Sediminibacillus albus sp. nov., a moderately halophilic, Gram-positive bacterium isolated from a hypersaline lake, and emended description of the genus *Sediminibacillus* Carrasco *et al.* 2008

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A moderately halophilic, Gram-positive-staining, endospore-forming, rod-shaped and strictly aerobic bacterium, designated strain NHBX5^T, was isolated from Lake Nanhuobuxun in China. Strain NHBX5^T was able to grow at NaCl concentrations of 0–22 % (w/v) (optimally at 7 %, w/v), at pH 6.0–9.0 (optimally at pH 7.5) and at temperatures of 10–45 °C (optimally at 37 °C). The cell-wall peptidoglycan of strain NHBX5^T contained *meso*-diaminopimelic acid as the diagnostic diamino acid. The predominant isoprenoid quinone was MK-7. The major cellular fatty acids were anteiso-C_{15:0} and anteiso-C_{17:0}. The polar lipids were phosphatidylglycerol, diphosphatidylglycerol and a glycolipid. The genomic DNA G + C content of strain NHBX5^T was 44.9 mol%. Phylogenetic analysis based on 16S rRNA gene sequence comparisons revealed that strain NHBX5^T was most closely related to *Sediminibacillus halophilus* EN8d^T (98.6 % gene sequence similarity). The level of DNA–DNA relatedness between strain NHBX5^T and *S. halophilus* CGMCC 1.6199^T was 34.6 %. Based on the data presented, strain NHBX5^T represents a novel species of the genus *Sediminibacillus*, for which the name *Sediminibacillus albus* sp. nov. is proposed. The type strain is NHBX5^T (=DSM 19340^T =CGMCC 1.6502^T). In addition, an emended description of the genus *Sediminibacillus* is presented.

Moderately halophilic bacteria that can grow optimally in media containing salt concentrations of 3–15 % (w/v) make up an extremely heterogeneous group of micro-organisms that are distributed widely in diverse saline habitats such as salt lakes, brines, saline soils, soda lakes, salted foods and some other hypersaline environments (Kushner, 1985; Ventosa *et al.*, 1998). The genus *Sediminibacillus*, which belongs to this moderately halophilic group of bacteria, was first proposed by Carrasco *et al.* (2008) to accommodate moderately halophilic, facultatively anaerobic, Gram-positive, rod-shaped bacteria related phylogenetically to members of the genera *Thalassobacillus* and *Halobacillus* within the family *Bacillaceae*. The genus *Sediminibacillus* comprises the single species *Sediminibacillus halophilus*, the type strain of which was isolated from sediment of Lake Erliannor in Inner Mongolia, China. During the course of an investigation of the diversity of halophilic micro-organisms in

Nanhuobuxun salt lake (36° 43' 14.7" N 95° 42' 41" E), a spore-forming, Gram-positive, moderately halophilic strain (NHBX5^T) was isolated that appeared to represent a novel species of the genus *Sediminibacillus*. In this study, the phenotypic, chemotaxonomic and genotypic characteristics of this moderately halophilic strain are presented.

Nanhuobuxun salt lake is located at 2700 m above sea level in the south-east Bieletan region of Qaidam basin of Qinghai province, China. It is a very typical chloride saline lake, with a high salinity of 31.8 % and a near-neutral pH of 6.7. Strain NHBX5^T was isolated from a sediment sample of Nanhuobuxun salt lake by enrichment in liquid SG medium (Sehgal & Gibbons, 1960) at 37 °C followed by serial dilution plating of enrichment cultures on SG agar medium until a pure colony was obtained. The SG medium (pH adjusted to 7.0–7.2 with 1 M NaOH before autoclaving at 121 °C for 20 min) contained the following (g l⁻¹): Casamino acids (Difco), 7.5; yeast extract (Difco), 10.0; sodium glutamate, 1.0; trisodium citrate, 3.0; MgSO₄·7H₂O, 20.0; KCl, 2.0; NaCl, 200; FeSO₄·7H₂O, 0.036; and MnCl₂·4H₂O, 0.00036. Strain NHBX5^T was cultured routinely on growth medium (GM; pH 7.0–7.5) containing the following (g l⁻¹): Casamino acids (Difco),

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain NHBX5^T is DQ989634.

A scanning electron micrograph of cells of strain NHBX5^T and the cellular fatty acid profiles of strain NHBX5^T and *S. halophilus* EN8d^T are available as supplementary material with the online version of this paper.

5.0; yeast extract (Difco), 5.0; NaCl, 70; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 5.0; KCl, 2.0; $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 0.036; and $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, 0.00036. Strain NHBX5^T was maintained in this medium supplemented with 30 % (v/v) glycerol at -80°C for long-term preservation. *S. halophilus* CGMCC 1.6199^T was obtained from the China General Microbiological Culture Collection and used as a reference strain for comparative phenotypic studies and DNA–DNA hybridization tests. The recommended medium and culture conditions for growth of this bacterium were used.

