Proposal of nine novel species of the *Bacillus cereus* group

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**Abstract**

Nine novel Gram-stain-positive bacteria were investigated by a polyphasic taxonomic approach. Based on the analysis of 16S rRNA gene sequences, these strains belonged to the *Bacillus cereus* group, sharing over 97% similarity with the known species of this group, and less than 95% similarity with other species of the genus *Bacillus*. Multilocus sequence typing analysis showed that they formed nine robust and well-separated branches from the known species. The digital DNA–DNA hybridization (DDDH) and average nucleotide identity (ANI) values between the nine strains were, respectively, below the 70 and 96% threshold values for species definition, and between each strain and the known type strains of this group were also below the two threshold values. On the basis of the phenotypic and phylogenetic data, along with low DDDH and ANI values among these bacteria, they are assigned to the following nine novel species of the *B. cereus* group: *Bacillus paranthracis* sp. nov., type strain Mn5T (=MCCC 1A00395T=KCTC 33714T=LMG 28873T); *Bacillus pacificus* sp. nov., type strain EB422T (=MCCC 1A06182T=KCTC 33858T); *Bacillus tropicus* sp. nov., type strain N24T (=MCCC 1A011406T=KCTC 33711T=LMG 28874T); *Bacillus albus* sp. nov., type strain N35-10-2T (=MCCC 1A02146T=KCTC 33710T=LMG 28875T); *Bacillus mobilis* sp. nov., type strain 0711P9-1T (=MCCC 1A05942T=KCTC 33717T=LMG 28877T); *Bacillus luti* sp. nov., type strain TD41T (=MCCC 1A00359T=KCTC 33716T=LMG 28872T); *Bacillus proteolyticus* sp. nov., type strain TD42T (=MCCC 1A00365T=KCTC 33715T=LMG 28870T); *Bacillus nitratireducens* sp. nov., type strain 4049T (=MCCC 1A00732T=KCTC 33713T=LMG 28871T); and *Bacillus paramycoides* sp. nov., type strain NH24A2T (=MCCC 1A04098T=KCTC 33709T=LMG 28876T).

The *Bacillus cereus* group, also called *Bacillus cereus sensu lato* (s.l.), is a subdivision of the genus *Bacillus* and currently comprises 12 closely related species, including the first species described *Bacillus anthracis*, along with *B. cereus*, *B. thuringiensis*, *B. mycoides*, *B. weihenstephanensis*, *B. pseudo- mycoides*, the recently identified *B. gaemokensis*, *B. manli- ponensis*, *B. cytotoxicus*, *B. toyonensis*, *B. bingmayongensis* [1] and *B. wiedmannii* [2] (the species names *B. gaemokensis*, *B. manliponensis* and *B. bingmayongensis* are effectively but not yet validly published and thus are given in quotation marks throughout this paper). Members of the *B. cereus* group are facultatively anaerobic, spore-forming bacteria that are ubiquitously distributed in diverse environments. In comparison with other species of this group, *B. anthracis* and *B. cereus* have received far more attention owing to their pathogenicity [3].

During the course of investigating the genetic diversity and population structure of bacteria of the *B. cereus* group from diverse marine environments, nine new taxa were identified that probably represented nine putative novel species [4]. Consequently, the objective of present study was to determine their phylogenetic status using a polyphasic taxonomic approach.

As listed in Table S1 (available in the online Supplementary Material), nine strains were isolated from different samples, including sediments and seawater, which were collected from diverse marine environments. They were maintained as aqueous glycerol suspensions (25%, v/v) at −80°C and then deposited at the Marine Culture Collection of China (MCCC). All type strains of species within the *B. cereus* group were used as reference strains in this study. Unless otherwise specified, the morphological, physiological and chemotaxonomic tests of these strains were performed on Luria–Bertani (LB) medium at 32°C.

Gram staining of cells was carried out using a Gram stain kit (Hangzhou Tianhe Microorganism Reagent) according to the manufacturer’s instructions. Cell morphology was...
observed by transmission electron microscopy using cells grown on LB medium at 32°C for 8 h (Fig. S1), and the presence of endospores was observed by phase-contrast microscopy (Fig. S2). Anaerobic growth was tested on LB medium in an anaerobic chamber (1029; Forma, N₂/CO₂/H₂, 86 : 7.7 %) at 32°C for 2 weeks. Catalase activity was determined by observing bubble production after the application of 3 % (v/v) hydrogen peroxide solution, a positive reaction indicated by the production of bubbles. Oxidase activity was evaluated via the oxidation of 1 % (w/v) p-aminodimethylaniline oxalate. Growth at different temperatures (4, 7, 10, 12, 15, 20, 25, 28, 32, 37, 39, 40, 42, 43, 45, 48 and 54°C) was investigated on LB medium for up to 2 weeks. The pH ranges for growth were confirmed in LB broth at pH 3.0–11.0 with increments of 1.0 pH unit, using appropriate biological buffers, citrate/phosphate (pH 3.0–7.0), Tris/HCl (pH 8.0–9.0) or sodium carbonate/sodium bicarbonate (pH 10.0–11.0). Salt tolerance was tested using modified LB broth containing 0, 0.5, 1, 2, 3, 5, 7, 9, 12 and 15 % (w/v) NaCl for 2 weeks. Hydrolysis of protein, starch, casein and CM-cellulose sodium were investigated on modified LB supplemented with 1 % skimmed milk powder, 1 % soluble starch, 1 % casein and 1 % CM-cellulose sodium, respectively. Other physiological and biochemical characteristics were confirmed using API 20E and API 50CHB strips (bio-Mérieux) following the manufacturer’s instructions. These characteristics of nine strains are listed in Table 1 and in the species descriptions.

