Reclassification of *Bacillus saliphilus* as *Alkalicoccus saliphilus* gen. nov., comb. nov., and description of *Alkalicoccus halolimnae* sp. nov., a moderately halophilic bacterium isolated from a salt lake

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**Abstract**

A Gram-stain-positive, coccoid-shaped, non-spore-forming and moderately halophilic bacterium, designed BZ-SZ-XJ29T, was isolated from a salt lake of China. On the basis of 16S rRNA gene sequence similarity, the closest phylogenetic relatives were *Bacillus saliphilus* 6AGT (97.3% 16S rRNA gene sequence similarity) and five other species of the genus *Bacillus* (95.4–96.3%). However, strain BZ-SZ-XJ29T shared only 89.5% 16S rRNA gene sequence similarity with *Bacillus subtilis* subsp. *subtilis* DSM 10T, indicating that this isolate might not be a member of the genus *Bacillus*. The genomic DNA G+C content was 40.0 mol% (Tm). The DNA–DNA relatedness value with *B. saliphilus* 6AGT was 45±2%. Strain BZ-SZ-XJ29T formed yellow pigment and grew in the presence of 0.74–4.15 M NaCl at pH 6.0–10.5 (optimum pH 7.5), and at 5–41 °C (optimum 33 °C). The predominant (≥10%) fatty acids were anteiso-C<sub>15:0</sub> and anteiso-C<sub>17:0</sub>. The dominant polar lipids consisted of diphosphatidylglycerol and the respiratory quinone was menaquinone-7 (MK-7). The peptidoglycan type of the cell wall was A1y, based on meso-diaminopimelic acid as the diagnostic diamino acid. On the basis of the combined phylogenetic data, phenotypic features and chemotaxonomic properties, it is proposed that *B. saliphilus* and strain BZ-SZ-XJ29T should be assigned to a single novel genus as two separate species, *Bacillus saliphilus* is reclassified in a new genus, *Alkalicoccus* gen. nov., as *Alkalicoccus saliphilus* comb. nov., and is the type species of the new genus; the type strain of the type species is 6AGT (=DSM 15402T=ATCC BAA-957T). Strain BZ-SZ-XJ29T (=DSM 29191T=JCM 30193T=CGMCC 1.12936T) is placed in the genus *Alkalicoccus* as a novel species, *Alkalicoccus halolimnae* sp. nov.

Moderately halophilic bacteria, where optimal growth occurs in media containing 0.5–2.5 mol l<sup>−1</sup> salts [equivalent to 3–15% (w/v) NaCl], are widely distributed in saline and/or alkaline environments [1]. These organisms are represented by heterogeneous physiological and taxonomic groups of both Gram-positive- and Gram-negative-staining organisms. They have a significant biotechnological potential for the production of compatible solutes, e.g. ectoine and glycine betaine, and may provide novel enzymes to meet rapidly growing industrial demands [1, 2]. Several Gram-stain-positive, coccoid-shaped, moderately halophilic taxa have been described, e.g. some members of the genera *Jeotgalicoccus* [3], *Marinococcus* [4], *Nesterenkonia* [5], *Planococcus* [6], *Salmicrobium* [7], *Salinicoccus* [8], *Serinicoccus* [9], *Sinococcus* [10] and *Tetragenococcus* [11]. In this study, we also report the isolation and characterization of a moderately halophilic Gram-stain-positive coccus, designed strain BZ-SZ-XJ29T, which was obtained from a mixture of water and sediment of a salt (−8.8 % NaCl, pH 8.3) 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. The investigation of endospore strain was isolated and characterized using the recommended standards for the description of novel aerobic, endospore-forming bacteria [12]. The collected 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-XJ29T was isolated from a combined water and sediment sample using 10-fold dilution-plating technique. The medium was modified as in a previous study [13] and was prepared with the following components (l<sup>−1</sup>):

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain BZ-SZ-XJ29T is KX618877.

