**Virgibacillus sediminis** sp. nov., a moderately halophilic bacterium isolated from a salt lake in China

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A Gram-positive, moderately halophilic, alkali-tolerant, strictly aerobic, oxidase- and catalase-positive, rod-shaped bacterium, strain YIM kkny3T, was isolated from a sediment sample collected from a salt lake in the Qaidam Basin of north-west China. Cells were motile by means of peritrichous flagella and formed ellipsoidal endospores lying in subterminal swollen sporangia. Growth occurred with 1–20 % (w/v) total salts (optimum, 5–10 %) and at pH 6.0–10.5 (optimum, pH 7.5–8.0) and 10–55 °C (optimum, 35–40 °C). It was unable to grow with NaCl as the only salt. meso-Diaminopimelic acid was present in the cell-wall peptidoglycan. The strain contained menaquinone 7 (MK-7) as the predominant respiratory quinone and diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine and an unidentified phospholipid as polar lipids. The major cellular fatty acids were anteiso-C15:0 and anteiso-C17:0. The DNA G+C content was 40.9 mol%. Phylogenetic analysis based on 16S rRNA gene sequences indicated that strain YIM kkny3T belonged to the genus *Virgibacillus*, and was most closely related to the type strains of *Virgibacillus olivae* (97.1 % similarity), *Virgibacillus marismortui* (97.0 %) and *Virgibacillus kekensis* (96.8 %). Levels of DNA–DNA relatedness between strain YIM kkny3T and the type strains of *V. olivae*, *V. marismortui* and *V. kekensis* were 12.4, 10.6 and 15.7 %, respectively. The combination of phylogenetic analysis, genotypic data, phenotypic characteristics and chemotaxonomic differences indicated that strain YIM kkny3T represents a novel species of the genus *Virgibacillus*, for which the name *Virgibacillus sediminis* sp. nov. is proposed. The type strain is YIM kkny3T (=CCTCC AA 207023T=DSM 19797T=KCTC 13193T).