To determine the phylogenetic positions of the nine strains, 16S rRNA gene sequences for them were amplified using the universal primers 27F and 1492R, and then sequenced by the ABI3730xl platform (Shanghai Majorbio Bio-pharm Technology). All 16S rRNA gene sequences of the nine strains were submitted to the GenBank database, and all accession numbers are shown in Table S1. Other 16S rRNA gene sequences of type strains within the B. cereus group were extracted from the EzTaxon-e database [5]. A phylogenetic tree based on 16S rRNA gene sequences was reconstructed using MEGA version 5.05 [6] after multiple alignments of the data using DNAMAN version 5.0 (Lynnons), with distance options according to the Kimura two-parameter model and clustering with the neighbour-joining (NJ) method [7]. Bootstrap analysis based on 1000 replications was used to estimate the confidence level of the tree topologies [8].

The nearly complete 16S rRNA gene sequences of the nine strains were determined (1509 bp). As shown in Table S2, these strains shared high 16S rRNA gene sequence similarities (>98 %), most higher than 99.5 %. Moreover, as illustrated in Fig. 1, these strains demonstrated close relationships with each other in the NJ tree with low bootstrap values. A 16S rRNA gene sequence similarity of 97 % is generally used as a threshold value for species definition in prokaryotes taxonomy [9]. Therefore, the 16S rRNA gene can only identify a bacterium to the B. cereus group, but cannot assign it accurately to a certain species according to its low discrimination.

Multilocus sequence typing (MLST) is an easy, reliable and robust approach for the identification of bacterial isolates as an alternative to 16S rRNA gene sequence analysis [10]. As a result, the taxonomic affiliations of these strains were analysed by MLST according to the P scheme (Table S3) [11]. To confirm the PCR results, seven housekeeping genes were also separately obtained from each genome sequence using a local BLAST search. The seven gene sequences from the PCR were the same as those from each genome on the basis of the local BLAST results. The phylogenetic tree for seven concatenated housekeeping gene sequences was determined according to the method as described above. As shown in Table S2, these strains shared low MLST similarities relative to those of 16S rRNA gene sequences. Meanwhile, as illustrated in Fig. 2, these strains formed well-separated branches with high bootstrap values. Based on the MLST analysis, the nine strains were clearly separated from recognized species, suggesting that they may represent potential novel species of the B. cereus group.

To further determine the taxonomic position, the genome sequences of the nine strains were determined by Tianjin Biochip Corporation using the Illumina/Solexa sequencing technology. All genome sequences of strains were submitted to the GenBank database, and all accession numbers are shown in Table S1. The genome sequences of 12 type strains as references were obtained from the GenBank database. Digital DNA–DNA hybridization (dDDH) values were calculated based on genome sequences using the genome-to-genome distance calculator website service (GGDC 2.0) (http://ggdc.dsmz.de/distcalc2.php) [12]. Average nucleotide identity (ANI) values were also calculated using the EzGenome web service (www.zejbiocloud.net/tools/ani). As listed in Table 2, the dDDH and ANI values between the nine strains were, respectively, below 70 and 96 %, and between each novel strain and each published type strain were also below the two threshold values, which were used as the standard for bacterial species delineation [13]. As a result, the nine strains are nine putative novel species of the B. cereus group. In addition, the DNA G+C contents of the nine strains ranged from 35.0 to 35.5 mol% based on the genome sequences, in accordance with the range of the 12 reference strains (34.8–37.1 mol%).

