**Paenibacillus taihuensis** sp. nov., isolated from an eutrophic lake

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Two Gram-stain-negative, facultatively anaerobic and endospore-forming rod-shaped bacterial strains, THMBG22T and R24, were isolated from decomposing algal scum. Phylogenetic analysis of 16S rRNA gene sequences showed that the two strains were closely related to each other (99.7% similarity) and that they were also closely related to *Paenibacillus sacheonensis* DSM 23054T (97–97.1%) and *Paenibacillus phyllosphaerae* DSM 17399T (96.1–96.4%). This affiliation was also supported by *rpoB*-based phylogenetic analyses. Growth was observed at 20–40 °C (optimum, 30–37 °C) and at pH 5.0–9.0 (optimum, pH 6.0–7.0). The cells contained MK-7 as the sole respiratory quinone and anteiso-C₁₅ : ₀ as the major cellular fatty acid. Their cellular polar lipids were composed of phosphatidylglycerol, diphosphatidylglycerol, phosphatidylethanolamine and 12 unidentified polar lipids. The diamino acid of their cell-wall peptidoglycan was meso-diaminopimelic acid. The DNA–DNA hybridization value between THMBG22T and R24 was 84%, and DNA–DNA relatedness to the most closely related species with a validly published name (*P. sacheonensis*) was 35–37%. These results supported the assignment of the new isolates to the genus *Paenibacillus* and also distinguished them from the previously described species of the genus *Paenibacillus*. Hence, it is proposed that strains THMBG22T and R24 represent a novel species of the genus *Paenibacillus*, with the name *Paenibacillus taihuensis* sp. nov. The type strain is THMBG22T (=CGMCC 1.10966T=NBRC 108766T).

Harmful algal blooms have been an increasingly serious problem affecting aquatic systems and environmental sustainability (Paerl et al., 2003). During the post-blooming stage, large quantities of algae cells accumulate and decompose, resulting in serious environmental and ecological consequences (Li et al., 2012). Recent studies have shown that various heterotrophic bacteria are associated with algal blooms and decomposition in eutrophic lakes (Berg et al., 2009; Grossart et al., 2006; Xing et al., 2011). Taihu lake, located in Jiangsu Province of China (30°55′40″–31°32′58″N 119°52′32″–120°36′10″E), has suffered from repeated algal blooms in the past years (Paerl et al., 2011). Previous studies on the microbial diversity and population dynamics during algal decomposition with molecular tools such as 16S rRNA gene sequencing showed that Clostridium-affiliated 16S rRNA gene sequences from members of the phylum Firmicutes were highly abundant in decomposing algal slurry (Xing et al., 2011). We attempted to cultivate bacterial isolates, and bacterial strains that are phylogenetically related to the genus *Paenibacillus* of the phylum Firmicutes were obtained.

The genus *Paenibacillus* was first proposed by Ash et al. (1993). At the time of writing, 143 species and four subspecies with validly published names are included in the genus *Paenibacillus* (http://www.bacterio.net/p/paenibacillus.html). Species of the genus *Paenibacillus* share common characteristics such as being rod-shaped, endospore-forming, aerobic or facultatively anaerobic. Their major isoprenoid quinone is MK-7; the diamino acid in the peptidoglycan is meso-diaminopimelic acid; and the major cellular fatty acid is anteiso-C₁₅ : ₀ (Ash et al., 1993; Shida et al., 1997). Members of this genus have been recently isolated from a variety of sources including soil (Wu et al., 2011), springs (Tang et al., 2011; Saha et al., 2010), plant roots (Kong et al., 2013), urban compost (Vaz-Moreira et al., 2010) and clinical samples (Glaeser et al., 2013; Kim et al., 2010). In this paper, two strains isolated from decomposing algal slurry and...
representing a novel species of the genus *Paenibacillus* are characterized and their taxonomy is reported on the basis of a polyphasic approach.

Algal bloom slurry was collected during the post-blooming stage from Lake Taihu (120.19067°N) in July 2010. Bacterial strains THMBG22T and R24 were isolated on R2A and RG agar plates, respectively, by using the serial dilution method. R2A was prepared according to the instructions from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ) (http://www.dsmz.de/microorganisms/medium). RG medium was modified from R2A agar, by using 0.8% Gelrite instead of agar as solidifying agent, considering its good plating efficiency (Shungu et al., 1983). Plates were incubated at 30 °C for 7 days. The isolates were then subcultured on E-R2A agar (five-strength enriched R2A) and then preserved at −80 °C as a suspension in E-R2A broth supplemented with 17% (w/v) glycerol.