The genus *Virgibacillus* was proposed by Heyndrickx et al. (1998). At the time of writing, the genus comprised 13 recognized species: *Virgibacillus pantothenticus* (Heyndrickx et al., 1998), *V. proomii* (Heyndrickx et al., 1999), *V. carmonensis*, *V. necropolis*, *V. marismortui* and *V. saleigens* (Heyman et al., 2003), *V. halodenitrificans* (Yoon et al., 2004), *V. dokdonensis* (Yoon et al., 2005), *V. koreensis* (Lee et al., 2006), *V. olivae* (Quesada et al., 2007), *V. halophilus* (An et al., 2007), *V. chiguensis* (Wang et al., 2008) and *V. kekensis* (Chen et al., 2008a). During a recent study of the microbial diversity of the Qaidam Basin in Qinghai Province, north-west China (Chen et al., 2007a, b, c, d), a moderately halophilic bacterium, designated strain YIM kkny3T, was isolated from a sediment sample collected from the Keke salt lake in August 2002. The lake is located at 36° 18′–36° 55′ N 99° 02′–99° 12′ E; the lake water was at 18 °C, had a pH of 6.5–7.5 and had a salinity of 298 g l⁻¹. Based on the results of the present polyphasic taxonomic study, strain YIM kkny3T is considered to represent a novel species of the genus *Virgibacillus*. Strain YIM kkny3T was isolated from a saline sediment sample by plating 1:10 serial dilutions of the sample on...
Difco marine agar 2216 (MA) supplemented with 5% (w/v) NaCl [containing approximately 7% NaCl and 8.4% total salts (hereafter referred to as MA5); pH 7.5] at 37 °C for 2 weeks. After primary isolation and purification, the isolate was kept as serial transfers on slants of MA5, as lyophilized cultures at 4 °C, or deep-frozen at −80 °C in Difco marine broth 2216 (MB) supplemented with 5% (w/v) NaCl and 20% (v/v) glycerol. Three reference strains, *V. marismortui* DSM 12325T, *V. halodenitrificans* DSM 10037T and *V. kekensis* YIM kknystT, were obtained from the DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen) and the YIM (Yunnan Institute of Microbiology). Unless otherwise indicated, morphological and physiological studies were performed with cells grown on MA5 (pH 7.5) at 37 °C. Cell morphology was examined by light microscopy (model BH2; Olympus). Gram staining was carried out by using the standard Gram reaction (Doetsch, 1981) combined with the KOH lysis test method (Gregersen, 1978). Flagella and endospores were stained according to the methods of Leifsson and Schaeffer-Fulton, respectively (Smibert & Krieg, 1994). For endospore observation, the novel strain was grown on MA5 supplemented with 10 mg MnSO₄ for 2 days at 30 °C and 7 days at room temperature (18–20 °C). Growth was tested at 5–60 °C (at increments of 5 °C) on MA5 and at pH 5.0–11.0 (with increments of 0.5 pH units) on MA5 and in MB supplemented with 5% (w/v) NaCl. Growth at different total salt concentrations [0 and 0.5% (w/v) and 1–30% (w/v) at increments of 1%] was determined on MA, prepared according to the formula of Atlas (1993). Growth was also tested with NaCl as the sole salt. Tests for methyl red, the Voges–Proskauer reaction, H₂S and indole production, hydrolysis of aesculin, nitrate and nitrite reduction were performed as recommended by Smibert & Krieg (1994). Hydrolysis of casein, gelatin, starch, Tweenes (20, 40, 60 and 80) and urea were determined as described by Cowan & Steel (1965). Growth under anaerobic conditions was determined on MA and in MB supplemented with 5% (w/v) NaCl and with or without 0.5% (w/v) glucose or 0.1% (w/v) nitrate, by using the GasPak Anaerobic System (BBL) according to the manufacturer’s instructions. Oxidation/fermentation of glucose and respiration on fumarate, nitrate and nitrite were studied according to the procedures described by Mata et al. (2002) by using MH medium (Quesada et al., 1983) with 7.5% sea salts solution (Rodriguez-Valera et al., 1981) as the basal medium. Nutritional tests were performed by using classical methods (Ventosa et al., 1982) in modified Koser medium (Koser, 1923) [containing (l⁻¹): 50 g NaCl, 2 g KCl, 0.2 g MgSO₄, 7H₂O, 1 g KNO₃, 1 g (NH₄)₂HPO₄ and 0.5 g KH₂PO₄]. When amino acids were used as substrates, the basal medium contained neither KNO₃ nor (NH₄)₂HPO₄. Organic compounds used as sole source of carbon and energy or as sole source of carbon, nitrogen and energy were tested at 0.2% (w/v). Motility, antimicrobial susceptibility and catalase and oxidase activities were determined as described by Chen et al. (2007b). Other enzymic activities and acid production from carbohydrates were carried out by using the API 20E, API ZYM and API 50CH systems (bioMérieux) according to the manufacturer’s instructions, except that the suspension medium was supplemented with artificial seawater [containing (w/v): 5% NaCl, 0.59% MgSO₄.7H₂O, 0.45% MgCl₂, 0.6% KCl and 0.13% CaCl₂; (Levring, 1946)]. Morphological, cultural, physiological and biochemical characteristics of strain YIM kknystT are summarized in the species description below and in Table 1.

DNA was isolated according to the method of Hopwood et al. (1985) and the G+C content was determined by using the HPLC method (Mesbah et al., 1989). Genomic DNA extraction, PCR-mediated amplification of the 16S rRNA gene and purification of PCR products were performed as described by Cui et al. (2001). Multiple alignments with sequences of the most closely related strains and the determination of the levels of similarity between sequences were carried out by using CLUSTAL_X (Thompson et al., 1997). Distances were calculated by using distance options according to the Kimura two-parameter model (Kimura, 1980). Phylogenetic trees were constructed by using three tree-making algorithms, the neighbour-joining (Saitou & Nei, 1987), maximum-likelihood (Felsenstein, 1981) and minimum-evolution (Felsenstein, 1997) methods, contained within the MEGA version 3.1 software package (Kumar et al., 2004). Confidence values for the branches of phylogenetic trees were determined by using bootstrap analysis (Felsenstein, 1985) based on 1000 resamplings. DNA–DNA hybridization was carried out by using photobiotin-labelled probes in microplate wells as described by Ezaki et al. (1989). A microplate spectrofluorometer (Gemini XPS; Molecular Devices) was employed for fluorescence measurements.

