Bacillus urumqiensis sp. nov., a moderately haloalkaliphilic bacterium isolated from a salt lake

Shanshan Zhang,[1] Zhaojun Li,[2] Yanchun Yan,[1] Chuanlun Zhang,[3] Jun Li[2] and Baisuo Zhao[1]

Correspondence
Baisuo Zhao
bszhao@live.com

[1]Graduate School, Chinese Academy of Agricultural Sciences, Beijing 100081, China

[2]Institute of Agricultural Resources and Regional Planning, Chinese Academy of Agricultural Sciences, Beijing 100081, China

[3]The School of Ocean and Earth Sciences, Tongji University, Shanghai 200092, China

A Gram-stain-positive, rod-shaped, aerobic and moderately haloalkaliphilic bacterium, designated BZ-SZ-XJ18^T, was isolated from the mixed water and sediment of a saline-alkaline lake located in the Xinjiang Uyghur Autonomous Region of China. Phylogenetic analysis based on 16S rRNA gene sequences indicated that strain BZ-SZ-XJ18^T was a member of the genus *Bacillus*. The closest phylogenetic relatives were *Bacillus saliphilus* 6AG^T (96.7 % 16S rRNA gene sequence similarity), '*Bacillus daqingensis*' X10-1 (96.6 %), *Bacillus luteus* JC167^T (96.5 %), *Bacillus daliensis* DLS13^T (96.2 %), *Bacillus chagannorensis* CG-15^T (95.2 %) and *Bacillus polygoni* YN-1^T (95.0 %). DNA–DNA relatedness between strain BZ-SZ-XJ18^T and the reference type strains of the related species of the genus *Bacillus* was lower than 27 %. The isolate formed yellow pigment and grew in the presence of 0.22–4.32 M Na^+ (equivalent to 1.3–25.3 %, w/v, NaCl) (optimum 1.08 M Na^+, equivalent to 6.3 %, w/v, NaCl), at pH 6.5–10.0 (optimum pH 8.5–9.5) and at 8–41 °C (optimum 37 °C). The major cellular fatty acids were anteiso-$C_{15:0}$ (43.0 %), $C_{16:0}$ (18.1 %), iso-$C_{15:0}$ (11.3 %), anteiso-$C_{17:0}$ (8.0 %) and iso-$C_{16:0}$ (7.0 %). The major polar lipids consisted of diphosphatidylglycerol and phosphatidylglycerol. The main respiratory quinone was menaquinone-7 (MK-7), and the peptidoglycan type of the cell wall was A1γ based on *meso*-diaminopimelic acid as the diagnostic diamino acid. The genomic DNA G+C content was 42.3 mol% (HPLC) or 41.4 mol% (T_m). On the basis of phenotypic, chemotaxonomic and phylogenetic features, strain BZ-SZ-XJ18^T is proposed to represent a novel species, *Bacillus urumqiensis* within the genus *Bacillus*. The type strain is BZ-SZ-XJ18^T (=DSM 29145^T=JCM 30195^T).

The genus *Bacillus* in the family *Bacillaceae*, originally proposed by Cohn (1872), is one of the largest bacterial genera (Logan *et al.*, 2007) and comprises more than 290 species at the time of writing (http://www.bacterio.net/index.html). Members of the genus *Bacillus* are usually obligate or facultative aerobes, endospore-forming, rod-shaped, catalase-positive, and have menaquinone-7 (MK-7) as the major respiratory quinone as well as iso-$C_{15:0}$ and anteiso-$C_{15:0}$ as the dominant fatty acids, and DNA G+C contents within the range of 32–66 mol% (Logan & De Vos, 2009). Some species of the genus *Bacillus* are halophilic and/or alkaliphilic, and were isolated from saline and/or alkaline environments, such as *Bacillus chagannorensis* (from a soda lake in China) (Carrasco *et al.*, 2007), *Bacillus ligniniphilus* (from sediments of the South China Sea; Zhu *et al.*, 2014), *Bacillus locisalis* (from a hypersaline and alkaline lake in China; Márquez *et al.*, 2011), *Bacillus polygoni* (from indigo balls in Japan; Aino *et al.*, 2008), *Bacillus rigiliprofundi* (from deep subseafloor basaltic crust; Sylvan *et al.*, 2015), *Bacillus saliphilus* (from a mineral pool in Italy; Romano *et al.*, 2005), *Bacillus taeanensis* (from a solar saltern in Korea; Lim *et al.*, 2006), *Bacillus halosaccharovorans, Bacillus iranensis* and *Bacillus salsus* (from the hypersaline lake Aran-Bidgol in Iran; Amoozegar *et al.*, 2013; Mehrshad *et al.*, 2013; Bagheri *et al.*, 2012).

Abbreviations: NJ, neighbour-joining; ME, minimum-evolution; ML, maximum-likelihood; PL, phospholipid; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; DPG, diphosphatidylglycerol; SQDG, sulfoquinovosyldiacylglycerol; MK-7, menaquinone–7.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain BZ-SZ-XJ18^T is KM066107.

Two supplementary figures and one supplementary table are available with the online Supplementary Material.

In this study, we report on the isolation and characterization of a novel moderately halophilic and alkaliphilic bacterium, designated strain BZ-SZ-XJ18^T, which was obtained from a mixture of water and sediment of a saline-alkaline (~8.8 % NaCl, ~pH 8.3, data not published) lake (43°24′35″N 88°6′39″E, 1072 m elevation) close to the 314 national road, 72 km from Urumqi city, Xinjiang Uyghur Autonomous Region of China, on the basis of the recommended standards for the description of new taxa (Logan *et al.*, 2009). The samples were immediately transferred to sterile serum bottles, tightly sealed with butyl rubber stoppers, kept at room temperature during transportation and then stored at 4 °C until further use.