One supplementary table and two supplementary figures are available with the online Supplementary Material.
100 g NaCl, 0.12 g MgSO₄·7H₂O, 0.061 g CaCl₂·2H₂O, 4.2 g NaHCO₃, 0.85 g NH₄Cl, 0.48 g K₂HPO₄, 0.021 g FeSO₄·7H₂O and 5 g yeast extract (Oxoid), and supplemented with 1 ml trace element solution (1⁻¹): 5 g MnSO₄·H₂O, 1 g CoCl₂·6H₂O, 1 g ZnSO₄·7H₂O, 0.1 g CuSO₄·2H₂O, 0.1 g KAl(SO₄)₂·12H₂O, 0.1 g H₂BO₃, 0.1 g Na₂MoO₄·2H₂O, 0.1 g pyridoxineHCl and 0.05 g thiamine-HCl·2H₂O. The pH was adjusted to 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 aerobically incubated at 37 °C for up to 5 days. Representative colonies were picked and repeatedly re-streaked on the same plate until a pure culture isolate, designated BZ-SZ-XJ29ᵀ, was obtained. Strain BZ-SZ-XJ29ᵀ was maintained on slant tubes every 3 weeks and stored at 4 °C for short-term preservation. Cryotubes were prepared using an equal volume of the liquid culture medium as described above and glycerol (30 %, v/v), and stored at –80 °C for long-term preservation. *Bacillus saliphilus* DSM 15402ᵀ (=6AGᵀ) and *Bacillus chagnonensis* DSM 18086ᵀ (=CG-15ᵀ), obtained from German Collection of Microorganisms and Cell Cultures (DSMZ), *Bacillus daliensis* CGMCC 1.10369ᵀ (=DLS13ᵀ), ‘Bacillus daqingensis’ CGMCC 1.12295ᵀ (=X10-1-1) and *B. subtilis* subsp. subtilis CGMCC 1.3358ᵀ (=DSM 10ᵀ) obtained from China General Microbiological Culture Collection Center (CGMCC), *Bacillus luteus* KCTC 33100ᵀ (=JC167ᵀ), obtained from Korean Collection for Type Cultures (KCTC), and *Bacillus urumqiensis* BZ-SZ-XJ18ᵀ obtained from this laboratory, were used as reference strains in this study.