The phylogenetic positions of the two isolates were determined with complete 16S rRNA and partial RNA polymerase β-subunit (rpoB) gene sequence analyses. Bacterial DNA preparation, PCR amplification and sequencing of the 16S rRNA and rpoB genes were carried out as previously described (Dahllof et al., 2000; Zhang et al., 2003). The almost complete 16S rRNA gene sequences (1509 nt) of the novel strains were used for calculating the similarities to their phylogenetic neighbours using the EzTaxon-e server version 2.1 (http://eztaxon-e.ezbiocloud.net/) (Kim et al., 2012). The phylogenetic analyses based on 16S rRNA and rpoB gene sequences of the two strains and the type strains of all species of the genus *Paenibacillus* with validly published names were performed by using the software package MEGA version 4.1 (Tamura et al., 2007); multiple alignments were performed using the CLUSTAL X program (version 1.64b) (Thompson et al., 1997). Phylogenetic trees were then reconstructed using maximum-parsimony and neighbour-joining methods (Fitch, 1971; Saitou & Nei, 1987) with Kimura’s two-parameter calculation model. The robustness of the topology in the phylogenetic trees was evaluated by bootstrap analyses based on 1000 resamplings (Felsenstein, 1985). Phylogenetic analyses based on rpoB gene sequences were performed using the same method as the 16S rRNA gene sequence analysis and genetic distances between strains THMBG22T and R24 and each species of the genus *Paenibacillus* were calculated on the basis of pairwise alignment according to the p-distance algorithm.

Based on the 16S rRNA gene sequence analysis, strains THMBG22T and R24 were phylogenetically related to members of the genus *Paenibacillus*. The two isolates shared 99.7% 16S rRNA gene sequence similarity, and they showed high sequence similarities to *Paenibacillus sacheonensis* (97.0–97.1%) (Moon et al., 2011), *Paenibacillus phyllosphaerae* (96.4–96.1%) (Rivas et al., 2005), *Paenibacillus pinihumi* (94.5–95.0%) (Kim et al., 2009) and the type species of the genus *Paenibacillus, Paenibacillus polymyxa* (91.7%) (Ash et al., 1993). The phylogenetic tree based on 16S RNA genes (Fig. 1a) indicated that both strains clustered with species of the genus *Paenibacillus*. In addition, the phylogenetic tree based on rpoB gene sequences (Fig. 1b) supported the hypothesis that strains THMBG22T and R24 fell into a coherent lineage with species of the genus *Paenibacillus*. Moreover, the partial rpoB gene sequence similarity values of strains THMBG22T and R24 was 99.5%, while similarities with *P. sacheonensis* DSM 23054T and *P. phyllosphaerae* DSM 17399T were 85.3–85.8% and 86.1%, respectively. Considering 85.5% sequence similarity of the rpoB gene as a cut-off for genera delineation and sequence similarity >97.7% corresponding to strains of the same species (Adékambi et al., 2008), those data suggested that strains THMBG22T and R24 belong to a novel species of the genus *Paenibacillus*, and the type strains of closely related species, *P. sacheonensis* DSM 23054T, *P. phyllosphaerae* DSM 17399T and *P. pinihumi* JCM 16419T, were used as the references for phenotypic studies.