The genomic DNA G+C content of strain YIM kknystT was 40.9 mol%. The almost-complete 16S rRNA gene sequence (1562 bp) of the organism was determined. Phylogenetic analysis based on 16S rRNA gene sequences revealed that strain YIM kknystT was related closely to members of the genus *Virgibacillus*, with sequence similarities of between 95.0% (to *V. koreensis* BH30097T) and 97.1% (to *V. olivae* E308T). Strain YIM kknystT showed 16S rRNA gene sequence similarities of 97.0, 96.8, 96.4 and 96.2% to the type strains of *V. marismortui*, *V. kekensis*, *V. proomii* and *V. halodenitrificans*, respectively. In the neighbour-joining, maximum-parsimony and minimum-evolution phylogenetic trees, strain YIM kknystT and the type strain of *V. salexigens* (16S rRNA gene sequence similarity of 95.5%) formed a subline supported by a low bootstrap resampling value (Fig. 1 and supplementary Fig. S1 available in IJSEM Online). Levels of DNA–DNA relatedness between strain YIM kknystT and the type strains of *V. olivae*, *V. marismortui* and *V. kekensis* were 12.4, 10.6, 12.8, 11.5 and 15.7%, respectively. These values were well below the threshold value of 70% recommended by Wayne et al. (1987) for assigning strains to the same species. It is therefore evident, based on phylogenetic analysis and
Table 1. Differential characteristics between strain YIM kkny3T and recognized species of the genus Virgibacillus

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<td>42.6</td>
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*E, ellipsoidal; s, spherical.
†C, central; ST, subterminal; T, terminal.

DNA–DNA hybridization data, that strain YIM kkny3T represents a novel species of the genus Virgibacillus (Wayne et al., 1987; Stackebrandt & Goebel, 1994).

Amino-acid and sugar analyses of whole-cell hydrolysates were performed as described by Hasegawa et al. (1983) and Stanek & Roberts (1974), respectively. Polar lipids were extracted according to the method of Minnikin et al. (1984) and were identified by two-dimensional TLC and spraying with appropriate detection reagents (Minnikin et al., 1984; Komagata & Suzuki, 1987). Isoprenoid quinones were analysed by HPLC as described by Groth et al. (1996). Fatty acids were determined for the new isolate as well as for three reference strains, V. marismortui DSM 12325T, V. halodenitrificans DSM 10037T and V. kekensis YIM kkny16T, as described by Sasser (1990), by using the Microbial Identification system (MIDI; Microbial ID). Cells for this purpose were grown in MB supplemen-
ted with 5 % (w/v) NaCl (pH 7.5) in flasks on a rotary shaker (shaking at 200 r.p.m.) at 37 °C for 3 days.

Chemotaxonomic data for strain YIM kkny3T were consistent with its assignment to the genus Virgibacillus (Heyndrickx et al., 1998; Heyrman et al., 2003). The peptidoglycan of strain YIM kkny3T contained meso-diaminopimelic acid. Galactose, glucose and ribose were present in the whole-cell hydrolysates. The fatty acid profile of strain YIM kkny3T was similar to those of members of the genus Virgibacillus (see Supplementary Table S1 in IJSEM Online). The major fatty acids of strain YIM kkny3T were anteiso-C₁₅ : ₀ (58.9 % of the total) and anteiso-C₁₇ : ₀ (17.3 %). Menaquinone 7 (MK-7; 97.7 %) was the predominant menaquinone, with MK-6 (2.0 %) and MK-8 (0.3 %) present in trace amounts. Diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine and an unknown phospholipid were found as polar lipids.
The results of the phylogenetic analysis and chemotaxonomic studies supported the view that strain YIM kkny3T should be assigned to the genus *Virgibacillus* (Heyndrickx et al., 1998; Heyrman et al., 2003). However, it could be distinguished from recognized species of the genus based on a number of phenotypic properties (Table 1) and by its discriminative fatty acid profile (Supplementary Table S1), although the latter may partly have resulted from the different culture conditions employed. The comparatively wide growth temperature range for strain YIM kkny3T (10–55 °C) (Table 1) and the high ratio of anteiso- to iso-branched fatty acids in its fatty acid profile (Supplementary Table S1) clearly differentiated the new isolate from recognized species of the genus *Virgibacillus*. Strain YIM kkny3T also differed from its closest phylogenetic relative, *V. olivae*, based on its ability to grow at pH 10 and to produce acids from D-glucose and D-mannose, and its inability to grow in the absence of NaCl or to hydrolyse starch (Table 1). In addition, strain YIM kkny3T could be differentiated from the type strain of *V. olivae* based on its much higher genomic DNA G+C content (7 mol% difference, Table 1) and its distinct cellular fatty acid pattern (the ratio of anteiso- to iso-branched fatty acids in the fatty acid profile of strain YIM kkny3T was approximately nine times higher than that of the type strain of *V. olivae* Supplementary Table S1). On the basis of the results of phylogenetic analysis, genotypic data (levels of DNA–DNA relatedness and genomic DNA G+C content), phenotypic characteristics and chemotaxonomic distinctiveness presented here, we suggest that strain YIM kkny3T represents a novel species of the genus *Virgibacillus*, for which the name *Virgibacillus sediminis* sp. nov. is proposed.