For 16S rRNA gene sequence analysis, genomic DNA of strain BZ-SZ-XJ29ᵀ 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 examined for integrity by agarose gel electrophoresis. A nearly full-length 16S rRNA gene sequence (1459 nt) was obtained using universal primers 8f and 1492r as described previously [14], and subsequently determined by sequencing the recombinant vector of PCR production and T·easy. The 16S rRNA gene sequence was compared with the available sequences and/or its close relatives from the *GenBank* database using the *BLAST* program (http://blast.ncbi.nlm.nih.gov/Blast.cgi) [15], SEQMATCH searches in the Ribosomal Database Project (http://rdp.cme.msu.edu/index.jsp) [16], and the Eztaxon-e server (www.ezbiocloud.net/eztaxon) [17]. 16S rRNA gene sequence analysis clearly revealed that strain BZ-SZ-XJ29ᵀ belonged to the order *Bacillales* of the phylum *Firmicutes*, and was most closely related to haloalkaliphile *Bacillus saliphilus* 6AGᵀ (97.3 % similarity) [18], alkaliphile *Bacillus daliensis* DLS13ᵀ (96.3 %) [19], alkaliphile *Bacillus luteus* JC167ᵀ (96.1 %) [20], haloalkaliphile ‘*Bacillus daqingensis*’ X10-1 (96.1 %) [21], moderate haloalkaliphile *Bacillus urumqiensis* BZ-SZ-XJ18ᵀ (95.8 %) [22] and moderate halophile *Bacillus chagnonensis* CG-15ᵀ (95.4 %) [23], and lower 16S rRNA gene sequence similarities were observed with other recognized members of the genus *Bacillus*. However, it exhibited a very low 16S rRNA gene sequence similarity of 89.5 % with the type species of the genus *Bacillus*, *Bacillus subtilis* subsp. *subtilis* DSM 10ᵀ, which is lower than with the representative species of the genera *Salisediminibacterium* (93.1 %), *Alterbacillus* (approximately 92.1 %) and *Halobacillus* (approximately 91.5 %). These values are clearly lower than the 98.65 % 16S rRNA gene sequence threshold for differentiating two bacterial species, recommended by Stackebrandt and Ebers [24] and recently reinforced by Kim *et al.* [25], without carrying out DNA–DNA hybridization. To delineate the phylogenetic position of strain BZ-SZ-XJ29ᵀ, 16S rRNA gene sequences were aligned using CLUSTAL X version 2.0 [26]. The bootstrap consensus trees were inferred from 1000 replicates with the minimum-evolution [27], maximum-parsimony [28], and neighbour-joining [29] algorithms in *MEGA* software package version 6.0 [30]. Evolutionary distances were calculated according to the algorithms of the Jukes–Cantor model [31]. The trees generated using the three methods were in good agreement among each other. The phylogeny of 16S rRNA gene sequences in Fig. 1 representatively showed that strain BZ-SZ-XJ29ᵀ shared a node and formed a coherent cluster with *B. saliphilus* 6AGᵀ with a relatively high bootstrap resampling value of 78–88 %. This cluster is independent from another cluster including *B. daliensis*DLS13ᵀ, ‘*B. daqingensis*’ X10-1 and *B. luteus* JC167ᵀ by a very low bootstrap support (31 %). The low sequence similarity between strain BZ-SZ-XJ29ᵀ and its neighbours indicated that this isolate was distinct from other members of the genus *Bacillus* [32]. The phylogenetic data based on 16S rRNA gene sequence divergence suggested that strain BZ-SZ-XJ29ᵀ and *B. saliphilus* 6AGᵀ constitute a separate taxon to *Bacillus* and should be assigned to a novel genus.

The G+C content of the genomic DNA of strain BZ-SZ-XJ29ᵀ was determined in duplicate using the thermal denaturation method (Tm) [33] with DNA from *Escherichia coli* K-12 as a control by the China General Microbiological Culture Collection Identification Service. The obtained G+C content of the genomic DNA was 40.0 mol%, which was lower than that of *B. saliphilus* and the closely related members of genus *Bacillus* (Table 1). DNA–DNA hybridization was carried out independently three times using the thermal denaturation and renaturation method [34] by China General Microbiological Culture Collection Identification Service. Strain BZ-SZ-XJ29ᵀ exhibited a DNA–DNA relatedness value of 45±2 % (means ±SD) with *B. saliphilus* DSM 15402ᵀ, which is clearly below the 70 % threshold currently widely accepted for bacterial species delineation [35].

Growth was tested at total Na⁺ concentration of 0.05–5.18 M [equivalent to 0.3–30.3 % (w/v) NaCl] at intervals of 2 % (w/v) NaCl and also at total Na⁺ concentration of 0.56 M (corresponding to 3.3 % (w/v) NaCl) and 4.32 M (25.3 %), as well as at 0–45 °C at intervals of 5 °C and at 3, 8, 33, 37, 41 and 43 °C. The pH range for growth was determined at
monitored by the measurement of turbidity at OD
medium as described above, the growth of the organism was
tests were performed in triplicate using the isolation
range from 5.5 to 11.0 with intervals of 0.5 pH units. All
need for Na
pH 7.5 at 1.42 M Na
7.5 and 33
optimum of 1.42 M Na
–
8.0), TAPS (pH 7.0
–
24.3 % (w/v) NaCl] with a relative broad
–
10.0) or CAPS (pH 10.0
4.32 M Na
+ [25.3 % (w/v) NaCl]. The pH
+ [8.3–12.3 % (w/v) NaCl] at pH
33 C or
+ [25.3 % (w/v) NaCl]. The pH
+ [3.3 % (w/v) NaCl] or ≥4.32 M Na
[25.3 % (w/v) NaCl]. The pH
7.5 and 33 C. No growth was found at
4.3–24.3 % (w/v) NaCl] with a relative broad
of strain BZ-SZ-XJ29 \(^\text{T}\) 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 because no growth was observed after incubation
for 7 days at the aforementioned conditions.