For the phenotypic characterization, minimal standards for the description of new taxa of aerobic, endospore-forming bacteria recommended by Logan et al. (2009) were followed. Strains THMBG22T and R24 were cultivated for two days at 30 °C on E-R2A agar for morphological observation by transmission (JEM-1400; JEOL) and scanning (Quanta 200; FEI) electron microscopy. Gram-staining was performed using a previously published staining method (Smibert & Krieg, 1994) with cells grown on E-R2A agar at 30 °C. Motility was observed with light microscopy and endospore formation was determined microscopically by staining with malachite green (Schaeffer & Fulton, 1933). Growth was tested in tryptic soy broth (TSB; Bacto), 1/2 strength TSB and R2A, as well as Luria–Bertani broth (LB; laboratory-prepared). The optimum and range of growth temperature were determined in E-R2A broth incubated for 3–7 days at 4, 8, 15, 20, 25, 30, 37, 40 and 45 °C. The pH range for growth was tested at pH 3, 4, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 10 and 11; KH2PO4/HCl, KH2PO4/K2HPO4 and K2HPO4/NaOH buffer systems were used to maintain the pH. Tolerance to NaCl was examined in E-R2A broth containing 0–5% (w/v, at intervals of 0.5%) NaCl. Physiological and biochemical properties and enzyme activities were tested using API 20 NE, API 50 CH and API ZYM kits (bioMérieux) according to the manufacturer’s instructions. Strains THMBG22T and R24 and their phylogenetic neighbours *P. sacheonensis* DSM 23054T, *P. phyllosphaerae* DSM 17399T and *P. pinihumi* JCM 16419T were harvested from TSB broth under the same incubation conditions. Anaerobic growth and oxidase and catalase activities were tested as previously described (Wu et al., 2013). Hydrolysis of casein, asesculin, gelatin, CM-cellulose, starch, urea and Tween 80 was tested as described by Lányi (1987) and Smibert & Krieg (1994). Voges–Proskauer reaction, methyl red test, hydrogen sulfide and indole production were observed according to the methods of Barrow & Feltham (1993). Susceptibility to antibiotics was determined on R2A plates incubated at 30 °C for 24 h, using antibiotic discs (Beijing Pharmaceutical Company) containing the following...
**Fig. 1.** Phylogenetic trees reconstructed with the neighbour-joining method based on 16S rRNA gene (a) and partial rpoB (b) gene sequences, showing that strains THMBG22$^T$ and R24 and the closely related species of the genus *Paenibacillus* are clustered together. GenBank accession numbers are given in parentheses. Confidence levels higher than 70% are shown from 1000-replicate bootstrap sampling. Nodes with filled circles indicate branches found in the phylogenetic trees reconstructed both with the neighbour-joining and maximum-parsimony methods. Bar, evolutionary distance ($K_{nuc}$) of 0.01(a) and 0.05 (b).
(µg per disc unless otherwise stated): penicillin G (10 IU), streptomycin (10), gentamicin (10), tetracycline (30), vancomycin (30), norfloxacin (10), novobiocin (5), ampicillin (10), chloramphenicol (30), erythromycin (15), kanamycin (30), doxycycline (30) and polymyxin B (300 IU).

Strains THMBG22T and R24 formed circular, flat, whitish-creamy colonies after 3 days at 30 °C. Cells were Gram-staining-negative, rod-shaped and motile by means of peritrichous flagella (Fig S1, available in IJSEM online). Subterminal endospores were formed in swollen sporangia. Growth was observed both under aerobic and anaerobic conditions in E-R2A broth. The strains were able to grow at 20–40 °C (optimum, 30–37 °C), at pH 5.0–9.0 (optimum, pH 6.0–7.0) and at NaCl concentrations lower than 1%. Both isolates grew well on TSB, 1/2 strength TSB, R2A and E-R2A, but did not grow on LB medium. Additional physiological and biochemical characteristics of strains THMBG22T and R24 are provided in the species description, and properties differentiating them from closely related species are detailed in Table 1.

For chemotaxonomic analyses, the biomass of the newly isolated strains and the reference type strains was harvested from TSB agar plates incubated at 30 °C for 2 days (stationary phase). Cellular fatty acids were extracted and methylated according to the standard protocol of MIDI (Sherlock Microbial Identification System, version 6.0), analysed by gas chromatography (GC 6890; Agilent) and identified using the TSBA6 database of the Microbial Identification System (Sasser, 1990). Isoprenoid quinones were extracted with chloroform/methanol (2:1, v/v) and purified by using TLC, and the identities of quinones were analysed by HPLC with an eclipse XDB-C18 column (4.6 × 150 mm; Agilent) (Collins, 1985). Polar lipids were extracted and separated by two-dimensional TLC and identified by spraying with appropriate detection reagents (Komagata & Suzuki, 1987; Minnikin et al., 1984). Cell-wall analysis was determined as described by Komagata & Suzuki (1987).