The morphology, pigmentation and size of colonies were observed on GM agar after 48 h incubation at 37°C . Cell morphology and motility were examined using light and scanning electron microscopes. Gram-type was determined by the staining method (Doetsch, 1981) and the KOH lysis method (Gregersen, 1978). The range of NaCl concentration for growth was determined in modified GM medium with different NaCl concentrations (0, 1, 3, 5, 7, 9, 12, 16, 20, 22 and 25 %, w/v). The pH range for growth was tested at intervals of 0.5 pH unit in liquid GM medium buffered with 20 mM MES (pH 5.0–6.5), PIPES (pH 6.5–7.5), HEPES (pH 7.0–8.0), Tricine (pH 7.5–9.0) and CHES (pH 9.0–10.0). Growth at different temperatures (4, 10, 15, 20, 25, 30, 37, 40, 45 and 50°C) was determined in GM medium. General biochemical tests (including the presence of oxidase and catalase, urease activity, H_2S production, nitrate reduction, NH_3 production, citrate utilization, indole production, Voges–Proskauer reaction, methyl red test and hydrolysis of Tweens 20, 40, 60 and 80, gelatin, aesculin, starch and casein) were performed according to previously described methods (Smibert & Krieg, 1981; Ventosa *et al.*, 1982). Anaerobic growth in the presence of nitrate was tested as described previously (Mancinelli & Hochstein, 1986). The utilization of glucose by oxidation or fermentation and of various substrates as sole carbon and energy sources were determined as described previously (Ventosa *et al.*, 1982). Unless otherwise indicated, all tests were carried out in triplicate in media containing 7 % (w/v) NaCl at 37°C . Growth was monitored by turbidity at OD_{600} . API 50CH test strips (Analytab Products; bioMérieux) were also used to examine assimilation of carbohydrates and the production of acid as recommended by the manufacturer, but with a modification that the suspension medium supplied by bioMérieux and used to resuspend cells of strain NHBX5^T was supplemented with 7 % (w/v) NaCl. Susceptibility to antimicrobial agents was tested by spreading exponential-phase cultures on GM agar medium plates with absorbent paper discs impregnated with antimicrobial agents. Zones of inhibition were determined after incubation at 37°C for 3 days.

Cells of strain NHBX5^T were aerobic rods and formed terminal ellipsoidal or spherical endospores after 48 h incubation at 37°C on GM agar supplemented with 5 mg $\text{MnSO}_4 \text{ l}^{-1}$ (see Supplementary Fig. S1, available in IJSEM Online). Anaerobic growth of strain NHBX5^T was not detected in the presence of glucose or nitrate. Nitrate reduction by strain NHBX5^T under aerobic conditions was

not observed. Strain NHBX5^T utilized glucose by oxidation. It was moderately halophilic, growing in modified GM medium with 0–22 % (w/v) NaCl (optimally at 7 %). Other phenotypic features are presented in the species description.

Preparation of the cell wall and determination of peptidoglycan structure were performed by the methods described by Schleifer & Kandler (1972) with the modification that TLC on cellulose sheets was used instead of paper chromatography. The amino acid composition of the cell wall hydrolysate was determined using one-dimensional descending film chromatography on cellulose sheets. Respiratory quinones were extracted according to the method of Collins *et al.* (1977) and analysed by reversed-phase HPLC (Groth *et al.*, 1996). Polar lipids were extracted and identified by one-dimensional TLC followed by spraying with the appropriate detection reagent (Kates, 1986). Fatty acids were extracted, methylated and analysed by GC using the standard Sherlock MIDI (Microbial Identification) system (Sasser, 1990; Kämpfer & Kroppenstedt, 1996). Genomic DNA of strain NHBX5^T was extracted using the method described by Marmur (1961). Cell mass used for procedures mentioned above was obtained by cultivating the strain at 37°C on GM agar. The genomic DNA G + C content was determined by the thermal denaturation method according to Marmur & Doty (1962).

Strain NHBX5^T possessed peptidoglycan type A1 γ with *meso*-diaminopimelic acid as the diagnostic diamino acid. The isoprenoid quinones detected in strain NHBX5^T were MK-7 (96.9 %) and MK-6 (3.1 %). The cellular polar lipids were diphosphatidylglycerol, phosphatidylglycerol and a glycolipid. The major fatty acids (>1 %) in cells of strain NHBX5^T were branched anteiso- $\text{C}_{15:0}$ (45.1 %), anteiso- $\text{C}_{17:0}$ (30.7 %), iso- $\text{C}_{15:0}$ (9.0 %), iso- $\text{C}_{16:0}$ (4.8 %) and iso- $\text{C}_{17:0}$ (1.4 %) and straight-chain $\text{C}_{16:0}$ (3.5 %). The genomic DNA G + C content of strain NHBX5^T was 44.9 mol%. These chemotaxonomic data are in accordance with those described for the genus *Sediminibacillus*.