Unless specified otherwise, the aforementioned medium
for growth of strain BZ-SZ-XJ29 \(^\text{T}\) in this study was used for the morphological, physiological and biochemistry analyses.
Cell morphology at the exponential growth phase was examined
by scanning electron microscopy at 15 kv (Quanta 200; FEI) and
transmission electron microscope at 80 kv (JEM-1400; JEOL).
Cultures for the investigation of endospore formation were grown at 33, 37 or 41 C for up to 7 days on
oligotrophic medium as follows (l
–
111 s sodium nitrite (5 mM), MnO
2 (20 mM), and
KHCO
3 in the absence of yeast extract. Strain BZ-
SZ-XJ29 \(^\text{T}\) was obligately dependent on Na
+ as no growth
occurred in the absence of Na\(^{+}\). Anaerobic growth was
3 C and 1.42 M Na
+ at a concentration of 50 mM MES
for pH 5.5–6.5), HEPES (pH 7.0–8.0), TAPS (pH 8.0–9.0),
CHES (pH 9.0–10.0) or CAPS (pH 10.0–11.0) over a pH
range from 5.5 to 11.0 with intervals of 0.5 pH units. All
tests were performed in triplicate using the isolation
medium as described above, the growth of the organism was
monitored by the measurement of turbidity at OD\text{600} using a portable spectrophotometer (DR 2800; HACH). Salinity
tolerance range for growth was 0.74–4.15 M Na
+ [corresponding to 4.3–24.3 % (w/v) NaCl] with a relative broad
of 1.42–2.10 M Na
+[8.3–12.3 % (w/v) NaCl] at pH
7.5 and 33 C. No growth was found at ≤0.56 M Na
+[3.3 % (w/v) NaCl] or ≥4.32 M Na
+[25.3 % (w/v) NaCl]. The pH
profile for growth occurred at pH 6.0–10.5 with optimum of
pH 7.5 at 1.42 M Na
+ and 33 C; growth was not observed at
pH 5.5 or at pH 11.0. The temperature range for growth
was 5–41 C with optimum of 33 C at 1.42 M Na
+ and pH
7.5, but no growth was observed at ≤3 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.

Fig. 1. Phylogenetic tree using neighbour-joining method based on 16S rRNA gene sequences and showing the relationship between
strain BZ-SZ-XJ29 \(^\text{T}\) 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 (%) are based on
1000 replicates and are shown for branches with >70 % bootstrap support. Closed circles indicate generic branches that were also
recovered when using the minimum-evolution and maximum-parismony algorithms. Bar, 0.01 substitutions per nucleotide position.
Table 1. Differential phenotypic characteristics that distinguish strain BZ-SZ-XJ29T from other closely related species of the genus Bacillus

Taxa: 1, Alkalicoccus halolimnae sp. nov. BZ-SZ-XJ29T (data from this study); 2, Bacillus saliphilus DSM 15402T [18]; 3, B. luteus KCTC 33100T [20]; 4, ′Bacillus daqingensis′ CGMCC 1.12295 [21]; 5, Bacillus daéliensis CGMCC 1.10369T [19]; 6, Bacillus chagnonensis DSM 18086T [23]; 7, Bacillus urumqiensis BZ-SZ-XJ18T [22]; 8, Bacillus subtilis subsp. subtilis CGMCC 1.3358T [20]. All strains are positive for Gram-staining and catalase activity, but negative for hydrolysis of Tween 20 and the methyl red test. +, Positive; −, negative; ND, not detected.