To determine the whole-cell fatty acid composition, cells of these strains were harvested from the third quadrants on triptase soy agar at 28°C. The cells were saponified, methylated and extracted using the standard MIDI (Sherlock Microbial Identification System, version 6.0B) protocol. The fatty acids were then analysed by GC (Agilent Technologies 6850) and identified using the TSBA6.0 database of the Microbial Identification System. As listed in Table 3, the major fatty acids of these strains were C₁₆:₀, iso-C₁₅:₀, summed feature 3 (C₁₆:₁ω₆c and/or C₁₆:₁ω₇c), anteiso-C₁₅:₀, iso-C₁₃:₀, iso-C₁₄:₀, iso-C₁₆:₀ and iso-C₁₇:₀.
| Characteristics | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
| Cell length (μm) | 2.3-3.1 | 1.2-1.6 | 0.5-0.7 | 0.8-1.0 | 0.7-1.0 | 0.5-0.6 | 0.8-1.0 | 0.5-0.6 | 0.7-1.0 | 0.8-1.0 | 0.5-0.6 | 0.7-1.0 | 0.8-1.0 | 0.5-0.6 | 0.7-1.0 | 0.8-1.0 | 0.5-0.6 | 0.7-1.0 | 0.8-1.0 | 0.5-0.6 |
| Cell width (μm)  | 2.3-3.1 | 1.2-1.6 | 0.5-0.7 | 0.8-1.0 | 0.7-1.0 | 0.5-0.6 | 0.8-1.0 | 0.5-0.6 | 0.7-1.0 | 0.8-1.0 | 0.5-0.6 | 0.7-1.0 | 0.8-1.0 | 0.5-0.6 | 0.7-1.0 | 0.8-1.0 | 0.5-0.6 | 0.7-1.0 | 0.8-1.0 | 0.5-0.6 |
| Methyl red       | +/+ | +/+ | +/+ | +/+ | +/+ | +/+ | +/+ | +/+ | +/+ | +/+ | +/+ | +/+ | +/+ | +/+ | +/+ | +/+ | +/+ | +/+ | +/+ | +/+ |
| Lipase          | +/+ | +/+ | +/+ | +/+ | +/+ | +/+ | +/+ | +/+ | +/+ | +/+ | +/+ | +/+ | +/+ | +/+ | +/+ | +/+ | +/+ | +/+ | +/+ | +/+ |
| Starch hydrolysis| +/+ | +/+ | +/+ | +/+ | +/+ | +/+ | +/+ | +/+ | +/+ | +/+ | +/+ | +/+ | +/+ | +/+ | +/+ | +/+ | +/+ | +/+ | +/+ | +/+ |
| API 50CHB results | +/+ | +/+ | +/+ | +/+ | +/+ | +/+ | +/+ | +/+ | +/+ | +/+ | +/+ | +/+ | +/+ | +/+ | +/+ | +/+ | +/+ | +/+ | +/+ | +/+ |

### Strains

- B. cereus
- B. manliponensis
- B. toyonensis
- B. wiedmannii

### API 50CHB Assays

- Arginine dihydrolase: +
- Oxidase/catalase: +
- Proskauer: +
- Fermentation/oxidation (glucose): +
- Methyl-α-D-mannopyranoside: -
- L-arbutin: -
- Aesculin ferric citrate: +
- Xylose: +
- L-fucose: +
- L-sorbose: -
- Xylopyranoside: -
- Lactose (bovine origin): +
- Melibiose: +
- Inulin: +
- Melezitose: +
- Raffinose: +
- Xylitol: +
- Gentiobiose: +
- Tagatose: -
- D-mannitol: +
- L-arabitol: -
Although the kinds of fatty acids of these strains were similar to each other, their proportions were different.

The phylogenetic analysis of 16S rRNA gene sequences suggested that the nine isolates belonged to the *B. cereus* group, and their chemotaxonomic features also corresponded to those of this group. Therefore, it is appropriate to regard the nine isolates as members of the *B. cereus* group. MLST analysis further demonstrated that they represented potential novel species of the *B. cereus* group based on the low MLST similarities and independent phylogenetic position of each strain in the MLST tree. Meanwhile, pairwise dDDH and ANI values were, respectively, less than 70 and 96 % (Table 2), and the physiological and biochemical characteristics of the strains could also be used to distinguish them from each other (Table 1). Therefore, we concluded that the nine isolates should be assigned to the following nine novel species of the *B. cereus* group: *Bacillus paranthracis* sp. nov. (type strain Mn5\textsuperscript{T}), *Bacillus pacificus* sp. nov. (type strain EB422\textsuperscript{T}), *Bacillus tropicus* sp. nov. (type strain N24\textsuperscript{T}), *Bacillus albus* sp. nov. (type strain N35-10-2\textsuperscript{T}), *Bacillus mobilis* sp. nov. (type strain 0711P9-1\textsuperscript{T}), *Bacillus luti* sp. nov. (type strain TD41\textsuperscript{T}), *Bacillus proteolyticus* sp. nov. (type strain TD42\textsuperscript{T}), *Bacillus nitratireducens* sp. nov. (type strain 4049\textsuperscript{T}), and *Bacillus paramycoides* sp. nov. (type strain NH24A2\textsuperscript{T}).

**DESCRIPTION OF BACILLUS PARANTHRACIS SP. NOV.**

*Bacillus paranthracis* (par.an.thra’cís. Gr. prep. para beside, alongside, near, like; N.L. gen. n. anthracis of anthrax, and also a specific epithet; N.L. gen. n. paranthracis near *Bacillus anthracis*).

