>
<tr>
<td>C_{16:0}</td>
<td>9.6</td>
<td>3.9</td>
<td>3.4</td>
<td>6.7</td>
</tr>
<tr>
<td>iso-C_{14:0}</td>
<td>4.3</td>
<td>4.8</td>
<td>4.6</td>
<td>–</td>
</tr>
<tr>
<td>iso-C_{15:0}</td>
<td>3.9</td>
<td>4.2</td>
<td>23.7</td>
<td>1.5</td>
</tr>
<tr>
<td>iso-C_{16:0}</td>
<td>20.2</td>
<td>18.5</td>
<td>11.0</td>
<td>7.0</td>
</tr>
<tr>
<td>iso-C_{17:0}</td>
<td>1.8</td>
<td>1.3</td>
<td>1.5</td>
<td>2.8</td>
</tr>
<tr>
<td>anteiso-C_{15:0}</td>
<td>47.2</td>
<td>55.9</td>
<td>25.7</td>
<td>45.4</td>
</tr>
<tr>
<td>anteiso-C_{17:0}</td>
<td>7.6</td>
<td>6.2</td>
<td>–</td>
<td>25.4</td>
</tr>
<tr>
<td>iso-C_{16:1} H</td>
<td>–</td>
<td>–</td>
<td>1.0</td>
<td>–</td>
</tr>
<tr>
<td>iso-C_{16:0} 3-OH</td>
<td>–</td>
<td>–</td>
<td>3.6</td>
<td>–</td>
</tr>
<tr>
<td>iso-C_{17:0} 3-OH</td>
<td>–</td>
<td>–</td>
<td>3.2</td>
<td>–</td>
</tr>
<tr>
<td>C_{18:0}9c</td>
<td>–</td>
<td>–</td>
<td>1.7</td>
<td>2.5</td>
</tr>
<tr>
<td>Summed feature 3*</td>
<td>1.2</td>
<td>1.5</td>
<td>4.6</td>
<td>3.2</td>
</tr>
<tr>
<td>Summed feature 8*</td>
<td>–</td>
<td>–</td>
<td>2.5</td>
<td>–</td>
</tr>
<tr>
<td>Summed feature 9*</td>
<td>–</td>
<td>–</td>
<td>4.8</td>
<td>–</td>
</tr>
</tbody>
</table>

*Summed features represent two or three fatty acids that cannot be separated by GLC using the MIDI system. Summed feature 3 comprises C_{16:1} 9c and/or C_{16:1} 7c; summed feature 8 comprises C_{18:0} 9c and/or C_{18:1} 9c; summed feature 9 comprises iso-C_{17:1} 9c and/or C_{16:0} 10-methyl.

**Fig. 2.** FH reduction by strain GSS02^T with different organic substances as electron donors under anaerobic conditions.
**Description of Paenibacillus guangzhouensis sp. nov.**

*Paenibacillus guangzhouensis* (guang.zhou.en’sis. N.L. masc. adj. guangzhouensis of or pertaining to Guangzhou, a city in Guangdong Province, from where the type strain was isolated).

Cells are Gram-reaction-variable. The Gram reaction of very young cultures (12 h) is negative and of older ones (up to 24 h old) is positive. Cells are rod-shaped, facultatively anaerobic, motile with peritrichous flagella (Fig. S1) and 0.4–0.7 μm in size. Spores form at 80 °C as cells exhibit heat resistance at this temperature for 10 min. Colonies on LA medium at 30 °C for 48 h are circular with slightly irregular edges, cream-coloured, smooth, convex and 0.5–1.5 mm in diameter. Growth occurs with 0–3 % (w/v) NaCl (optimum 1 %), at pH 6.5–10.0 (optimum pH 7.0–8.0) and at 16–40 °C (optimum 35 °C). Cells cannot grow on MacConkey agar. Catalase and oxidase are produced and nitrate is reduced. Voges–Proskauer test is negative. Gelatin and casein are hydrolysed but starch, Tween 20 and Tween 80 are not. Positive for aesculin, ferric citrate and β-galactosidase but negative for arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, urease and tryptophan deaminase. Indole is produced but dihydrolase, lysine decarboxylase, ornithine decarboxylase, L-histidine, L-proline, L-fucose, melibiose, L-rhamnose, sucrose, d-arabitol, d-xylitol, L-arabinose, L-sorbose, dulcitol, inositol, trisodium citrate, D-lyxose, D-tagatose, L-fucose, d-arabitol, L-arabitol or xylitol. ASSimilates glycogen, l-histidine, L-proline, L-fucose, melibiose, D-sorbitol, L-arabinose, lactic acid, sodium acetate and propionic acid but not d-ribose, capric acid, valeric acid, trisodium citrate, sucrose, malate, d-glucose, salicyc, d-mannit, N-acetylglucosamine, inositol, itaconic acid, suberic acid, sodium malonate, L-alanine, potassium 5-ketogluconate, L-serine, 3-hydroxybenzoic acid, potassium 2-ketogluconate, 3-hydroxybutyric acid or 4-hydroxybenzoic acid. The predominant menaquinone is MK-7. The major cellular fatty acids are anteiso-C<sub>15</sub>:0 and iso-C<sub>16</sub>:0. The diagnostic diamino acid in the cell-wall peptidoglycan is meso-diaminopimelic acid.

The type strain, GSS02<sup>T</sup> (=KCTC 33171<sup>T</sup>=CCTCC AB 2013236<sup>1</sup>), was isolated from a forest soil sample taken from Huolu Mountain in Guangzhou, Guangdong Province, China. The DNA G+C content of the type strain is 53.4 mol%.

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