Strain BZ-SZ-XJ18^T was isolated from a combined water and sediment sample using a 10-fold dilution-plating technique. The medium, modified as previously reported (Zhao & Chen, 2012), was prepared as follows (l^{-1}): 100 g NaCl, 0.12 g $MgSO_4{\cdot}7H_2O$, 0.061 g $CaCl_2{\cdot}2H_2O$, 4.2 g $NaHCO_3$, 0.85 g NH_4Cl, 0.48 g K_2HPO_4, 0.021 g $FeSO_4{\cdot}7H_2O$, 0.015 g nitrilotriacetic acid and 5 g yeast extract (Oxoid), and supplemented with 1 ml trace element solution (l^{-1}): 5 g $MnSO_4{\cdot}H_2O$, 1 g $CoCl_2{\cdot}6H_2O$, 1 g $ZnSO_4{\cdot}7H_2O$, 0.1 g $CuSO_4{\cdot}2H_2O$, 0.1 g $KAl(SO_4)_2{\cdot}12H_2O$, 0.1 g H_3BO_3, 0.1 g $Na_2MoO_4{\cdot}2H_2O$, 0.1 g pyridoxine hydrochloride and 0.05 g thiamine hydrochloride dihydrate. Subsequently, the pH was adjusted to pH 8.0 with 2 M HCl and 1.6 % agar was added to the medium. After autoclaving at 121 °C for 45 min, 0.2 % (w/v) filter-sterilized α-D-glucose was added to the liquid medium before pouring plates. The plates were incubated aerobically at 37 °C for up to 5 days. Representative colonies were then picked and repeatedly re-streaked on the same medium until a pure culture isolate, named BZ-SZ-XJ18^T, was obtained. Strain BZ-SZ-XJ18^T was maintained on slant tubes, subcultured every 3 weeks and stored at 4 °C for short-term preservation. Cryotubes were prepared using an equal volume of the liquid culture medium described above and glycerol (30 %, v/v) and stored at −80 °C for long-term preservation. *B. saliphilus* DSM 15402^T and *Bacillus chagannorensis* DSM 18086^T, obtained from German Collection of Microorganisms and Cell Cultures (DSMZ), '*Bacillus daqingensis*' CGMCC 1.12295, *Bacillus daliensis* CGMCC 1.10369^T and *Bacillus subtilis* subsp. *subtilis* CGMCC 1.3358^T, obtained from China General Microbiological Culture Collection Center (CGMCC), and *Bacillus luteus* KCTC 33100^T, obtained from Korean Collection for Type Cultures (KCTC), were used as reference strains in this study. These strains were grown in the liquid medium described by Zhilina *et al.* (2004) with the following composition (l^{-1}): 70 g NaCl, 20 g Na_2CO_3, 10 g glucose, 5 g yeast extract (Oxoid), 5 g peptone (Oxoid), 1 g KH_2PO_4 and 0.2 g $MgSO_4.7H_2O$. The pH was adjusted to pH 9.0 with 2 M HCl before autoclaving.

For 16S rRNA gene sequence analysis, genomic DNA of strain BZ-SZ-XJ18^T was extracted using a Microbial DNA isolation kit according to the manufacturer's instructions (New Industry). DNA concentrations were quantified with a Nanodrop 1000 spectrophotometer (Thermo Scientific), and DNA was examined for integrity by agarose gel electrophoresis. A nearly full-length 16S rRNA gene sequence (1530 high-quality nucleotide positions) was obtained by using universal primers 8f and 1492r as described previously (Zhao *et al.*, 2010) and sequencing the recombinant vector of PCR product cloned in pGEM-T easy vector (Promega). The 16S rRNA gene sequence was compared with available sequences and/or those of close relatives from the GenBank database (http://blast.ncbi.nlm.nih.gov/Blast.cgi) using the BLASTN program, from the Ribosomal Database Project (http://rdp.cme.msu.edu/index.jsp) using SEQMATCH searches, and from the EzTaxon-e server (http://www.ezbiocloud.net/eztaxon) (Kim *et al.*, 2012) using the global alignment algorithm of Myers & Miller (1988). 16S rRNA gene sequence analysis revealed that strain BZ-SZ-XJ18^T belonged to the genus *Bacillus* of the family *Bacillaceae*, and was most closely related to haloalkaliphile *B. saliphilus* 6AG^T (96.7 % similarity) (Romano *et al.*, 2005), haloalkaliphile '*B. daqingensis*' X10-1 (96.6 % similarity) (Wang *et al.*, 2014), alkaliphile *Bacillus luteus* JC167^T (96.5 % similarity) (Subhash *et al.*, 2014), alkaliphile *Bacillus daliensis* DLS13^T (96.2 %) (Zhai *et al.*, 2012), moderate halophile *Bacillus chagannorensis* CG-15^T (95.2 %) (Carrasco *et al.*, 2007), moderate halophile *B. polygoni* YN-1^T (95.0 %) (Aino *et al.*, 2008); lower similarities were observed with other recognized members of the genus *Bacillus*. Additionally, strain BZ-SZ-XJ18^T exhibited very low 16S rRNA gene sequence similarity of 90.3 % with the type species, *B. subtilis* subsp. *subtilis* DSM 10^T, of the genus *Bacillus*. The low sequence similarity between strain BZ-SZ-XJ18^T and its nearest neighbour, strain 6AG^T, indicated that the isolate was distinct from other type species of the genus *Bacillus* on the basis of the widely accepted criterion that strains with <97 % 16S rRNA gene sequence similarity between each other are considered as belonging to different species (Horner-Devine *et al.*, 2004; Stackebrandt & Goebel, 1994).

To delineate the phylogenetic position of strain BZ-SZ-XJ18^T within the genus *Bacillus*, 16S rRNA gene sequences were aligned using CLUSTAL X version 2.0 (Larkin *et al.*, 2007). Bootstrap consensus trees were inferred from 1000 replicates with the neighbour-joining (Saitou & Nei, 1987), minimum-evolution (Rzhetsky & Nei, 1992) and maximum-likelihood (Felsenstein, 1981) algorithms in MEGA version 6.0 (Tamura *et al.*, 2013). Evolutionary distances were calculated according to the algorithms of the Jukes–Cantor model (Jukes & Cantor, 1969). The trees generated using the three methods were in good agreement among each other (Fig. 1). The phylogeny of 16S rRNA gene sequences representatively showed that strain BZ-SZ-XJ18^T formed a distinct branch with the recognized type strains of species of the genus *Bacillus* (i.e. *B. saliphilus* 6AG^T, *B. daliensis* DLS13^T and *B. chagannorensis* CG-15^T) with high levels of bootstrap support (95 or 96 %), which suggested that the isolate should be placed in a novel species within the genus *Bacillus*.