Cells are Gram-stain-positive, facultatively anaerobic, non-motile, rod-shaped, 1.2–1.4 µm in width and 2.8–3.3 µm in length. A central elliptical endospore is observed. Colonies are off-white, circular, non-translucent and 2–3 mm in diameter after incubation at 32 °C for 48 h on LB medium. Catalase and oxidase are positive. Growth occurs at 15–45 °C (optimum 30 °C), at pH 5–10 (optimum pH 7–8) and with 0–9 % (w/v) NaCl (optimum 1–2 %). Hydrolyses skimmed milk and casein. In API 20E tests, positive for arginine dihydrolase, citrate utilization, acetoin production (Voges–Proskauer) and gelatinase; negative for β-galactosidase, lysine decarboxylase, ornithine decarboxylase, H₂S production, urease, tryptophan desaminase, indole production, and acid production from glucose, mannitol, inositol, sorbitol, rhamnose, sucrose, melibiose, amygdalin and arabinose. In API 50CHB tests, acid production from d-ribose, d-glucose, d-fructose, N-acetylglucosamine, aesculin ferric citrate, maltose, sucrose and trehalose is positive; no acid production from glycerol, erythritol, d-arabinose, l-arabinose, d-xylose, l-xylose, L-choronitol, methyl β-D-xylpyranoside, D-galactose, D-mannose, L-sorbosé, l-rhamnose, dulcitol, inositol, D-mannitol, D-sorbitol, methyl α-D-mannopyranoside, methyl α-D-gluco.pyranoside, amygdalin, arbutin, salicin, cellobiose, lactose (bovine
Cells are Gram-stain-positive, facultatively anaerobic, non-motile, rod-shaped, 1.2–1.6 µm in width and 3.0–4.0 µm in length. A central elliptical endospore is observed. Colonies are white, circular, non-translucent and 2–3 mm in diameter after incubation at 32°C for 48 h on LB medium. Catalase and oxidase are positive. Growth occurs at 15–45°C (optimum 30°C), at pH 5–10 (optimum pH 6) and with 0–9 % (w/v) NaCl (optimum 1 %). Hydrolyses skimmed milk and casein. In API 20E tests, positive for arginine dihydrolase, citrate utilization, acetoin production (Voges-Proskauer) and gelatinase; negative for β-galactosidase, lysine decarboxylase, ornithine decarboxylase, H₂S production, urease, tryptophan desaminase, indole production, and acid production from glucose, mannitol, inositol, sorbitol, rhamnose, sucrose, melibiose, amygdalin and arabinobase. In API 50CHB tests, acid production from D-ribose, D-xylene, D-glucose, D-fructose, N-acetylglucosamine, arbutin, aesculin ferric citrate, maltose, sucrose and trehalose is positive; no acid production from glycerol, erythritol, D-arabinose, L-arabinose, L-xylene, D-xylose, methyl β-D-xylypyranoside, D-galactose, D-mannose, L-sorbose, L-rhamnose, dulcitol, inositol, D-mannitol, D-sorbitol, methyl α-D-mannopyranoside, methyl α-D-glucopyranoside, amygdalin, salicin, cello-biose, lactose (bovine origin), melibiose, inulin, melezitose, raffinose, starch, glycogen, xylitol, gentiobiose, turanose, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, potassium gluconate, potassium 2-ketogluconate and potassium 5-ketogluconate. The principal fatty acids are C₁₁:0 and iso-C₁₅:0.

The type strain, Mn5T (=MCCC 1A00395T=KCTC 33714T =LMG 28873T), was isolated from sediment of the Pacific Ocean. The DNA G+C content of the type strain is 35.2 mol%.

**DESCRIPTION OF BACILLUS PACIFICUS SP. NOV.**

*Bacillus pacificus* (pa.ci’fi.cus. L. masc. adj. *pacificus* peaceful, pertaining to the Pacific Ocean).

The type strain, EB422T (=MCCC 1A06182T=KCTC 33858T), was isolated from sediment of the Pacific Ocean. The DNA G+C content of the type strain is 35.2 mol%.
**DESCRIPTION OF BACILLUS TROPICUS SP. NOV.**

*Bacillus tropicus* (tro´pi.cus. L. masc. adj. tropical, referring to the tropical region).