<table>
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<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
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<tbody>
<tr>
<td><strong>Sampling site</strong></td>
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<td>Algal mat</td>
<td>Saline-alkaline soil</td>
<td>Agricultural soil</td>
<td>Soda lake</td>
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<td>Soil</td>
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<td>Cocci</td>
<td>Rods</td>
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<tr>
<td><strong>Colony colour</strong></td>
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<td>Yellow</td>
<td>Yellow</td>
<td>Orange</td>
<td>Yellow</td>
<td>Yellow-orange</td>
<td>Yellow</td>
<td>White to cream</td>
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<td><strong>O2 requirement</strong></td>
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<td>Strictly aerobic</td>
<td>Strictly aerobic</td>
<td>Strictly aerobic</td>
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<td><strong>Motility</strong></td>
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<td>−</td>
<td>+</td>
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<td><strong>NaCl concentration for growth (%) (w/v)</strong></td>
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<td>1–20</td>
<td>0–16</td>
<td>0–6</td>
<td>0–8</td>
<td>3–20</td>
<td>1.3–25.3</td>
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<td>3</td>
<td>0–3</td>
<td>2</td>
<td>7</td>
<td>6.3</td>
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<td><strong>Growth pH</strong></td>
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<td>7.5–11.0</td>
<td>6.8–9.8</td>
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<td>9</td>
<td>10.0</td>
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<td>37</td>
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<td>25–37</td>
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<td>37</td>
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<td>37–40</td>
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<td>+</td>
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<td>+</td>
<td>−</td>
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<td><strong>Casein</strong></td>
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<td>+</td>
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<td>+</td>
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<td><strong>Acid from</strong></td>
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<td><strong>d-Fructose</strong></td>
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<td>+</td>
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<td><strong>Voges–Proskauer reaction</strong></td>
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<td><strong>Quinone composition</strong></td>
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<td>MK-7, DMMK-7</td>
<td>MK-7</td>
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<tr>
<td><strong>DNA G+C content (mol%)</strong></td>
<td>40.0</td>
<td>48.8</td>
<td>47.7</td>
<td>53.4</td>
<td>43.9</td>
<td>53.8</td>
<td>41.7</td>
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</table>

*Data for all strains obtained in this study.

1.0 g peptone, 100 g (or 150, 200, 230 g) NaCl, 0.2 g (NH4)2SO4, 0.2 g MgSO4, 7H2O, 1.0 g K2HPO4, 1.0 g glucose and 20 g agar, and the pH was adjusted to 7.5 with 2 M NaOH. Endospores were observed with a light microscope (LEICA DM750; Leica Microsystems) under ×100 (oil immersion) objectives (NA 1.5150) according to the Schaeffer-Fulton staining method [36], and negatively stained with 1.0% (w/v) uranyl acetate for transmission electron microscopy at 80 kv (JEM-1400; JEOL). Intracellular polyhydroxyalkanoate (PHA) granules accumulation was examined using transmission electron microscopy (JEM-1400; JEOL) and by Sudan Black B staining methods according to the literature [37, 38]. Exopolysaccharide (EPS) was investigated by staining with Alcian Blue 8GX [36]. Cells were coccus-shaped (approx. 1.2–1.5 μm) (Fig. S1a, available in the online Supplementary Material) and endospore formation was not
detected under oligotrophic conditions tested including the heat resistance and salinity stress as described above. These two important phenotypic features of cellular morphology and the inability to form endospores are consistent with those of \textit{B. saliphilus} 6AG\textsuperscript{T}, which are differentiating features to those of members of the genus \textit{Bacillus}. Therefore, it appears to be appropriate that strain BZ-SZ-XJ29\textsuperscript{T} and \textit{B. saliphilus} 6AG\textsuperscript{T} should be placed in one novel genus. Strain BZ-SZ-XJ29\textsuperscript{T} was confirmed as Gram-staining-positive by using the standard Gram reaction [39]. No flagellum was observed by negatively stained with 1.0\% (w/v) uranyl acetate (Fig. S1b).