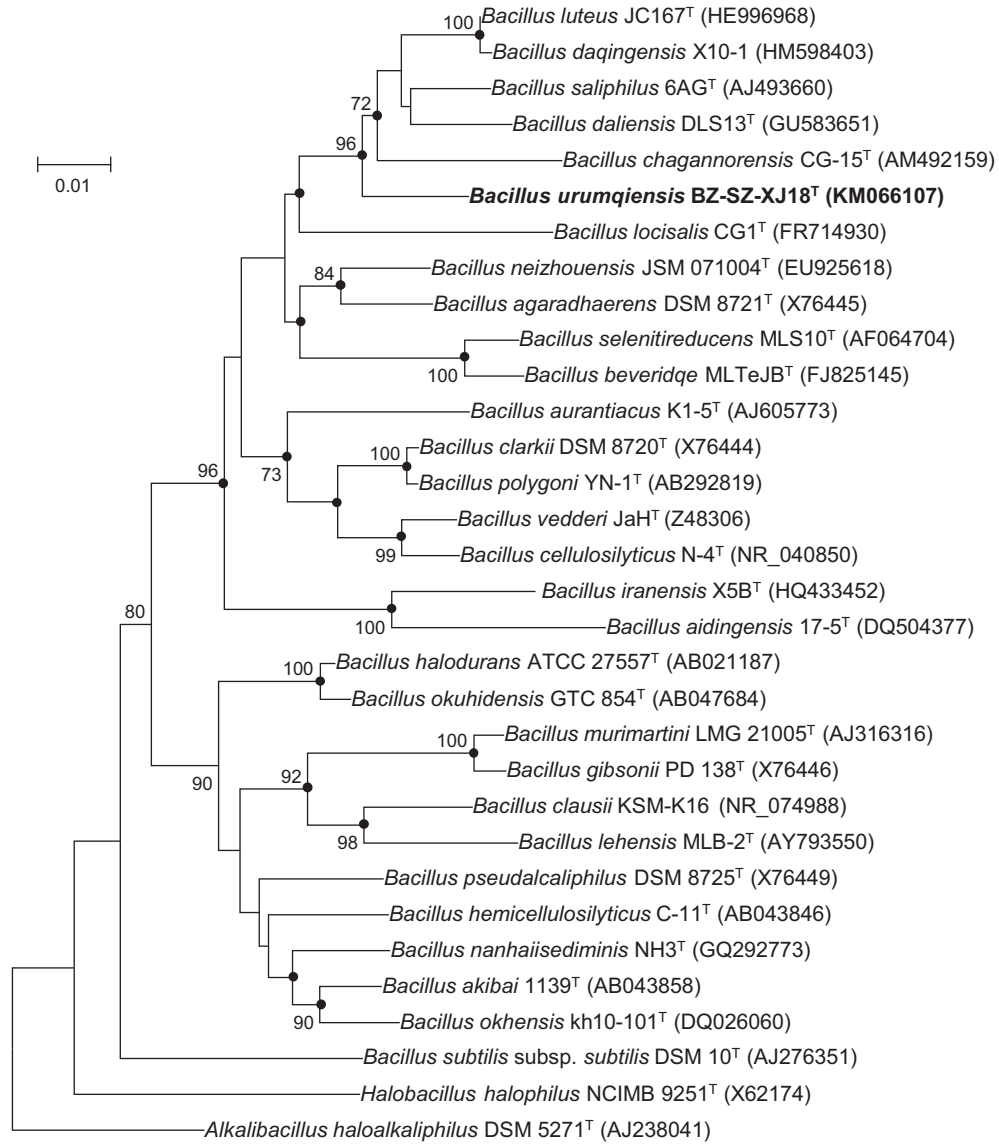

Fig. 1. Phylogenetic tree using the neighbour-joining method based on 16S rRNA gene sequences showing the relationship between strain BZ-SZ-XJ18^T and related species from the genus *Bacillus* and related genera. Sequence data of reference strains were obtained from the GenBank/EMBL and/or RDP databases. Accession numbers are indicated in parentheses. Bootstrap values (percentages) are based on 1000 replicates and are shown for branches with more than 70 % bootstrap support. Filled circles indicate generic branches that were also recovered when using the minimum-evolution and maximum-likelihood algorithms. Bar, 0.01 substitutions per nucleotide position.

DNA–DNA hybridization was carried out independently three times using the thermal denaturation and renaturation method (Zakrzewska-Czerwińska *et al.*, 1988) by China General Microbiological Culture Collection Identification Service. Strain BZ-SZ-XJ18^T exhibited levels of DNA–DNA relatedness of 27±2 % (mean±SD), 22±1 %, 19 ±2 %, 17±1 % and 12±1 % with *B. luteus* KCTC 33100^T, '*B. daqingensis*' CGMCC 1.12295, *B. saliphilus* DSM 15402^T, *B. daliensis* CGMCC 1.10369^T and *B. chagannorensis* DSM 18086^T, respectively, which are clearly below the 70 % threshold currently widely accepted as criterion for bacterial species delineation (Stackebrandt & Goebel, 1994). The G +C content of genomic DNA of strain BZ-SZ-XJ18^T was duplicately determined by the thermal denaturation method (T_m) (Gerhardt *et al.*, 1994) using DNA from *Escherichia coli* K-12 as a control and the HPLC method (Mesbah *et al.*, 1989; Mesbah & Whitman, 1989) by China General Microbiological Culture Collection Identification Service. The mol% DNA G+C content of the genomic DNA obtained was 42.3 (HPLC) or 41.4 (T_m), both of which were lower

than those of closely related members of the genus *Bacillus* (Table 1), and this value is within the range for the genus *Bacillus* (32–66 mol%) (Logan & De Vos, 2009).

