A novel Gram-stain-positive, slightly halophilic, catalase-positive, oxidase-negative, endospore-forming, motile, facultatively anaerobic, rod-shaped bacterium, designated strain JSM 081004T, was isolated from non-saline forest soil in Xiaoxi National Natural Reserve, China. Growth occurred with 0.5–20 % (w/v) NaCl (optimum 2–4 %), at pH 6.0–10.5 (optimum pH 8.0) and at 5–40 °C (optimum 25–30 °C). meso-Diaminopimelic acid was present in the cell-wall peptidoglycan. The major cellular fatty acids were iso-C15 : 0 and anteiso-C15 : 0. Strain JSM 081004T contained MK-7 as the predominant respiratory quinone, and diphosphatidylglycerol, phosphatidylethanolamine and phosphatidylglycerol as the major polar lipids. The genomic DNA G+C content of strain JSM 081004T was 40.1 mol%. Phylogenetic analysis based on 16S rRNA gene sequence comparisons revealed that strain JSM 081004T should be assigned to the genus Bacillus and was most closely related to the type strains of Bacillus lehensis (sequence similarity 97.8 %), Bacillus oshimensis (97.8 %) and Bacillus patagoniensis (97.3 %). Phylogenetic analysis, DNA–DNA relatedness values, phenotypic characteristics and chemotaxonomic data all support the proposal of strain JSM 081004T as a representative of a novel species of the genus Bacillus, for which the name Bacillus xiaoxiensis sp. nov. is proposed; the type strain is JSM 081004T (=CCTCC AA 208057T =DSM 21943T).
cultures at 4 °C and also deep-frozen at −80 °C in 20 % (v/v) glycerol. Three type strains, *Bacillus lehensis* DSM 19099^T*, *Bacillus oshimensis* DSM 18940^T* and *Bacillus patagoniensis* DSM 16117^T*, obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ, Braunschweig, Germany), were used as reference strains for comparison. Unless otherwise indicated, morphological, physiological, molecular and chemotaxonomic studies were performed with cells grown on MA (pH 8.0) at 30 °C.

Cell morphology was examined by using light microscopy (model DM3000; Leica). Gram staining and KOH lysis tests were carried out according to Smibert & Krieg (1994) and Gregersen (1978), respectively. Flagella and endospores were examined according to the methods of Leifson and Schaeffer–Fulton, respectively (Smibert & Krieg, 1994). Growth was tested at various temperatures (4, 5–55 °C, in increments of 5 °C) and pH (5.0–11.0, in increments of 0.5 units) on MA as well as in nutrient broth (NB) supplemented with 2.5 % (w/v) NaCl. The buffer solutions described by Chen et al. (2007) were used for pH experiments. Growth in the absence of NaCl was investigated on nutrient agar (NA) and in NB prepared in the presence of 2–4 % (w/v) NaCl, at pH 8.0 and at 35 °C. Cultures at 4 °C and also deep-frozen at −80 °C in 20 % (v/v) glycerol. Three type strains, *Bacillus lehensis* DSM 19099^T*, *Bacillus oshimensis* DSM 18940^T* and *Bacillus patagoniensis* DSM 16117^T*, obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ, Braunschweig, Germany), were used as reference strains for comparison. Unless otherwise indicated, morphological, physiological, molecular and chemotaxonomic studies were performed with cells grown on MA (pH 8.0) at 30 °C.

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Cells of strain JSM 081004^T* were Gram-stain-positive, endospore-forming, motile, slightly halophilic, facultatively anaerobic, straight rods, with optimum growth occurring in the presence of 2–4 % (w/v) NaCl, at pH 8.0 and at 25–30 °C. Colonies were yellow-pigmented, flat, opaque with smooth, glistening surfaces and circular/slightly irregular margins, and 2–3 mm in diameter after incubation for 3–4 days at 30 °C on MA. Detailed phenotypic properties that differentiate strain JSM 081004^T* from related species of the genus *Bacillus* are summarized in Table 1 and also mentioned in the species description below.