Cells are Gram-stain-positive, facultatively anaerobic, motile by means of peritrichous flagella, rod-shaped, 1.3–1.6 µm in width and 2.2–2.6 µm in length. A central elliptical endospore is observed. Colonies are off-white, circular, non-translucent and 2–3 mm in diameter after incubation at 32 °C for 48 h on LB medium. Catalase and oxidase are positive. Growth occurs at 15–45 °C (optimum 30 °C), at pH 5–9 (optimum pH 6) and with 0–9 % (w/v) NaCl (optimum 0–0.5 %). Hydrolyses starch, skimmed milk, casein and CM-cellulose sodium. In API 20E tests, positive for arginine dihydrolase, citrate utilization, acetoin production (Voges-Proskauer) and gelatinase; negative for β-galactosidase, lysine decarboxylase, ornithine decarboxylase, H₂S production, urease, tryptophan desaminase, indole production, and acid production from glucose, mannitol, inositol, sorbitol, rhamnose, sucrose, melibiose, amygdalin and arabinose. In API 50CHB tests, acid production from glycerol, d-ribose, d-fructose, aesculin ferric citrate, salicin, cellobiose, maltose, trehalose, starch, glycogen and potassium gluconate is positive; no acid production from erythritol, d-arabinose, l-arabinose, d-xylene, l-xylene, d-adenitol, methyl β-d-xylopyranoside, d-galactose, d-glucose, D-mannose, L-sorbose, L-rhamnose, dulcitol, inositol, d-mannitol, d-sorbitol, methyl α-d-mannopyranoside, methyl α-d-glucopyranoside, N-acetylglucosamine, amygdalin, arbutin, lactose (bovine origin), melibiose, sucrose, inulin, melezitose, raffinose, xylitol, gentiobiose, turanose, d-lyxose, d-tagatose, d-fucose, L-fucose, L-arabitol, L-arabinol, potassium 2-keto-gluconate and potassium 5-keto-gluconate. The principal fatty acids are C₁₆:₀ and iso-C₁₅:₀.

The type strain, N₂⁴ᵀ (=MCCC 1A01406ᵀ=KCTC 33711ᵀ=LMG 28874ᵀ), was isolated from sediment of the South China Sea. The DNA G+C content of the type strain is 35.2 mol%.

**DESCRIPTION OF BACILLUS ALBUS SP. NOV.**

*Bacillus albus* (al´bus. L. masc. adj. albus white).

Cells are Gram-stain-positive, facultatively anaerobic, non-motile, rod-shaped, 1.3–1.5 µm in width and 2.8–3.2 µm in length. A central elliptical endospore is observed. Colonies are white, circular, non-translucent and 2–3 mm in diameter.
after incubation at 32 °C for 48 h on LB medium. Catalase and oxidase are positive. Growth occurs at 15–40 °C (optimum 30 °C), at pH 5–10 (optimum pH 7) and with 0–9 % (w/v) NaCl (optimum 0.5–1 %). Hydrolyses starch, skimmed milk and casein. In API 20E tests, positive for arginine dihydrolase, citrate utilization, acetoin production (Voges–Proskauer) and gelatinase; negative for β-galactosidase, lysine decarboxylase, ornithine decarboxylase, H2S production, urease, tryptophan desaminase, indole production, and acid production from glucose, mannitol, inositol, sorbitol, rhamnose, sucrose, melibiose, amygdalin and arabinose. In API 50CHB tests, acid production from D-ribose, D-glucose, D-fructose, N-acetylglucosamine, arbutin, aesculin ferric citrate, salicin, cellobiose, maltose, sucrose, trehalose, starch and glycogen is positive; no acid production from glycerol, erythritol, D-arabinose, L-arabinose, D-xylose, L-xylene, D-adonitol, methyl β-D-xylopyranoside, D-galactose, D-mannose, L-sorbosel, rhamnose, dulcitol, inositol, D-mannitol, D-sorbitol, methyl α-D-mannopyranoside, methyl α-D-glucopyranoside, amygdalin, lactose (bovine origin), melibiose, inulin, melezitose, raffinose, xyitol, gentiobiose, turanose, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, potassium gluconate, potassium 2-ketogluconate and potassium 5-ketogluconate. The principal fatty acids are C16:0 iso-C15:0 and summed feature 3 (C16:1ω6c and/or C16:1ω7c).

The type strain, N35-10-2T (≡MCCC 1A02146T=KCTC 33710T=LMG 28875T), was isolated from sediment of the South China Sea. The DNA G+C content of the type strain is 35.0 mol%.