Unless specified otherwise, the aforementioned medium for growth of strain BZ-SZ-XJ18^T in this study was used for the morphological, physiological and biochemical analyses. Cell morphology during the exponential growth phase was examined by scanning electron microscopy at 15 kV (FEI Quanta 200) and transmission electron microscopy at 80 kV (JEOL JEM-1400). Cells for the investigation of endospore formation were grown at 37 °C (or 40 °C) for up to 7 days on oligotrophic medium as follows (l^{-1}): 0.7 g yeast extract, 1.0 g peptone, 60 g (or 150 g, 200 g) NaCl, 0.2 g $(NH_4)_2SO_4$, 0.2 g $MgSO_4.7H_2O$, 1.0 g K_2HPO_4, 1.0 g glucose and 20 g agar, and the pH was adjusted to pH 8.5 with 2 M NaOH. Endospores were observed with a light microscope (LEICA DM750) under ×100 (oil immersion) objectives (NA 1.5150) according to the Schaeffer–Fulton staining method (Murray *et al.,* 1994) and negatively stained with 1.0 % (w/v) uranyl acetate for observation by transmission electron microscope at 80 kV (JEOL JEM-1400). Intracellular polyhydroxyalkanoate granule accumulation was examined using transmission electron microscopy (JEOL JEM-1400) and by the Sudan Black B staining method according to the literature (Burdon 1946; Lee & Choi, 2004). Exopolysaccharide was investigated by staining with Alcian Blue 8GX (Murray *et al.,* 1994). Cells were flexible rod-shaped, about 0.6–0.7 μm in width and from 1.2 to 2.1 μm in length (Fig. S1a, available in the online Supplementary Material). A single subpolar flagellum attached to cells was observed by negative staining with 1.0 % (w/v) uranyl acetate (Fig. S1b), and Gram-positive staining was confirmed by using the standard Gram reaction (Doetsch *et al.,* 1981). Endospore-formation was not observed under the oligotrophic conditions tested including heat resistance and salinity stress, which is inconsistent with an important feature of the genus *Bacillus*.

Growth at salinities (total Na^+) of 0.05–5.18 (0–30 %, w/v, at intervals of 2 % additional NaCl), 0.06 (0.05 %, w/v, additional NaCl), 0.14 (0.5 %), 0.19 (0.8 %), 0.22 (1 %) and 4.32 M (25 %) and at temperatures of 0–50 °C (at intervals of 5 °C), 8, 33, 37, 41 and 43 °C was examined. The pH range for growth was determined at 37 °C and 1.08 M Na^+ with 50 mM MES (pH 5.5–6.5), HEPES (pH 7.0–8.0), TAPS (pH 8.0–9.0), CHES (pH 9.0–10.0), and CAPS (pH 10.0–11.0) over a pH range from pH 5.5 to 11.0 with intervals of 0.5 pH units. All the above tests were investigated in triplicate in the isolation medium above by the measurement of maximum OD_{600} using a portable spectrophotometer (HACH DR 2800). The salinity range for growth was 0.22–4.32 M Na^+ (corresponding to 1.3–25.3 %, w/v, NaCl) with an optimum of 1.08 M Na^+ (6.3 %, w/v, NaCl) at pH 8.5 and 37 °C. No growth was found at ≥4.84 M Na^+. Growth occurred at pH 6.5–10.0 with a broad optimum pH 8.5–9.5 at 1.08 M Na^+ and 37 °C, but not at pH equal to or lower than pH 6.0 or at pH equal to or high than pH 10.5. The temperature range for growth was 8–41 °C with optimum 37 °C at 1.08 M Na^+ and pH 8.5, but not at ≤5 °C or ≥43 °C. The need for Na^+ was tested in liquid medium where NaCl and $NaHCO_3$ were replaced with equimolar concentrations of KCl and $KHCO_3$ in the absence of yeast extract. Strain BZ-SZ-XJ18^T was obligately dependent on Na^+ as no growth occurred in the absence of Na^+. Anaerobic growth was carried out in Hungate tubes containing 5 ml anaerobic broth supplemented with 0.2 % yeast extract as an electron donor and sodium thiosulfate (20 mM), sodium nitrate (20 mM), sodium nitrite (5 mM), MnO_2 (10 mM) or fumarate (20 mM) as a potential electron acceptor. The isolate was strictly aerobic as demonstrated by no growth after incubation for 7 days under the aforementioned conditions.

For the utilization of sole carbon and energy sources, cultures of various organic substrates (0.5 %, w/v) were incubated in 5 ml broth containing 6 % NaCl (pH 8.5) for up to 72 h at 37 °C and growth was recorded after the third successive transfer. When an amino acid as substrate was being investigated, the medium was prepared without $(NH_4)_2SO_4$. To assess acid production from sugars (1.0 %, w/v), 15 μl bromocresol purple (1.0 %, w/v) was added to a final concentration of 0.03 gl^{-1}. Tests for hydrolysis of casein, gelatin, hippurate, starch, tyrosine, Tween 20 and Tween 80, production of indole and H_2S, and phenylalanine deamination were carried out as described by Mata *et al.* (2002). Nitrate and nitrite reduction, and the methyl red and Voges–Proskauer tests were carried out as described by Lányí (1987). Catalase activity was tested by bubble production in a 3 % (v/v) hydrogen peroxide solution and oxidase activity was determined by oxidation of 1.0 % *p*-aminodimethylaniline oxalate. Presence of arginine, lysine and ornithine decarboxylases was determined by the observation of a colour change of bromocresol purple to purple after 24 h of incubation (Cowan *et al.*, 1993). Antibiotic sensistivity was examined by spreading culture suspension on solid medium agar plates (1.6 %) and using sensi discs (Hangzhou Microbial Reagent) as described by Mata *et al.* (2002) with incubation for 5 days. Large zones of inhibition, i.e. >8 mm including 6 mm diameter of the sensi discs, indicated that the micro-organism was susceptible while small or no zones of inhibition indicated resistance. Strain BZ-SZ-XJ18^T was sensitive to the following antimicrobial agents (μg per disc unless otherwise stated): cefalotin (30), cefoxitin (30), chloramphenicol (30), clarithromycin (15), clindamycin (2), erythromycin (15), polymyxin B (300 U), rifampicin (30), sulfamethoxazole (300), and vancomycin (30), and resistant to amoxicillin (10), ampicillin (50), carbenicillin (100), cefotaxime (30), gentamicin (10), kanamycin (50), nalidixic acid (30), neomycin(30), penicillin (10 IU), streptomycin (300) and tetracycline (30). Other detailed results of morphological features, nutritional and physiological characteristics, and biochemical tests are shown in the species description. Several differential phenotypic features are shown in Table 1 between strain BZ-SZ-XJ18^T and six closely related species, including the type species *B. subtilis* subsp. *subtilis*, in the genus *Bacillus*, including