The predominant cellular fatty acids of THMBG22T and R24 were anteiso-C15:0, iso-C15:0 and iso-C15:0. A full comparison of the fatty acid profiles of THMBG22T and R24 and reference strains is given in Table S1. The predominant isoprenoid quinone of THMBG22T and R24 was determined to be MK-7. The polar lipid profile of THMBG22T consisted of phosphatidylglycerol (PG), diphosphatidylglycerol (DPG), phosphatidylethanolamine (PE), four glycolipids (GL), three aminophospholipids (APL), two aminolipids (AL), two phospholipids (PL) and one unidentified polar lipid (Fig. S2). Strain THMBG22T shared common polar lipids, PE, PG and DPG, with the type species P. polymyxa (Kämpfer et al., 2006). In addition, strain THMBG22T possessed GLs that had been observed in Paenibacillus motobuensis (Saha et al., 2010) and APLs that had been found in Paenibacillus typhae (Kong et al., 2013), Paenibacillus wooponensis, Paenibacillus humicus, P. pinihumi (Baik et al., 2011), P. motobuensis

Table 1. Phenotypic characteristics of Paenibacillus taihuensis sp. nov. and related species of the genus Paenibacillus

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
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<td>+</td>
<td>NA</td>
<td>+</td>
<td>−</td>
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<tr>
<td>Catalase</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>−</td>
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<td>−</td>
<td>−</td>
<td>−</td>
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<td>−</td>
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<tr>
<td>H₂S production</td>
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<td>+ †</td>
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<td>+</td>
<td>+ †</td>
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<td>+ †</td>
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<td>+</td>
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<tr>
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<td>+</td>
<td>w†</td>
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<tr>
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<td>w</td>
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<tr>
<td>Melezitose</td>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>+ †</td>
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<tr>
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<td>+</td>
<td>+</td>
<td>w</td>
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<td>−</td>
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<tr>
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<td>−</td>
<td>−</td>
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<td>−</td>
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<tr>
<td>DNA G+C content (mol%)†</td>
<td>55.2</td>
<td>57.6</td>
<td>56.1‡</td>
<td>50.7‡</td>
<td>49.5</td>
</tr>
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</table>

*Data from Moon et al. (2011); Rivas et al. (2005) and Kim et al. (2009).
†Different results were reported by Moon et al. (2011); Rivas et al. (2005) and Kim et al. (2009).
‡Data derived using HPLC method, which is different from the method used in this study.

All strains were positive for hydrolysis of starch, CM-cellulose and asculin; assimilation of N-acetylglucosamine, maltose and glucuronate; activities of oxidase, alkaline phosphatase, esterase lipase (C8), leucine arylamidase, trypsin, α-galactosidase and β-galactosidase. All strains produced acid from d-glucose, N-acetylglucosamine, amygdalin, arbutin, ascinul ferric citrate, cellobiose, maltose, lactose, d-melibiose, sucrose, trehalose, raffinose, turanose, starch, glycogen and gentiobiose. All tested strains were negative for acetoin production and indole production; hydrolysis of casein and Tween 80; assimilation of caprate, adiuate, malate, citrate and penylacetate; tryptophan deaminase, arginine dihydrolase, lipase (C14), β-glucuronidase and x-mannosidase activities.
as meso-diaminopimelic acid. These results supported the assignment of THMBG22<sup>T</sup> and R24 to the genus Paenibacillus (Ash et al., 1993).

The DNA base composition was determined by thermal denaturation (Marmur & Doty, 1962) and Escherichia coli K-12<sup>T</sup> was used as reference. DNA–DNA hybridizations between THMBG22<sup>T</sup> and R24 and the type strain of the most closely phylogenetically related species (P. sacheonensis DSM 23054<sup>T</sup>) were performed by the thermal denaturation and renaturation method according to the protocol of De Ley et al. (1970), with some modifications by Huss et al. (1983) using a Lambda 35 UV/VIS spectrophotometer (Perkin Elmer) with a thermal controller. The DNA G+C contents of strains THMBG22<sup>T</sup> and R24 were 55.2 and 57.6 mol%, respectively. DNA–DNA relatedness between strains THMBG22<sup>T</sup> and R24 was 84%, and they showed 35–37% DNA–DNA relatedness to the most closely phylogenetically related species, P. sacheonensis DSM 23054<sup>T</sup>, respectively.