**Description of *Virgibacillus sediminis* sp. nov.**

*Virgibacillus sediminis* (se.di’m.in.is. L. gen. n. *sediminis* of sediment).

Cells are Gram-positive, catalase- and oxidase-positive, strictly aerobic rods, approximately 0.4–0.7 μm wide and 2.5–4.0 μm long, occurring singly, in pairs or in short chains. Cells are motile by means of peritrichous flagella. They bear ellipsoidal endosporules that lie in subterminally swollen sporangia. After 3 days on MA supplemented with 5 % (w/v) NaCl at 37 °C, colonies are 2–3 mm in diameter, circular to slightly irregular, raised, translucent and creamy white to pale yellow. No soluble pigments are produced. Growth occurs with 1–20 % (w/v) total salts (optimum, 5–10 %) and at 10–55 °C (optimum, 35–40 °C) and pH 6.0–10.5 (optimum, pH 7.5–8.0). Unable to grow with NaCl as sole salt. Aesculin and gelatin are hydrolysed, but casein, starch, Tween-20, 40, 60 and 80 and urea are not. Positive for nitrate reduction and oxidation of glucose, but negative for fermentation of glucose, nitrite reduction, H2S and indole production, and methyl red and Voges–Proskauer tests. Respiration on nitrate, nitrite and fumarate under anaerobic conditions is not observed. Acids are produced from cellobiose, D-glucose, D-fructose, D-mannose and turanose (API 50CH); other organic substrates included in the API 50CH panels are not metabolized. The following compounds are utilized as sole sources of carbon.

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source of carbon and energy or sole source of carbon, nitrogen and energy: celllobiose, dextrin, D-glucose, maltose, D-mannose, D-ribose, trehalose, acetate, gluconate and L-alanine. The following substances are not utilized: L-arabinose, D-fructose, D-galactose, D-lactose, melezitose, melibiose, raffinose, L-rhamnose, D-saline, sucrose, D-xylose, adonitol, D-arabinitol, glycerol, myo-inositol, D-mannitol, D-sorbitol, butyrate, citrate, fumarate, propionate, succinate, L-asparagine, L-cysteine, L-glutamic acid, D-glycine, L-histidine, hydroxy-L-proline, L-isoleucine, L-leucine, L-methionine, L-proline, L-serine and L-valine. Constitutive enzymes expressed are acid phosphatase, esterase (C4), gelatinase, α-glucosidase, β-glucoronidase, leucine arylamidase and naphthol-AS-BI-phosphohydrolase, but alkaline phosphatase, N-acetyl-β-glucosaminidase, arginine dihydrolase, α-chymotrypsin, cystine arylamidase, esterase lipase (C8), α-fucosidase, α-galactosidase, β-galactosidase, β-glucosidase, lipase (C14), lysis decarboxylase, α-mannosidase, ornithine decarboxylase, trypsinodeaminase, trypsin and valine arylamidase are not produced (API 20E and ZYM). Cells are resistant to ampicillin (30 μg), gentamicin (10 μg) and nalidixic acid (20 μg), but susceptible to chloramphenicol (30 μg), kanamycin (30 μg), licomycin (2 μg), novobiocin (30 μg), polymyxin B (30 μg), rifampicin (5 μg), streptomycin (10 μg) and tetracycline (30 μg). Whole-cell hydrolysates contain meso-diaminopimelic acid and galactose, glucose and ribose. The predominant menaquinone is MK-7. The polar lipids consist of diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine and an unknown phospholipid. The major cellular fatty acids of the type strain (comprising 86.2% of the total) are anteiso-C₁₅:₀ and anteiso-C₁₇:₀. The DNA G+C content of the type strain is 40.9 mol%.

The type strain, YM kkn5^T (=CCTCC AA 207023^T = DSM 19797^ =KCTC 131935^), was isolated from a saline sediment sample collected from the Keke salt lake in the Qaidam Basin in Qinghai Province, north-west China.

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