Table 1. Differential phenotypic characteristics that distinguish strain BZ-SZ-XJ18^T from other closely related species of the genus *Bacillus*

Strains: 1, *B. urumqiensis* sp. nov. BZ-SZ-XJ18^T; 2, *B. saliphilus* DSM 15402^T (data from Romano *et al.*, 2005); 3, '*B. daqingensis*' CGMCC 1.12295 (Wang *et al.*, 2014); 4, *B. luteus* KCTC 33100^T (Subhash *et al.*, 2014); 5, *B. daliensis* CGMCC 1.10369^T (Zhai *et al.*, 2012); 6, *B. chagannorensis* DSM 18086^T (Carrasco *et al.*, 2007); 7, *B. subtilis* subsp. *subtilis* CGMCC 1.3358^T (Subhash *et al.*, 2014). +, Positive; −, negative; W, weakly positive. All strains are positive for Gram-staining and catalase activity, but negative for hydrolysis of Tween 20 and the methyl red test.

Characteristic	1	2	3	4	5	6	7
Cell morphology	Rods	Cocci	Rods	Small rods	Rods	Rods	Rods
Colony colour	Yellow	Yellow	Yellow	Orange	Yellow	Yellow–orange	White to cream
O_2 requirement	Strictly aerobic	Strictly aerobic	Strictly aerobic	Strictly aerobic	Facultively anaerobic	Facultively anaerobic	Strictly aerobic
Endospore formation	−	−	−	+	+	+	+
Motility	+	−	−	−	+	+	+
NaCl concentration for growth (%,w/v)							
Range	1.3–25.3	1–20	0–16	0–6	0–8	3–20	0–5
Optimum	6.3	15	3	0–3	2	7	0
pH for growth							
Range	6.5–10.0	7.0–10.0	7.5–11.0	6.8–9.8	7.5–11.0	6.0–11.0	6.0–8.5
Optimum	8.5–9.5	9	10.0	8.0–9.0	9	8.5	7.0–8.5
Temperature for growth (°C)							
Range	8-41	10–50	10–50	10–45	10–45	10–40	10–47
Optimum	37	37	35	25–37	30	37	37–40
Oxidase activity	+	+	+	+	+	−	+
Hydrolysis of:							
Casein	+	−	+	−	+	−	+
Gelatin	+	+	+	+	−	−	−
Starch	+	−	+	+	−	−	+
Tween 80	−	−	−	+	−	−	−
Acid production from:							
Cellobiose	+	−	+	−	+	+	−
D-Fructose	+	+	+	−	+	+	+
D-Galactose	−	+	−	+	+	+	+
D-Mannose	+	+	+	−	+	+	−
Indole production	−	+	−	−	+	−	+
H_2S production	−	+	−	−	+	−	+
Nitrate reduction	−	−	−	−	−	+	+
Phenylalanine deamination	+	+	+	−	−	+	−
Voges–Proskauer reaction	+	+	+	+	+	−	+
Quinone composition	MK-7	MK-7, DMK-7	MK-7	MK-7	MK-7	MK-7	MK-7
DNA G+C content (mol%)	41.7	48.8	47.7	53.4	43.9	53.8	42.8
Sampling site	Salt lake	Algal mat	Saline-alkaline soil	Agricultural soil	Soda lake	Soda lake	Soil

*Data obtained in this study are shaded in grey except that those data are from the relevant references indicated in parentheses.

those concerning endospore-formation, motility, physiological features, oxidase activity, hydrolysis of gelatin, H_2S production, phenylalanine deamination, Voges–Proskauer reaction and acid production.

For cellular fatty acid analysis, the bacterial biomass was harvested after incubation for 48 h at 37 °C in liquid medium (pH 9.0) as descibed in a previous study (Zhilina *et al.,* 2004). Total fatty acids were prepared and analysed by GC/MS according to the instructions of the Microbial

Identification System (MIDI). Peak areas were integrated automatically and fatty acid names and percentages were determined using the Microbial Identification standard software package (Sasser, 1990). The fatty acids making up more than 5 % of the total were anteiso-$C_{15:0}$ (43.0 %), $C_{16:0}$ (18.1 %), iso-$C_{15:0}$ (11.3 %), anteiso-$C_{17:0}$ (8.0 %) and iso-$C_{16:0}$ (7.0 %). The minor fatty acids included iso-$C_{14:0}$ (4.1 %), $C_{14:0}$ (2.6 %), $C_{18:0}$ (2.2 %) and iso-$C_{17:0}$ (2.4 %). The major fatty acids of strain BZ-SZ-XJ18^T were saturated straight-chain ($C_{16:0}$) and saturated iso- and anteiso-branched-chain fatty acids (iso-$C_{15:0}$, iso-$C_{16:0}$, anteiso-$C_{15:0}$ and anteiso-$C_{17:0}$) similar to the fatty acid composition of the related type strains of the genus *Bacillus* (Table S1). No hydroxy fatty acidwas detected from all strains tested. The relative concentration of straight-chain $C_{16:0}$ in strain BZ-SZ-XJ18^T was significantly approximately twofold higher than that of the related taxa except for strain CG-15^T whereas the level of branched-chain $C_{16:0}$ was lower by 1.7–2.0-fold than that of the type strains that were compared. In brief, although the fatty acid profiles determined were similar, the proportions of fatty acids of strain BZ-SZ-XJ18^T clearly differentiated it from its closest phylogenetic relatives.