**DESCRIPTION OF BACILLUS MOBILIS SP. NOV.**

*Bacillus mobilis* (mo’bi.lis. L. adj. mobilis mobile). Cells are Gram-stain-positive, facultatively anaerobic, motile by means of peritrichous flagella, rod-shaped, 1.2–1.6 μm in width and 2.5–3.2 μm in length. A central elliptical endospore is observed. Colonies are milk white, circular, non-translucent and 2–3 mm in diameter after incubation at 32 °C for 48 h on LB medium. Catalase and oxidase are positive. Growth occurs at 10–39 °C (optimum 30 °C), at pH 5–9 (optimum pH 7) and with 0–9 % (w/v) NaCl (optimum 0 %, w/v). Hydrolyses starch, skimmed milk and casein. In API 20E tests, positive for β-galactosidase, citrate utilization, acetoin production (Voges–Proskauer) and gelatinase; negative for arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, H2S production, urease, tryptophan desaminase, indole production, and acid production from glucose, mannitol, inositol, sorbitol, rhamnose, sucrose, melibiose, amygdalin and arabinose. In API 50CHB tests, acid production from D-ribose, D-glucose, arbutin, aesculin ferric citrate, salicin, maltose, starch (starch) and glycogen is positive; no acid production from glycerol, erythritol, D-arabinose, L-
arabinose, D-xylene, L-xylene, D-adenitol, methyl β-D-xylopyranoside, D-galactose, D-fructose, D-mannose, L-sorbose, L-rhamnose, dulcitol, inositol, D-mannitol, D-sorbitol, methyl α-D-mannopyranoside, methyl α-D-glucopyranoside, N-acetylglucosamine, amygdalin, cellobiose, lactose (bovine origin), melibiose, sucrose, trehalose, inulin, melizitose, raffinose, xyitol, gentiobiose, turanose, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, potassium gluconate, potassium 2-ketogluconate and potassium 5-ketogluconate. The principal fatty acids are C_{16:0}, iso-C_{15:0} and iso-C_{16:1}.

**Table 3.** Cellular fatty acid compositions of the nine novel strains and the type strains of species within the *B. cereus* group

| Fatty acid | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 |
|------------|---|---|---|---|---|---|---|---|---|----|----|----|----|----|----|----|----|----|----|----|----|----|
| C_{12:0}   | 1.6 | 1.7 | 1.0 | 2.7 | 1.8 | 1.3 | 3.3 | 4.7 | 1.5 | 1.4 | 1.0 | 2.7 | 2.5 | 1.4 | 2.3 | 5.9 | TR | ND | ND | TR |
| C_{14:0}   | 5.4 | 6.8 | 5.5 | 7.9 | 3.6 | 6.9 | 6.5 | 7.7 | 3.9 | 4.1 | 4.1 | 3.7 | 3.6 | 3.2 | 5.0 | 9.0 | 2.4 | 3.2 | 4.1 | 3.3 |
| C_{16:0}   | 14.6 | 19.9 | 12.0 | 16.4 | 16.7 | 14.8 | 33.3 | 30.5 | 33.0 | 12.5 | 10.3 | 15.6 | 18.0 | 9.0 | 12.5 | 21.0 | 10.8 | 5.6 | 9.8 | 7.3 |
| C_{16:1ω7c} | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| C_{16:1ω11c} | 1.0 | 1.7 | 1.0 | 1.2 | 1.3 | 1.6 | 2.1 | 2.4 | 1.2 | TR | TR | 1.2 | 1.1 | ND | ND | ND | ND | ND | ND |
| C_{18:2ω7c, 9c} | TR | TR | TR | TR | TR | TR | TR | TR | TR | TR | TR | TR | TR | TR | TR | TR | TR |
| C_{18:0} | 1.8 | 2.0 | TR | 6.1 | 3.4 | 2.6 | 5.3 | 9.2 | 5.5 | TR | TR | 1.6 | 1.3 | TR | 2.7 | 5.0 | ND | ND | 1.7 | TR |
| C_{18:1ω9c} | 2.4 | 3.0 | TR | 2.0 | 3.0 | 2.0 | 1.0 | 1.0 | TR | TR | TR | TR | TR | TR | 1.4 | 1.7 | TR | ND | TR | ND | TR |
| iso-C_{14:0} | 1.4 | 1.2 | TR | 1.3 | 1.9 | 1.1 | 2.3 | 2.2 | 1.5 | 1.9 | 1.5 | 2.6 | 2.9 | 8.7 | 3.0 | 4.9 | TR | TR | TR | TR |
| iso-C_{15:0} | 7.0 | 6.4 | 7.1 | 6.9 | 4.9 | 6.8 | 8.8 | 8.5 | 7.9 | 20.3 | 18.5 | 21.9 | 22.3 | 12.6 | 7.9 | 5.0 | 7.0 | 7.1 | 7.7 | 6.9 |
| iso-C_{16:0} | 5.5 | 3.5 | 5.9 | 4.2 | 5.4 | 4.0 | 2.7 | 1.8 | 1.9 | 4.8 | 5.2 | 3.4 | 3.5 | 5.5 | 5.9 | 6.3 | 5.0 | 2.3 | 2.9 | 5.1 |
| iso-C_{17:0} | 14.7 | 13.8 | 18.9 | 1 | 10.8 | 14.9 | 8.3 | 5.5 | 9.0 | 20.2 | 21.8 | 12.5 | 12.6 | 13.3 | 10.6 | 4.0 | 36.5 | 38.6 | 21.0 | 27.6 |
| iso-C_{17:1ω9c} | ND | TR | TR | 1.2 | TR | TR | TR | TR | TR | TR | TR | ND | ND | ND | ND | TR | TR | ND | ND | ND |
| iso-C_{18:0} | 7.4 | 5.3 | 7.8 | 4.6 | 9.1 | 5.6 | 3.4 | 3.2 | 3.6 | 3.3 | 3.7 | 2.8 | 2.9 | 8.3 | 5.3 | 5.7 | 6.7 | 5.1 | 3.6 | 9.1 |
| iso-C_{18:1ω9c} | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| iso-C_{18:2ω7c, 9c} | 6.5 | 5.2 | 9.6 | 3.8 | 6.0 | 6.6 | 4.3 | 2.5 | 8.5 | 6.7 | 6.9 | 7.5 | 6.6 | 7.0 | 5.3 | 2.7 | 8.2 | 11.4 | 11.5 | 10.1 |
| iso-C_{17:0} | 2.7 | 3.2 | 6.5 | 4.2 | TR | TR | ND | ND | TR | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| iso-C_{17:1ω9c} | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | TR | TR |
| iso-C_{17:1ω11c} | 1.7 | 1.7 | 3.5 | TR | 2.4 | 1.8 | 1.6 | 1.8 | 1.1 | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| iso-C_{18:0} | 2.1 | 1.0 | 2.6 | 1.3 | ND | 2.7 | TR | TR | TR | TR | TR | TR | ND | ND | ND | ND | ND | ND | ND |
| antiso-C_{16:0} | 1.7 | 2.0 | 1.0 | 1.3 | 1.8 | 1.4 | 2.9 | 2.4 | 2.5 | 4.0 | 2.8 | 3.9 | 5.8 | 4.9 | 3.5 | 3.3 | 1.8 | ND | 2.2 | 1.0 |
| antiso-C_{16:1ω9c} | 5.6 | 6.7 | 4.4 | 4.4 | 6.5 | 4.6 | 3.9 | 2.4 | 4.2 | 6.5 | 5.3 | 3.8 | 5.4 | 3.6 | 5.5 | 3.3 | 10.8 | 3.1 | 7.4 | 4.0 |
| antiso-C_{17:0} | 2.2 | 2.7 | 1.7 | 1.6 | 3.2 | 1.8 | 1.5 | 1.1 | 2.0 | 1.5 | 1.1 | 1.7 | 1.6 | 2.0 | 1.5 | 3.4 | ND | 2.8 | 1.5 |
| antiso-C_{17:1ω9c} | 1.0 | TR | TR | TR | 1.0 | TR | TR | TR | TR | TR | TR | TR | TR | TR | TR | TR | TR | TR | TR |
| Summed feature 2 | 1.7 | 1.9 | TR | 1.9 | TR | 1.3 | TR | TR | TR | TR | TR | TR | TR | TR | TR | TR | TR | TR |
| Summed feature 3 | 9.0 | 8.0 | 9.9 | 10.3 | 6.6 | 11.6 | 5.5 | 5.6 | 6.7 | ND | ND | ND | ND | 15.2 | 10.3 | ND | 11.5 | 15.1 | ND |
| Summed feature 4 | 3.8 | 4.6 | TR | 7.9 | 5.6 | 3.7 | 2.1 | 4.1 | 1.8 | ND | ND | ND | ND | 4.0 | 6.4 | ND | ND | ND | ND |