Genomic DNA was isolated according to Hopwood et al. (1985) and the G+C content was determined using the HPLC method (Mesbah et al., 1989). The 16S rRNA gene was amplified by PCR and sequenced as described by Cui et al. (2001). Pairwise sequence similarities were calculated using a global alignment algorithm, implemented at the EzTaxon server (Chun et al., 2007). Phylogenetic analysis was performed by using the software package MEGA version 4.1 (Tamura et al., 2007) after multiple alignment of sequence data by CLUSTAL X (Thompson et al., 1997). Distances were calculated using distance options according to Kimura’s two-parameter model (Kimura, 1980) and clustering was performed by the neighbour-joining method (Saitou & Nei, 1987). Maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Kluge & Farris, 1969) trees were generated by using the tree-making algorithms contained in the PHYLIP package (Felsenstein, 2002). Bootstrap analysis was used to evaluate tree topology by means of 1000 resamplings (Felsenstein, 1985). After the DNA was purified to an absorbance ratio of A₂₆₀ versus A₂₈₀ higher than 1.8, DNA–DNA hybridization experiments were performed according to the optical renaturation method (De Ley et al., 1970; Huß et al., 1983; Jahneke, 1992) using a UV-1206 spectrophotometer (Shimadzu) equipped with a TB-85 thermo-bath. Every hybridization experiment was repeated five times and the highest and lowest values in each experiment were excluded. DNA–DNA relatedness values were expressed as the means of the remaining three values.

The DNA G+C content of strain JSM 081004^T* was 40.1 mol%. The almost-complete 16S rRNA gene sequence (1467 bp) was determined. Phylogenetic analysis based on 16S rRNA gene sequences revealed that strain JSM 081004^T* should be assigned to the genus *Bacillus* and was related most closely to *B. lehensis* MLB2^T* (16S rRNA gene sequence similarity of 97.8 %; Ghosh et al., 2007), *B. oshimensis* K11^T* (97.8 %; Yumoto et al., 2005) and *B. patagoniensis* PAT 05^T* (97.3 %; Oliviera et al., 2005); sequence similarities less than 95.5 % were observed with other species of the genus *Bacillus*. The neighbour-joining phylogenetic tree further confirmed that strain JSM 081004^T* was closely related phylogenetically to members of the genus *Bacillus* and formed a robust lineage with the type strains of *B. lehensis*, *B. oshimensis*, *B. patagoniensis* and *Bacillus clausii* (95.3 % similarity; Nielsen et al., 1995) (Fig. 1). Topology was similar to those of the phylogenetic trees reconstructed by using maximum-likelihood and maximum-parsimony methods (Supplementary Fig. S1, available in IJSEM Online). Levels of DNA–DNA relatedness of strain JSM 081004^T* with *B. lehensis* DSM 19099^T*, *B. oshimensis* DSM 18940^T* and *B. patagoniensis* DSM 16117^T* were 18.6 % (SD of 1.8 %), 17.9 % (SD of 1.5 %) and 16.4 % (SD of 1.7 %), respectively, values that are well below the threshold value (70 %) recommended by Wayne et al. (1987) (Table 1 and also mentioned in the species description below).
Therefore, it would appear that, on the basis of phylogenetic and DNA–DNA hybridization data, strain JSM 081004T represents a novel species of the genus *Bacillus* according to accepted criteria (Wayne et al., 1987; Stackebrandt & Goebel, 1994).

Amino acids of whole-cell hydrolysates were analysed by TLC as described by Hasegawa et al. (1983). Isoprenoid quinones were analysed by HPLC as described by Groth et al. (1996). Polar lipids were extracted according to the method of Minnikin et al. (1979) and identified by two-dimensional TLC; total lipid material and specific functional groups were detected using Dittmer–Lester reagent (phosphate-containing lipids), ninhydrin (free amino groups), Dragendorff reagent (quaternary nitrogen) and anisaldehyde/sulfuric acid (glycolipids) (Dittmer & Lester, 1964; Vaskovsky et al., 1975; Ryu & MacCoss, 1979; Collins & Jones, 1980). Fatty acids were determined according to Sasser (1990) using the Microbial Identification System (Microbial ID) with cells grown in marine broth 2216 (Difco) in flasks on a rotary shaker (with shaking at 200 r.p.m.) at 30 °C for 2 days.

Chemotaxonomic data for strain JSM 081004T were consistent with assignment of the strain to the genus *Bacillus*. The strain possessed a cell-wall type based on *meso*-diaminopimelic acid as the diagnostic diamino acid.