In order to complete the chemotaxonomic characterization of strain BZ-SZ-XJ18^T, analyses of the polar lipids, respiratory quinones and cell-wall peptidoglycan were performed by the Identification Service, Leibniz-Institut DSMZ-Deutsche Sammlung von Microorganismen und Zellkulturen (Braunschweig, Germany). The methods for analysis followed the protocols online (https://www.dsmz.de/services/services-microorganisms/identification.html) and previous studies (Schuman 2011; Tindall 1990a, 1990b; Tindall *et al.*, 2007). The predominant lipids of strain BZ-SZ-XJ18^T (Fig. S2) were diphosphatidylglycerol and phosphatidylglycerol, in accordance with the lipid composition of *B. saliphilus* 6AG^T, '*B. daqingensis*' X10-1, *B. luteus* JC167^T, *B. daliensis* DLS13^T, *B. chagannorensis* CG-15^T and the type species, *B. subtilis* (Kosowski *et al.*, 2014; Subhash *et al.*, 2014; Wang *et al.*, 2014; Zhai *et al.*, 2012). Phosphatidylethanolamine was detected as minor amounts of polar lipid in strain BZ-SZ-XJ18^T; however, it was abundant in the closely related taxa. Additionally, moderate to minor amounts of sulfoquinovosyldiacylglycerol and two unidentified phospholipids from strain BZ-SZ-XJ18^T were also found whereas these were missing in the related type strains compared. The main respiratory quinone was menaquinone-7 (MK-7), which is in agreement with the most closely related species of the genus *Bacillus* yielding MK-7 as the major compound. The cell-wall peptidoglycan was of the A1$_\gamma$ type, containing *meso*-diaminopimelic acid as the diagnostic diamino acid. The major polar lipids, the main respiratory quinone and the peptidoglycan type of the cell wall of strain BZ-SZ-XJ18^T were typical of those observed in members of the genus *Bacillus* (Carrasco *et al.*, 2007; Subhash *et al.*, 2014; Zhai *et al.*, 2012).

On the basis of morphological, physiological and chemotaxonomic properties, 16S rRNA gene sequence analysis, together with DNA–DNA relatedness, strain BZ-SZ-XJ18^T is a representative of a novel specie in the genus *Bacillus*, for which the name *B. urumqiensis* sp. nov. is proposed.

Description of *Bacillus urumqiensis* sp. nov.

Bacillus urumqiensis (u.rum.qi.en′sis. N.L. masc. adj. *urumqiensis* pertaining to Urumqi, in Xinjiang Uyghur Autonomous Region of China, where the species was isolated).

Cells are Gram-stain-positive, strictly aerobic, motile and rod-shaped (0.6–0.7×1.2–2.1 μm). Endospores are not observed under the aforementioned conditions. Colonies are yellow-pigmented, opaque, convex and circular but have untidy edges and are approximately 1–2 mm in diameter after incubation at 37 °C for 2–3 days on plates containing the modified solid medium. Growth occurs at the total Na^+ concentration of 0.22–4.32 M (equivalent to 1.3–25.3 % NaCl) with an optimum at 1.08 M Na^+ (equivalent to 6.3 % NaCl), at pH 6.5–10.0 with an optimum at pH 8.5–9.5, at temperatures of 8–41 °C with an optimum of 37 °C. Does not yield growth without NaCl or with sodium thiosulfate, sodium nitrate, sodium nitrite, MnO_2 or fumarate as an electron acceptor under anaerobic conditions. Does not accumulate polyhydroxyalkanoate granules or produce exopolysaccharide. Positive for the hydrolysis of gelatin, casein and starch, phenylalanine deamination, Voges–Proskauer test, catalase, oxidase, lysine decarboxylase and ornithine decarboxylase, but negative for the hydrolysis of hippurate, Tween 20 and Tween 80, nitrate and nitrite reduction, indole production, H_2S production, tyrosine decomposition, methyl red test and arginine decarboxylase. As sole carbon and energy sources, utilizes D-ribose, D-xylose, D-fructose, D-fucose, D-galactose, α-D-glucose, D-mannose, L-rhamnose, D-cellobiose, α-lactose, D-maltose, sucrose, D-melezitose and D-raffinose; does not utilize L-arabinose. As sole carbon, nitrogen and energy sources, utilizes L-alanine, L-arginine, L-histidine, L-serine, D-serine, L-aspartic acid, L-glutamic acid, glucuronamide, γ-aminobutyric acid, glycyl-L-proline and L-pyroglutamic acid, but does not utilize *N*-acetyl-D-galactosamine, *N*-acetyl-D-glucosamine, or *N*-acetyl-β-D-mannosamine. Acid is produced from D-ribose, D-xylose, D-fructose, D-fucose, α-D-glucose, D-mannose, D-cellobiose, α-D-lactose, D-maltose, sucrose, D-melezitose and D-raffinose, but not from L-arabinose, D-galactose or L-rhamnose. The fatty acid profile includes anteiso-$C_{15:0}$, $C_{16:0}$, iso-$C_{15:0}$, anteiso-$C_{17:0}$ and iso-$C_{16:0}$ as the major fatty acids (>5 %), followed by iso-$C_{14:0}$, $C_{14:0}$, iso-$C_{17:0}$ and $C_{18:0}$. The predominant polar lipids are diphosphatidylglycerol and phosphatidylglycerol. The main respiratory quinone is menaquinone-7 (MK-7) and the peptidoglycan type is A1$_\gamma$, with *meso*-diaminopimelic acid as the diagnostic diamino acid.

The type strain is BZ-SZ-XJ18^T (=DSM 29145^T=JCM 30195^T), isolated from a mixture of water and sediment of a saline-alkaline lake close to Urumqi city, Xinjiang Uyghur

Autonomous Region of China. The DNA G+C content of the genomic DNA of the type strain is 42.3 mol% (HPLC) or 41.4 mol% (T_m).

Acknowledgements

This work was supported by the National Science Foundation of China (NSFC), grants 31570110 and 31370158, and the Foundation of Graduate School of Chinese Academy of Agricultural Sciences (CAAS), grant 0042014011.