The type strain, 0711P9-1^T (=MC55 1A05942^T=KCTC 33717^T=LMG 28877^T), was isolated from sediment of the Indian Ocean. The DNA G+C content of the type strain is 35.3 mol%.

**DESCRIPTION OF BACILLUS LUTI SP. NOV.**

*Bacillus luti* (lu’ ti. L. neut. gen. n. luti of mud).

Cells are Gram-stain-positive, facultatively anaerobic, motile by means of peritrichous flagella, rod-shaped, 1.2–1.3 μm in width and 2.2–2.5 μm in length. A central
elliptical endospore is observed. Colonies are white, circular, non-translucent and 2–3 mm in diameter after incubation at 32 °C for 48 h on LB medium. Catalase and oxidase are positive. Growth occurs at 10–39 °C (optimum 30 °C), at pH 5–10 (optimum pH 7) and with 0–7 % (w/v) NaCl (optimum 0.5 %). Hydrolyses skimmed milk and casein. In API 20E tests, positive for citrate utilization, acetoin production (Voges–Proskauer) and gelatinase; negative for β-galactosidase, arginine dihydrolase, lactose (bovine origin), melibiose, sucrose, inulin, melezitose, raffinose, xylitol, gentiobiose, turanose, D-lyxose, D-tagatose, D-fucose, L-fucose, L-arabinose, L-ribitol, potassium gluconate, potassium 2-ketogluconate and potassium 5-ketogluconate. The principal fatty acids are C16:0 and iso-C15:0.

The type strain, TD41 (=LMG 28872) (=KCTC 33716T), was isolated from sediment of the Pacific Ocean. The DNA G+C content of the type strain is 35.5 mol%.