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### Table 1. Characteristics used to distinguish strain JSM 081004T from the type strains of phylogenetically related species of the genus *Bacillus*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colony pigmentation</td>
<td>Yellow</td>
<td>Creamy yellow</td>
<td>Creamy</td>
<td>Creamy white</td>
</tr>
<tr>
<td>Spore shape</td>
<td>Ellipsoidal</td>
<td>Oval</td>
<td>Ellipsoidal</td>
<td>Oval</td>
</tr>
<tr>
<td>Spore position</td>
<td>Central to subterminal</td>
<td>Subterminal</td>
<td>Terminal</td>
<td>Subterminal</td>
</tr>
<tr>
<td>Sporangium</td>
<td>Slightly swollen</td>
<td>Unswollen</td>
<td>Unswollen</td>
<td>Swollen</td>
</tr>
<tr>
<td>Motility</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Facultatively anaerobic</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>Oxidase</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Urease</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Citrate utilization</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Growth conditions</td>
<td></td>
<td></td>
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<tr>
<td>NaCl range (%, w/v)</td>
<td>0.5–20</td>
<td>0.5–15</td>
<td>0–18</td>
<td>1–25</td>
</tr>
<tr>
<td>NaCl optimum (%, w/v)</td>
<td>2–4</td>
<td>5–8</td>
<td>2–4</td>
<td>6–10</td>
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<tr>
<td>pH range</td>
<td>6.0–10.5</td>
<td>6.5–10.5</td>
<td>7.0–11.0</td>
<td>6.5–10.5</td>
</tr>
<tr>
<td>pH optimum</td>
<td>8.0</td>
<td>8.0</td>
<td>8.5</td>
<td>7.5–8.0</td>
</tr>
<tr>
<td>Temperature range (°C)</td>
<td>5–40</td>
<td>10–40</td>
<td>10–40</td>
<td>5–40</td>
</tr>
<tr>
<td>Temperature optimum (°C)</td>
<td>25–30</td>
<td>30</td>
<td>25–30</td>
<td>25–30</td>
</tr>
<tr>
<td>Hydrolysis of:</td>
<td></td>
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</tr>
<tr>
<td>DNA</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Aesculin</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tween 40</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tween 60</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Tween 80</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Polar lipids*</td>
<td>DPG, PG, PE, PL</td>
<td>DPG, PG, PE, 2PL</td>
<td>DPG, PG, PE, PI, PIM, PL</td>
<td>DPG, PG, PE, PI, PIM, PL</td>
</tr>
<tr>
<td>DNA G + C content (mol%)†</td>
<td>40.1</td>
<td>41.4</td>
<td>40.8</td>
<td>39.7</td>
</tr>
</tbody>
</table>

*DPG, Diphasphatidylglycerol; PG, phosphatidylglycerol; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PIM, phosphatidylinositol mannoside; PL, unidentified phospholipid.

†Data for the type strains of *B. lehensis*, *B. oshimensis* and *B. patagoniensis* were obtained from Ghosh et al. (2007), Yumoto et al. (2005) and Olivera et al. (2005), respectively.
Strain JSM 081004T contained MK-7 (96.4 %) as the predominant menaquinone, with MK-6 (1.1 %) and MK-8 (2.5 %) present in minor amounts. The polar lipids of this strain consisted of diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol and one unknown phospholipid (Table 1). The fatty acid profile of strain JSM 081004T was similar to those of the type strains of the three phylogenetically related species of the genus *Bacillus*, although there were differences in the proportions of some components (Table 2). The fatty acid profile of strain JSM 081004T contained the major compounds iso-C_{15:0} (75.4 %) and anteiso-C_{15:0} (11.5 %), which are characteristic of numerous members of the genus *Bacillus* (Kampfer, 1994).

The results of the phylogenetic analysis and of morphological and chemotaxonomic investigations supported the affiliation of strain JSM 081004T to the genus *Bacillus*. However, the yellow pigmentation of strain JSM 081004T, as well as the ability to grow under anaerobic conditions and reduce nitrate to nitrite, together with several other phenotypic characteristics and chemotaxonomic data, differentiated the isolate clearly from its phylogenetic relatives (Tables 1 and 2). In conclusion, phylogenetic analysis based on 16S rRNA gene sequences, DNA–DNA relatedness results, and phenotypic and chemotaxonomic data presented here support the proposal that strain JSM 081004T represents a novel species of the genus *Bacillus*, *Bacillus xiaoxiensis* sp. nov.