References

Subhash, Y., Sasikala, C. & Ramana, C. (2014). *Bacillus luteus* sp. nov., isolated from soil. *Int J Syst Evol Microbiol* **64**, 1580–1586.

Aino, K., Hirota, K., Matsuno, T., Morita, N., Nodasaka, Y., Fujiwara, T., Matsuyama, H., Yoshimune, K. & Yumoto, I. (2008). *Bacillus polygoni* sp. nov., a moderately halophilic, non-motile obligate alkaliphile isolated from indigo balls. *Int J Syst Evol Microbiol* **58**, 120–124.

Amoozegar, M. A., Didari, M., Bagheri, M., Fazeli, S. A., Schumann, P., Spröer, C., Sánchez-Porro, C. & Ventosa, A. (2013). *Bacillus salsus* sp. nov., a halophilic bacterium from a hypersaline lake. *Int J Syst Evol Microbiol* **63**, 3324–3329.

Bagheri, M., Didari, M., Amoozegar, M. A., Schumann, P., Sánchez-Porro, C., Mehrshad, M. & Ventosa, A. (2012). *Bacillus iranensis* sp. nov., a moderate halophile from a hypersaline lake. *Int J Syst Evol Microbiol* **62**, 811–816.

Burdon, K. L. (1946). Fatty material in bacteria and fungi revealed by staining dried, fixed slide preparations. *J Bacteriol* **52**, 665–678.

Carrasco, I. J., Márquez, M. C., Xue, Y., Ma, Y., Cowan, D. A., Jones, B. E., Grant, W. D. & Ventosa, A. (2007). *Bacillus chagannorensis* sp. nov., a moderate halophile from a soda lake in inner mongolia, china. *Int J Syst Evol Microbiol* **57**, 2084–2088.

Cohn, F. (1872). Untersuchungen über bakterien. *Beitr Biol Pflanz* **1**, 127–244.

Cowan, S. & Steel, K. (1993). *Manual for the Identification of Medical Bacteria*, 3rd edn. Edited and revised by G. I. Barrow & R. K. A. Feltham. London: Cambridge University Press.

Doetsch, R. N., Murray, R. G. E., Costilow, R. N., Nester, E. W., Wood, W. A., Krieg, N. R. & Phillips, G. H. (1981). Determinative methods of light microscopy. In *Manual of Methods for General Bacteriology*. pp. 21–33. Edited by P. Gerhardt. Washington: American Society for Microbiology.

Felsenstein, J. (1981). Evolutionary trees from DNA sequences: A maximum likelihood approach. *J Mol Evol* **17**, 368–376.

Gerhardt, P., Murray, R. G. E., Wood, W. A. & Krieg, N. R. (1994). *Methods for General and Molecular Bacteriology*. Washington, DC: American Society for Microbiology.

Horner-Devine, M. C., Lage, M., Hughes, J. B. & Bohannan, B. J. M. (2004). A taxa–area relationship for bacteria. *Nature* **432**, 750–753.

Jukes, T. H. & Cantor, C. R. (1969). Evolution of protein molecules. In *Mammalian Protein Metabolism*, vol. 3, pp. 21–132. Edited by H. N. Munro. New York: Academic Press.

Kim, O. S., Cho, Y. J., Lee, K., Yoon, S. H., Kim, M., Na, H., Park, S. C., Jeon, Y. S., Lee, J. H. & other authors (2012). Introducing EzTaxon-e: a prokaryotic 16S rRNA gene sequence database with phylotypes that represent uncultured species. *Int J Syst Evol Microbiol* **62**, 716–772.

Kosowski, K., Schmidt, M., Pukall, R., Hause, G., Kämpfer, P. & Lechner, U. (2014). *Bacillus pervagus* sp. nov. and *Bacillus andreesenii* sp. nov., isolated from a composting reactor. *Int J Syst Evol Microbiol* **64**, 88–94.

Larkin, M. A., Blackshields, G., Brown, N. P., Chenna, R., McGettigan, P. A., McWilliam, H., Valentin, F., Wallace, I. M., Wilm, A. & other authors (2007). CLUSTAL W and CLUSTAL_X version 2. *Bioinformatics*. **23**, 2947–2948.

Lee, S. Y. & Choi, J. I. (2004). Polyhydroxyalkanoates: biodegradeable polymer. In *Manual of Indusrial Microbiology and Biotechnology*, 2nd edn, pp. 616–624. Edited by A. L. Demain, J. E. Davies, R. M. Atlas, G. Cohen, C. L. Hershberger, W. S. Hu, D. H. Sherman, R. C. Willson & J. H. David. Washington: American Society of Microbiology.

Lim, J. M., Jeon, C. O. & Kim, C. J. (2006). Bacillus taeanensis sp. nov., a halophilic gram-positive bacterium from a solar saltern in korea. *Int J Syst Evol Microbiol* **56**, 2903–2908.

Logan, N. A., Popovitch, T. J. & Hoffmaster, A. (2007). Bacillus and related genera. In *Manual of Clinical Microbiology*, 9th edn, vol. 1, pp. 445–460. Edited by P. R. Murray, E. J. Baron, J. H. Jorgensen, M. A. Pfaller & R. H. Yolken. Washington, DC: American Society for Microbiology.

Logan, N. A., Berge, O., Bishop, A. H., Busse, H. J., De Vos, P., Fritze, D., Heyndrickx, M., Kämpfer, P., Rabinovitch, L. & other authors (2009). Proposed minimal standards for describing new taxa of aerobic, endospore-forming bacteria. *Int J Syst Evol Microbiol* **59**, 2114–2121.

Logan, N. A. & De Vos, P. (2009). Genus Bacillus Cohn 1872. In *Bergey's Manual of Systematic Bacteriology*, 2nd edn, vol. 3, pp. 21–218. Edited by P. De Vos, G. Garrity, D. Jones, N. R. Krieg, W. Ludwig, F. A. Rainey, K.-H. Schleifer & W. B. Whitman. New York: Springer-Verlag.

Lányí, B. (1987). Classical and rapid identification methods for medically important bacteria. *Methods Microbiol* **19**, 1–67.