For the utilization of sole carbon and energy sources, cultures containing various organic substrates (0.5\%, w/v) were incubated for up to 72 h in 5 ml broth containing 8.3\% NaCl, at 33 °C and pH 7.5, and growth was recorded after the third successive transfer. When an amino acid as substrate was being investigated, the medium was prepared without (NH\textsubscript{4})\textsubscript{2}SO\textsubscript{4}. Acid production from sugars (1.0\%, w/v) was assayed by the oxidation of bromocresol purple added to a final concentration of 0.03 g l\textsuperscript{-1}. Tests for hydrolysis of casein, gelatin, hippurate, starch, tyrosine, Tween 20 and Tween 80, production of indole and H\textsubscript{2}S, and phenylalanine deamination were carried out as described by Mata \textit{et al.} [40]. Nitrate and nitrite reduction, and the methyl red and Voges–Proskauer tests were carried out as described by Lányi [41]. Catalase activity was tested by bubble production in a 3\% hydrogen peroxide solution, and oxidase activity was determined by oxidation of 1.0\% \textit{p}-aminodimethylaniline oxalate. The presence of arginine, lysine and ornithine decarboxylases was determined by the observation of the colour change of bromocresol purple to purple after incubation for 24 h [42]. Susceptibility of strain BZ-SZ-XJ29\textsuperscript{T} to antibiotics were performed by plating the culture suspension on solid medium agar plates (1.6\%) using the sensi discs as described by Mata \textit{et al.} [40] and incubating at 33°C for 5 days. Large zones of inhibition, i.e. >8 mm including the 6 mm diameter of the sensi discs, indicated that the micro-organism was susceptible, while small or no zone of inhibition indicated resistance. Strain BZ-SZ-XJ29\textsuperscript{T} was sensitive to the following antimicrobial agents (µg per disc unless otherwise stated): amoxicillin (10), ampicillin (50), carbenicillin (100), cefotaxime (30), cefoxitin (30), chloramphenicol (30), clarithromycin (15), clindamycin (2), erythromycin (15), penicillin (10 IU), rifampicin (30), sulfamethoxazole (300), tetracycline (30) and vancomycin (30), and resistant to gentamicin (10), kanamycin (50), nalidixic acid (30), neomycin (30) and streptomycin (300). Other detailed results of morphological features, nutritional and physiological characteristics, and biochemical tests are shown in the species description. Several differential phenotypic features between strain BZ-SZ-XJ29\textsuperscript{T} and \textit{B. saliphilus} 6AG\textsuperscript{T} as well as the other closely related species of the genus \textit{Bacillus}, are shown in Table 1, including those concerning endospore formation, motility, physiological features, oxidase activity, hydrolysis of gelatin, H\textsubscript{2}S production, phenylalanine deamination and Voges–Proskauer reaction, and acid production, etc.