### Description of *Bacillus xiaoxiensis* sp. nov.

*Bacillus xiaoxiensis* (xia.o.xi.en’sis. N.L. masc. adj. xiaoxiensis pertaining to Xiaoxi National Natural Reserve, Strain JSM 081004T contained MK-7 (96.4 %) as the predominant menaquinone, with MK-6 (1.1 %) and MK-8 (2.5 %) present in minor amounts. The polar lipids of this strain consisted of diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol and one unknown phospholipid (Table 1). The fatty acid profile of strain JSM 081004T was similar to those of the type strains of the three phylogenetically related species of the genus *Bacillus*, although there were differences in the proportions of some components (Table 2). The fatty acid profile of strain JSM 081004T contained the major compounds iso-C_{15:0} (75.4 %) and anteiso-C_{15:0} (11.5 %), which are characteristic of numerous members of the genus *Bacillus* (Kampfer, 1994).

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### Description of *Bacillus xiaoxiensis* sp. nov.

*Bacillus xiaoxiensis* (xia.o.xi.en’sis. N.L. masc. adj. xiaoxiensis pertaining to Xiaoxi National Natural Reserve,
China, the source of the sample from which the type strain was isolated.

Cells are Gram-stain-positive, catalase-positive, oxidase-negative, slightly halophilic, facultatively anaerobic, straight rods, approximately 0.6–1.2 μm wide and 3.0–5.0 μm long, occurring singly, as pairs or as short chains, producing ellipsoidal endospores that lie in central to subterminal, slightly swollen sporangia. Motile by means of peritrichous flagella. Colonies are yellow-pigmented, flat and opaque, have smooth, glistening surfaces and circular/slightly irregular margins, and are 2–3 mm in diameter on MA. No diffusible pigments are produced. Growth occurs with 0.5–20 % (w/v) NaCl (optimum 2–4 %), at pH 6.0–10.5 (optimum pH 8.0) and at 5–40 °C (optimum 25–30 °C). Nitrate is reduced to nitrite, but nitrite is not further reduced. Negative for egg yolk reaction, methyl red, Voges–Proskauer, H2S and indole production tests. Aesculin, casein, gelatin, starch and Tween 20 are hydrolysed, but cellulose, DNA, ONPG, and Tweens 40, 60 and 80 are not. Acids are produced from amygdalin, D-glucose, glycerol, glycogen, maltose, D-mannitol, melibiose, raffinose, starch and sucrose, but not from N-acetylglucosamine, adonitol, L-arabinose, cellobiose, dulcitol, D-fructose, D-galactose, myo-inositol, lactose, D-mannose, melezitose, L-rhamnose, D-ribose, D-salicin, D-sorbitol, trehalose or D-xyllose. The following compounds are utilized as sole sources of carbon: D-arabitol, D-galactose, lactose, maltose, melezitose, melibiose, raffinose, L-arabinose, cellobiose, dextrin, D-fructose, D-galactose, lactose, maltose, melezitose, melibiose, raffinose, L-rhamnose, D-ribose, sucrose, trehalose, adonitol, D-arabitol, myo-inositol, D-mannitol, D-sorbitol, butyrate, citrate, gluconate, propionate, succinate, N-acetylglucosamine, L-alanine, L-arginine, L-glutamic acid, glycine, L-histidine, hydroxy L-proline, L-isoleucine, L-leucine, L-methionine, L-phenylalanine, L-proline, L-serine and L-valine. Alkaline phosphatase, α-chymotrypsin, esterase (C4), esterase lipase (C8), leucine arylamidase and naphthol-AS-BI-phosphohydrolase are expressed constitutively; acid phosphatase, arginine dihydrolase, cystine arylamidase, x-fucosidase, x- and β-galactosidase, x- and β-glucosidase, N-acetyl-β-glucosaminidase, β-glucuronidase, lipase (C14), lysine decarboxylase, α-mannosidase, ornithine decarboxylase, phenylalanine deaminase, trypsin, urease and valine arylamidase activities are not observed. meso-Diaminopimelic acid is present in the cell-wall peptidoglycan as the diagnostic diaminoglycine. Possesses MK-7 as the major polar lipids. Major fatty acids are iso-C15:0 and anteiso-C15:0.

The type strain is DSM 21943T (= CCTCC AA 208057T =DSM 21943T), isolated from non-saline forest soil in Xiaoxi National Natural Reserve, China. The DNA G+C content of the type strain is 40.1 mol% (HPLC method).

Acknowledgements

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