Mata, J. A., Martínez-Cánovas, J., Quesada, E. & Béjar, V. (2002). A detailed phenotypic characterisation of the type strains of halomonas species. *Syst Appl Microbiol* **25**, 360–375.

Mehrshad, M., Amoozegar, M. A., Didari, M., Bagheri, M., Fazeli, S. A., Schumann, P., Spröer, C., Sánchez-Porro, C. & Ventosa, A. (2013). *Bacillus halosaccharovorans* sp. nov., a moderately halophilic bacterium from a hypersaline lake. *Int J Syst Evol Microbiol* **63**, 2776–2781.

Mesbah, M, Premachandran, U. & Whitman, W. B. (1989). Precise measurement of the G+C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int J Syst Bacteriol* **39**, 159–167.

Mesbah, M. & Whitman, W. B. (1989). Measurement of deoxyguanosine/thymidine ratios in complex mixtures by high-performance liquid chromatography for determination of the mole percentage guanine + cytosine of DNA. *J Chromatogr A* **479**, 297–306.

Murray, R. G. E., Doetsch, R. N. & Robinow, C. F. (1994). Determinative and cytological light microscopy. In *Methods for General and Molecular Bacteriology*, 2nd edn, pp. 21–41. Edited by P. Gerhardt, R. G. E. Murray, W. A. Wood & N. R. Krieg. Washington, DC, USA: American Society for Microbiology.

Myers, E. W. & Miller, W. (1988). Optimal alignments in linear space. *Comput Appl Biosci* **4**, 11–17.

Márquez, M. C., Carrasco, I. J., de la Haba, R. R., Jones, B. E., Grant, W. D. & Ventosa, A. (2011). *Bacillus locisalis* sp. nov., a new haloalkaliphilic species from hypersaline and alkaline lakes of china, kenya and tanzania. *Syst Appl Microbiol* **34**, 424–428.

Romano, I., Lama, L., Nicolaus, B., Gambacorta, A. & Giordano, A. (2005). *Bacillus saliphilus* sp. nov., isolated from a mineral pool in campania, italy. *Int J Syst Evol Microbiol* **55**, 159–163.

Rzhetsky, A. & Nei, M. (1992). A simple method for estimating and testing minimum-evolution trees. *Mol Biol Evol* **9**, 945–967.

Saitou, N. & Nei, M. (1987). The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.

Sasser, M. (1990). Identification of bacteria by gas chromatography of cellular fatty acids. *MIDI Technical Note No. 101, MIDI*. NEWARK: DE. May (Revised Feb 1997).

Schumann, P. (2011). Peptidoglycan structure. *Methods Microbiol* **38**, 101–129.

Stackebrandt, E. & Goebel, B. M. (1994). Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int J Syst Bacteriol* **44**, 846–849.

Sylvan, J. B., Hoffman, C. L., Momper, L. M., Toner, B. M., Amend, J. P. & Edwards, K. J. (2015). *Bacillus rigiliprofundi* sp. nov., an endospore-forming, mn-oxidizing, moderately halophilic bacterium isolated from deep subseafloor basaltic crust. *Int J Syst Evol Microbiol* **65**, 1992–1998.

Tamura, K., Stecher, G., Peterson, D., Filipski, A. & Kumar, S. (2013). MEGA6: Molecular evolutionary genetics analysis version 6.0. *Mol Biol Evol* **30**, 2725–2729.

Tindall, B. J. (1990a). A comparative study of the lipid composition of halobacterium saccharovorum from various sources. *Syst Appl Microbiol* **13**, 128–130.

Tindall, B. J. (1990b). Lipid composition of *Halobacterium lacusprofundi*. *FEMS Microbiol Letts* **66**, 199–202.

Tindall, B. J., Smibert, R. A., Sikorski, J. & Krieg, N. R. (2007). Phenotypic characterization and the principles of comparative systematics. In *Methods for General and Molecular Microbiology*, 3rd edn, vol. 1, pp. 330–393. Edited by C. A. Reddy, T. J. Beveridge, J. A. Breznak, G. Marzluf, T. M. Schmidt & L. R. Snyder. Washington DC, USA: ASM Press.

Wang, S., Sun, L., Wei, D., Zhou, B., Zhang, J., Gu, X., Zhang, L., Liu, Y., Li, Y. & other authors (2014). *Bacillus daqingensis* sp. nov., a halophilic, alkaliphilic bacterium isolated from saline-sodic soil in Daqing, China. *J Microbiol* **52**, 548–553.

Zakrzewska-Czerwinska, J., Mordarski, M. & Goodfellow, M. (1988). DNA base composition and homology values in the classification of some rhodococcus species. *J Gen Microbiol* **134**, 2807–2813.

Zhai, L., Liao, T., Xue, Y. & Ma, Y. (2012). Bacillus daliensis sp. nov., an alkaliphilic, gram-positive bacterium isolated from a soda lake. *Int J Syst Evol Microbiol* **62**, 949–953.

Zhao, B., Wang, H., Li, R. & Mao, X. (2010). Thalassospira xianhensis sp. nov., a polycyclic aromatic hydrocarbon-degrading marine bacterium. *Int J Syst Evol Microbiol* **60**, 1125–1129.

Zhao, B. & Chen, S. (2012). *Alkalitalea saponilacus* gen. nov., sp. nov., an obligately anaerobic, alkaliphilic, xylanolytic bacterium from a meromictic soda lake. *Int J Syst Evol Microbiol* **62**, 2618–2623.

Zhilina, T. N., Appel, R., Probian, C., Brossa, E. L., Harder, J., Widdel, F. & Zavarzin, G. A. (2004). *Alkaliflexus imshenetskii* gen. nov. sp. nov., a new alkaliphilic gliding carbohydrate-fermenting bacterium with propionate formation from a soda lake. *Arch Microbiol* **182**, 244–253.

Zhu, D., Tanabe, S. H., Xie, C., Honda, D., Sun, J. & Ai, L. (2014). Bacillus ligniniphilus sp. nov., an alkaliphilic and halotolerant bacterium isolated from sediments of the South China Sea. *Int J Syst Evol Microbiol* **64**, 1712–1717.