For cellular fatty acid analysis, the bacterial biomass was harvested after incubation for 48 h at 37 °C in the liquid medium as previous study [43]. 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 [44]. The fatty acids constituting 10\% or more of the total were anteiso-C\textsubscript{15:0} (59.5\%), and anteiso-C\textsubscript{17:0} (22.3\%), followed by iso-C\textsubscript{15:0} (5.3\%), iso-C\textsubscript{16:0} (5.1\%), C\textsubscript{14:0} (4.5\%), iso-C\textsubscript{17:0} (1.6\%) and iso-C\textsubscript{14:0} (1.0\%). No hydroxy fatty acid was detected from all tested strains. The major fatty acids of strain BZ-SZ-XJ29\textsuperscript{T} were anteiso saturated branched-chain fatty acids (anteiso-C\textsubscript{15:0} and anteiso-C\textsubscript{17:0}), which were present in significantly higher amounts than in \textit{B. saliphilus} 6AG\textsuperscript{T} and the related type strains of the genus \textit{Bacillus} (Table S1). The relative concentrations of iso-C\textsubscript{15:0} iso-C\textsubscript{16:0} and C\textsubscript{16:0} of strain BZ-SZ-XJ29\textsuperscript{T} were apparently lower than those of other members. In brief, although the determined profiles of the fatty acids were similar, the proportions of fatty acids of strain BZ-SZ-XJ29\textsuperscript{T} clearly differentiates the strain from its closest relatives. In order to complete the chemotaxonomic characterization of strain BZ-SZ-XJ29\textsuperscript{T}, the polar lipids profile by TLC, respiratory quinones using HPLC methods and cell-wall peptidoglycan type were determined by the Identification Service of the Leibniz-Institut DSMZ-Deutsche Sammlung von Microorganismen und Zellkulturen (Braunschweig, Germany). The methods for analysis were used following the protocol online (www.dsmz.de/services/services-microorganisms/identification.html) and as previously described [45–48]. The predominant polar lipid (Fig. S2) of strain BZ-SZ-XJ29\textsuperscript{T} was diphosphatidylglycerol in accordance with the polar lipid composition of strain \textit{B. saliphilus} 6AG\textsuperscript{T}, \textit{B. chagnonorensis} CG-15\textsuperscript{T}, \textit{B. dalisensis} DLS1\textsuperscript{T}, \textit{B. luteus} JC167\textsuperscript{T}, \textit{B. urumqiensis} BZ-SZ-XJ18\textsuperscript{T}, \textit{B. daqingensis} X10-1\textsuperscript{T} and \textit{B. subtilis} [19–21, 49]. Moderate to minor amounts of phosphatidylethanolamine, phosphatidylglycerol, a phospholipid, and most likely sulfoquinovosylglycerol were also found in strain BZ-SZ-XJ29\textsuperscript{T}. The respiratory quinone observed was menaquinone-7 (MK-7), which is in agreement with other relatives yielding MK-7 as the major compound. Demethylmenaquinone 7 (DMK-7) found in \textit{B. saliphilus} DSM 15402\textsuperscript{T} was not detected in strain BZ-SZ-XJ29\textsuperscript{T}. The cell-wall peptidoglycan was of the A1\textsubscript{v} type, containing meso-diaminopimelic acid as the diagnostic diamino acid. The major polar lipids, respiratory quinone and the peptidoglycan type of the cell wall of strain BZ-SZ-XJ29\textsuperscript{T} were typical of those observed in \textit{B. saliphilus} and the other members of the genus \textit{Bacillus} [19, 20, 23].

On the basis of morphological, physiological and chemotaxonomic properties, 16S rRNA gene sequence analysis together with DNA–DNA relatedness indicated that the
species *Bacillus saliphilus* should be reclassified in a novel genus, *Alkalicoccus* gen. nov., as *Alkalicoccus saliphilus* comb. nov. (type species of the new genus). Strain BZ-SZXJ29T represents a novel species of the genus *Alkalicoccus*, for which the name *Alkalicoccus halolimnae* sp. nov., is proposed.

**DESCRIPTION OF *ALKALICOCCUS GEN. NOV.***

*Alkalicoccus* [Al.ka.li. coc’cus. N.L. n. alkali (from Arabic article al the; Arabic n. qalby ashes of saltwort) alkali; L. fem. n. coccus sphere; M.L. masc. n. Alkalicoccus a coccus living in basic surroundings].