The 16S rRNA gene was amplified by PCR using universal bacterial primers (8F and 1495R) as described previously (Duckworth *et al.*, 1996). The almost-complete nucleotide sequence (1468 bp) was determined by direct sequencing and compared with available 16S rRNA gene sequences in GenBank by using the BLAST program. Multiple alignment with sequences from closely related species was performed by using the program CLUSTAL_X (Thompson *et al.*, 1997). Ambiguous and unalignable bases were manually omitted and then the phylogenetic tree was constructed from the evolutionary distance matrix calculated by using the neighbour-joining, minimum-evolution and maximum-parsimony methods in the program MEGA version 3.1 (Kumar *et al.*, 2004). The robustness of the resultant tree topology was evaluated by bootstrap resampling analysis with 1000 replicates (Felsenstein, 1985). A comparative analysis using 16S rRNA gene sequences available in the database revealed that strain NHBX5^T was most closely

related to *S. halophilus* EN8d^T, with a sequence similarity of 98.6 %, and had less than 96.7 % gene sequence similarity to other known species of the closely related genera, such as 94.4–96.7 % to members of the genus *Virgibacillus*, 95.2–96.3 % to members of the genus *Halobacillus*, 95.2 % to the type strain of *Thalassobacillus devorans* (the sole species of the genus *Thalassobacillus*), 94.4–95.3 % to members of the genus *Gracilibacillus* and 93.9–95.2 % to members of the genus *Amphibacillus*. The phylogenetic tree (Fig. 1) based on the neighbour-joining method showed that strain NHBX5^T clustered with *S. halophilus* EN8d^T with significant bootstrap support (100 %). Topologies of phylogenetic trees constructed using the minimum-evolution and maximum-parsimony algorithms were similar to that of the tree constructed using neighbour-joining analysis (data not shown). DNA–DNA hybridization was performed by the spectrophotometric renaturation rate method (Huß *et al.*, 1983; De Ley *et al.*, 1970) to determine the level of genomic DNA relatedness between strain NHBX5^T and *S. halophilus* CGMCC 1.6199^T. The level of DNA–DNA relatedness between strain NHBX5^T and *S. halophilus* CGMCC 1.6199^T was 34.6 % (mean of three independent experiments, which did not differ by more than 4 %). This low value indicates that strain NHBX5^T is not related genotypically to *S. halophilus* CGMCC 1.6199^T.

Several phenotypic features that also distinguish strain NHBX5^T from *S. halophilus* EN8d^T are shown in Table 1. Strain NHBX5^T was strictly aerobic, whereas *S. halophilus* EN8d^T was able to grow under anaerobic conditions. Cells of *S. halophilus* EN8d^T did not display endospores, whereas strain NHBX5^T easily formed ellipsoidal or spherical endospores, causing swelling of sporangia. Differences in other features such as pigmentation, range of temperature for growth, nitrate reduction and H₂S production and the genomic DNA G + C content enable strain NHBX5^T to be distinguished from *S. halophilus* EN8d^T.

Therefore, on the basis of the polyphasic evidence above, strain NHBX5^T represents a novel species of the genus *Sediminibacillus*. The name *Sediminibacillus albus* sp. nov. is proposed for this species. Based on differential phenotypic characteristics of strain NHBX5^T not reported in the

description of the genus *Sediminibacillus* by Carrasco *et al.* (2008), an emended description of the genus is presented.

Emended description of the genus *Sediminibacillus* Carrasco *et al.* 2008

The description of the genus *Sediminibacillus* is as given by Carrasco *et al.* (2008) with the following amendments. Endospores may be formed terminally in swollen sporangia or not be observed. Cells are aerobic or facultatively anaerobic. Nitrate may or may not be reduced. The genomic DNA G + C content ranges from 44.9 to 47.5 mol%. The type species is *Sediminibacillus halophilus*.

Description of *Sediminibacillus albus* sp. nov.

Sediminibacillus albus (al'bus. L. masc. adj. *albus* white).