To further distinguish the two isolates among the related species, random amplified polymorphic DNA (RAPD)-PCR was applied to produce fingerprint patterns of THMBG22<sup>T</sup> and R24 and closely related species according to the method of Ronimus et al. (1997). As shown in Fig. S3, strains THMBG22<sup>T</sup> and R24 produced identical RAPD patterns with primers OP2R and OP2R0 and showed differences with primer OP1R6, indicating they belong to the same species.

Based on the phylogenetic, chemotaxonomic, physiological and biochemical features, strains THMBG22<sup>T</sup> and R24 represent a member of the genus Paenibacillus and clearly could be distinguished from other species of the genus Paenibacillus. Therefore we propose that strains THMBG22<sup>T</sup> and R24 represent a novel species of the genus Paenibacillus, for which the name Paenibacillus taihuensis sp. nov. is proposed and the type strain is THMBG22<sup>T</sup>.

**Description of Paenibacillus taihuensis sp. nov.**

*Paenibacillus taihuensis* (tai.hu.en’sis. N.L. masc. adj. *taihuensis* of or pertaining to Lake Taihu in Jiangsu province, China, where the type strain was isolated).

Cells are Gram-staining-negative, rod-shaped (0.8–1.5 × 2–4 μm), facultatively anaerobic and motile by means of peritrichous flagella. Subterminal endospores are observed in swollen sporangia. Colonies on E-R2A agar are white, sarcoid, flat, circular and usually 1–3 mm in diameter after 3 days of incubation at 30 °C. Growth occurs at 20–40 °C (optimum, 30–37 °C), at pH 5.0–9.0 (optimum, pH 6.0–7.0) and at NaCl concentrations lower than 1%. Oxidase and catalase activities are positive. Hydrogen sulfide is produced. Starch, aesculin and CM-cellulose are hydrolysed, while gelatin, casein and Tween 80 are not. Methyl red test and Voges–Proskauer reaction are negative. In API 20NE strips, nitrate is not reduced to nitrite, glucose is not fermented and indole is not produced. Urease, trypsin, deaminase and arginine dihydrolase activities are absent. The following substrates are assimilated: D-glucose, D-mannose, N-acetylgalactosamine, malose and gluconate but not L-arabinose, D-mannitol, caprate, citrate, adipate, malate and phenylacetate. In the API 50CH strip, acid is produced from D-xylene, methyl β-D-xylpyranoside, D-glucose, methyl α-D-mannopyranoside, methyl α-D-glucopyranoside, N-acetylgalactosamine, amygdalin, arbutin, aesculin ferrer citrate, salicin, cellobiose, maltose, lactose, D-melibiose, sucrose, trehalose, raffinose, starch, glycogen, gentiobiose, turanose, potassium 5-ketogluconate, D-mannose, D-fructose and melezitose; acid is not produced from the other substrates. In the API ZYM strip tests, alkaline phosphatase, esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, z-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, z-galactosidase, β-galactosidase, z-glucosidase and β-galactosidase activities are positive, while esterase (C4), lipase (C14), β-glucuronidase, N-acetyl-β-glucosaminidase, α-mannosidase and β-fucosidase activities are negative. Cells cannot grow with penicillin G (10 IU), streptomycin (10 μg), gentamicin (10 μg), tetracycline (30 μg), vancomycin (30 μg), norfloxacin (10 μg), novobiocin (5 μg), ampicillin (10 μg), chloramphenicol (30 μg), erythromycin (15 μg) or kanamycin (30 μg). The major cellular fatty acids (>9%) are anteiso-C<sub>15 : 0</sub>, iso-C<sub>16 : 0</sub> and iso-C<sub>15 : 0</sub>. The major respiratory quinone is MK-7. The polar lipid profile contains PG, DPG, PE, four GLs, three APLs, two aminolipids, two phospholipids and one unidentified polar lipid. The diaminoc acid in the cell-wall peptidoglycan is meso-diaminopimelic acid.

The type strain is THMBG22<sup>T</sup> (=CGMCC 1.10966<sup>T</sup> = NBRC 108766<sup>T</sup>), isolated from lake Taihu, PR China. The DNA G+C content of the type strain is 55.2 mol%.

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**References**