Cells are strictly aerobic, Gram-stain-positive, non-endospore-forming, coccus-shaped and non-motile. Catalase-positive. Moderately halophilic, growing over a wide range of NaCl concentrations. No growth occurs in the absence of Na+. The major fatty acids are mainly composed of anteiso saturated branched-chain fatty acids, e.g. anteiso-C15:0 and anteiso-C17:0. The respiratory quinone is (MK-7 and the dominant polar lipids consist of diphosphatidylglycerol. The DNA G+C content is 40.0–48.8 mol%.

The type species is *Alkalicoccus saliphilus*.

**DESCRIPTION OF *ALKALICOCCUS SALIPHILUS* (ROMANO ET AL. 2005) COMB. NOV.**

*Alkalicoccus saliphilus* (sa.li’phi.lus. L. n. sal salt; Gr. adj. philos loving; N.L. masc. adj. saliphilus loving salt).


The description is the same as that given by Romano et al. [18].

The type strain is 6AGT (=DSM 15402T=ATCC BAA-957T).

**DESCRIPTION OF *ALKALICOCCUS HALOLIMNAE* SP. NOV.**

*Alkalicoccus halolimnae* (ha.lo.li’ni.nea. Gr. n. hals, halos salt; Gr. n. limne lake; N.L. gen. n. halolimnae of a salt lake).

Cells are coccus-shaped, approximately 1.2–1.5 µm. Colonies are yellow-pigmented, opaque, convex and circular with approximately 1–2 mm diameter after incubation at 33 °C for 3 days on plates containing the modified solid medium. Growth occurs at total Na+ concentrations of 0.74–4.15 M Na+ [equivalent to 4.3–24.3 % (w/v) NaCl] with an broad optimum at 1.42–2.10 M Na+ [equivalent to 8.3–12.3 % (w/v) NaCl], at pH 6.0–10.5 (optimum pH 7.5) and at temperatures of 5–41 °C (optimum 33 °C). Does not yield growth without NaCl or with sodium thiosulfate, sodium nitrate, sodium nitrite, MnO2 or fumarate as an electron acceptor under anaerobic conditions. Does not accumulate polyhydroxylkanoate granules or produce exopolysaccharide. As sole carbon and energy sources, utilizes l-arabinose, D-ribose, D-xylene, D-fructose, D-fucose, D-galactose, α-D-glucose, D-mannose, maltose, sucrose and D-melezitose; does not utilize cellobiose, D-galactose, raffinose and stachyose. As sole carbon, nitrogen and energy sources, utilizes γ-aminoobutyric acid and N-acetyl-D-glucosamine, but does not utilize L-alanine, L-arginine, L-histidin, L-serine, D-serine, L-aspartic acid, L-glutamic acid, glucuronamide, glycylo-proline and L-pyrogam glutamic acid. Acid is produced from D-ribose, D-xylene, D-fructose, D-fucose, α-D-glucose, D-mannose, maltose, sucrose and D-melezitose, but not from L-arabinose, D-galactose and L-rhamnose. Positive for catalase activity, hydrolysis of casein and starch, phenylalanine deamination and Voges–Proskauer tests, but negative for oxidase, hydrolysis of hippurate, Tween 20 and Tween 80, nitrate and nitrite reduction, indole production, H2S production, tyrosine decomposition, methyl red test, arginine decarboxylase, lysine decarboxylase and ornithine decarboxylase. The major fatty acids are anteiso-C15:0, anteiso-C17:0, iso-C15:0 and iso-C16:0 followed by C16:0 and iso-C14:0. The peptidoglycan type is A1y, with meso-diaminopimelic acid as the diagnostic diamino acid.

The type strain is BZ-SZXJ29T (=DSM 29191T=JCM 30193T =CGMCC 1.12936T), isolated from a mixture of water and sediment of a saline-alkaline lake close to Urumqi city, Xinjiang Uyghur Autonomous Region of China. The G+C content of the genomic DNA of the type strain is 40.0 mol% (Tm).

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**Conflicts of interest**

The authors declare no conflicts of interest regarding this manuscript.

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