Cells are Gram-positive-staining rods (0.4–0.6 × 2.0–4.0 µm), strictly aerobic, spore-forming and motile by means of peritrichous flagella. Ellipsoidal or spherical endospores are formed terminally in swollen sporangia. Cells occur singly, in pairs or in short chains. After cultivation on GM agar at 37 °C for 2 days, colonies are circular, white, opaque and slightly convex, 1.5–2.0 mm in diameter. Grows at 10–45 °C (optimum 37 °C) and at pH 5.5–9.0 (optimum pH 7.5). Can grow at NaCl concentrations between 0 and 22 % (w/v) with optimum growth at 7 % (w/v) NaCl. Positive for oxidase, catalase, methyl red reaction, H₂S production from thiosulfate, NH₃ production from peptone, indole production, citrate utilization and hydrolysis of gelatin, aesculin and Tweens 20, 40, 60 and 80, but is negative for the Voges–Proskauer reaction, nitrate reduction, urease production and casein hydrolysis. Produces acids from D-glucose by oxidation. Produces acid in the API 50CH gallery from glycerol, L-arabinose, D-xylose, D-glucose, D-galactose, D-fructose, D-mannose, L-sorbose, L-rhamnose, mannitol, amygdalin, arbutin, salicin, starch, cellobiose, maltose, lactose, melibiose, trehalose, gentiobiose and N-acetylglucosamine, but not from D-sorbitol, sucrose, erythritol, D-ribose, turanose, D-arabinose, D-adonitol, methyl β-D-xylopyranoside, dulcitol, inositol, methyl-α-D-glucopyranoside, inulin, melezi-

Fig. 1. Phylogenetic tree showing the relationship between strain NHBX5^T and related strains based on 16S rRNA gene sequences. Numbers at nodes represent the levels of bootstrap support (%) based on a neighbour-joining analysis of 1000 resampled datasets. Accession numbers of nucleotide sequences are given in parentheses. Bar, 1 % sequence divergence.

Table 1. Characteristics that distinguish strain NHBX5^T from *S. halophilus* EN8d^T

Data for *S. halophilus* EN8d^T were taken from Carrasco *et al.* (2008). Both strains are rod-shaped, motile and positive for Gram-staining reaction, oxidase, catalase and the Voges–Proskauer reaction. Both strains hydrolyse aesculin, gelatin and Tween 80, but not casein or starch. Both strains produce acid from D-glucose, D-galactose, D-mannose, trehalose, D-fructose and glycerol. The following compounds are utilized as sole carbon and energy sources: D-arabinose, cellobiose, D-galactose and D-lactose. The following compounds are not utilized as sole carbon and energy sources: D-mannose and D-sorbitol. The following compounds are not used as sole carbon, nitrogen and energy sources: L-arginine, aspartic acid, L-cysteine, glutamic acid, L-methionine and phenylalanine. +, Positive; –, negative; ND, not observed.

Characteristic	Strain NHBX5 ^T	<i>S. halophilus</i> EN8d ^T
Colony pigmentation	White	Cream
Size of cells (μm)	0.4–0.6 × 2.0–4.0	0.9 × 1.5–7.0
Spore formation	+	ND
Anaerobic growth	–	+
pH range for growth	5.5–9.0	5.0–9.5
Temperature range for growth (°C)	10–45	20–55
NaCl concentration for growth (% w/v)		
Range	0–22	0–20
Optimum	7.0	5.0–7.5
Indole test	+	–
Nitrate reduction	–	+
H ₂ S production	+	–
Utilization of maltose, melibiose, raffinose and D-xylose as sole carbon and energy sources	+	–
Utilization of L-alanine and L-serine as sole carbon, nitrogen and energy sources	–	+
DNA G + C content (mol%)	44.9	47.5

tose, raffinose, glycogen, xylitol, L-xylose, D-tagatose, D- or L-fucose, D- or L-arabitol, gluconate, 5- or 2-ketogluconate or methyl α-D-mannopyranoside. The following compounds are not used as sole carbon, nitrogen and energy sources: L-alanine, L-serine, L-arginine, aspartic acid, L-cysteine, glutamic acid, L-methionine and phenylalanine. Susceptible to ciprofloxacin (5 μg), chloramphenicol (30 μg), kanamycin (30 μg), erythromycin (15 μg), tetracycline (30 μg), ampicillin (10 μg), novobiocin (5 μg), rifampicin (5 μg), vancomycin (30 μg) and penicillin (5 μg), but resistant to norfloxacin (10 μg), bacitracin (0.04 U) and streptomycin (10 μg). The major cellular fatty acids are anteiso-C_{15:0} and anteiso-C_{17:0}. The major cellular polar lipids are diphosphatidylglycerol, phosphatidylglycerol and a glycolipid. The major menaquinone type is MK-7. The cell-wall peptidoglycan contains *meso*-diaminopimelic acid as the diagnostic diamino acid.

The type strain is NHBX5^T (=DSM 19340^T =CGMCC 1.6502^T), isolated from a sediment sample of Lake Nanhuobuxun in Qinghai province, China. The genomic DNA G + C content of strain NHBX5^T is 44.9 mol% (*T_m* method).

Acknowledgements

This work was supported by grants from the Ministry of Science and Technology of China (973 programs 2003CB716001 and 2004CB719605; 863 programs 2006AA020201 and 2007AA021306)

and the Chinese Academy of Sciences (Knowledge Innovation program, KSCX2-YW-G-011).

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