DESCRIPTION OF BACILLUS NITRATIREDCENS SP. NOV.

Bacillus nitratireducens (ni.trat.i.re.du’cens. N.L. n. nitrates; L. part. adj. reducens converting to a different state; N.L. part. adj. nitratireducens reducing nitrate).

Cells are Gram-stain-positive, facultatively aerobic, non-motile, rod-shaped, 1.0–1.5 μm in width and 2.7–3.0 μm in length. A central elliptical endospore is observed. Colonies are off-white, rhizoidal and non-translucent after incubation at 32 °C for 48 h on LB medium. Catalase and oxidase are positive. Growth occurs at 7–39 °C (optimum 30 °C), at pH 5–9 (optimum pH 7) and with 0–9 % (w/v) NaCl (optimum 0 %). Hydrolyses skimmed milk and casein. In API 20E tests, positive for arginine dihydrolase, citrate utilization, acetoin production (Voges–Proskauer) and gelatinase; negative for β-galactosidase, lysine decarboxylase, ornithine decarboxylase, urease, tryptophan desaminase, indole production, and acid production from glucose, mannitol, inositol, sorbitol, rhamnose, sucrose, melibiose, amygdalin and arabinose. In API 50CHB tests, acid production from D-ribose, D-glucose, D-fructose, N-acetylglucosamine, arbutin, aesculin ferric citrate, salicin, cellobiose, maltose, trehalose, starch and glycogen is positive; no acid production from glycerol, erythritol, D-arabinose, L-arabinose, D-xylose, L-lyxose, D-xylose, D-adonitol, methyl β-D-xylopyranoside, D-galactose, D-mannose, L-sorbose, L-rhamnose, dulcitol, inositol, D-mannitol, D-sorbitol, methyl α-D-mannopyranoside, methyl α-D-glucopyranoside, amygdalin, lactose (bovine origin), melibiose, sucrose, inulin, melezitose, raffinose, xylitol, gentiobiose, turanose, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, potassium gluconate, potassium 2-ketogluconate and potassium 5-ketogluconate. The principal fatty acids are C16:0 and iso-C15:0 and summed feature 3 (C16:1ω6c and/or C16:1ω7c).

The type strain, TD42 (=MCCC 1A00365T=KCTC 33715T =LMG 28870T), was isolated from sediment of the Pacific Ocean. The DNA G+C content of the type strain is 35.2 mol%.

DESCRIPTION OF BACILLUS PARAMYCOIDES SP. NOV.

Bacillus paramycoides (pa-ra.my.co.i’des. Gr. prep. para beside, alongside, near, like; N.L. masc. adj. mycoides fungus-like, and also a specific epithet; N.L. masc. adj. paramycoides near Bacillus mycoides).
Cells are Gram-stain-positive, facultatively anaerobic, non-motile, rod-shaped, 0.8–1.2 μm in width and 1.8–2.2 μm in length. Endospores are not observed. Colonies are waxy, circular, non-translucent and 2–3 mm in diameter after incubation at 32°C for 48 h on LB medium. Catalase and oxidase are positive. Growth occurs at 15–39°C (optimum 30°C), at pH 5–9 (optimum pH 7) and with 0–5% (w/v) NaCl (optimum 0.5%). Hydrolyses starch, skimmed milk and casein. In API 20E tests, positive for citrate utilization, acetoin production (Voges–Proskauer), gelatinase, and acid production from glucose; negative for β-galactosidase, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, H₂S production, urease, tryptophan desaminase, indole production, and acid production from mannitol, inositol, sorbitol, rhamnose, sucrose, melibiose, amygdalin and arabinose. In API 50CHB tests, acid production from D-ribose, D-galactose, D-glucose, D-fructose, D-mannose, N-acetylglucosamine, amygdalin, arbutin, aesculin, ferric citrate, salicin, cellobiose, maltose, trehalose, starch and glycogen is positive; no acid production from glycerol, erythritol, lactose (bovine origin), melibiose, fructose, D-xylose, L-xylose, D-xylitol, D-glucose, D-xylose, D-fructose, D-fructose, D-mannose, L-arabinose, D-arabinose, L-arabinose, L-rhamnose, dulcitol, inositol, D-mannitol, D-sorbitol, methyl-α-D-mannopyranoside, methyl α-D-glucopyranoside, lactose (bovine origin), melibiose, fructose, D-manno-oligosaccharides, potassium gluconate, potassium gluconate, potassium 2-ketogluconate and potassium 5-ketogluconate. The principal fatty acid is C₁₆:₀.

The type strain, NH24A2T (≡MCCC 1A04098T=KCTC 33709T=LMG 28876T), was isolated from sediment of the South China Sea. The DNA G+C content of the type strain is 35.2 mol%.

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Conflicts of interest
The authors declare that there are no conflicts of interest.

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