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Short communication

Bacillus locisalis sp. nov., a new haloalkaliphilic species from hypersaline and alkaline lakes of China, Kenya and Tanzania☆

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ABSTRACT

A polyphasic taxonomic study was performed on seven *Bacillus*-like bacteria isolated from three hypersaline and alkaline lakes located in China, Kenya and Tanzania. All strains were moderately halophilic and alkaliphilic, Gram positive, motile rods. The DNA G+C content from the seven isolates ranged from 42.2 to 43.4 mol% and their major fatty acid was anteiso-$C_{15:0}$. Strain CG1^T, selected as representative strain of the isolates, possesses *meso*-diaminopimelic acid in the cell wall peptidoglycan, MK-7 as the predominant menaquinone and diphosphatidylglycerol, phosphatidylglycerol and phosphatidylethanolamine as the major polar lipids. Comparative 16S rRNA gene sequence analysis indicated that the isolates belonged to the genus *Bacillus*. The seven isolates shared 97.7–99.9% 16S rRNA gene sequence similarity, and formed a branch that was distinct from the type strains of the recognized species of the genus *Bacillus*. They were most closely related to *Bacillus agaradhaerens* DSM 8721^T (92.6–93.8% 16S rRNA sequence similarity). DNA–DNA hybridization values between the seven isolates were 85–100%. According to the polyphasic characterization, the strains represent a novel species, for which the name *Bacillus locisalis* sp. nov. is proposed. The type strain is CG1^T (CCM 7370^T = CECT 7152^T = CGMCC 1.6286^T = DSM 18085^T).

1. Scope of the paper: systematics

Haloalkaliphilic bacteria are extremophilic microorganisms that are widely distributed in different hypersaline and alkaline habitats with a variable (up to saturation) salt concentration and high pH values. The genus *Bacillus* was proposed by Cohn in 1872 [6] and since then it has undergone substantial taxonomic changes. Currently, this genus groups near 200 species [10] with some of them having a moderately halophilic and alkaliphilic/alkalitolerant response, such as the case of *Bacillus oshimensis* (from soil in Japan) [28], *Bacillus saliphilus* (from an algal mat from a mineral pool in Italy) [24], *Bacillus chagannorensis* (from a soda lake in China) [3], *Bacillus aurantiacus* (from an extremely shallow soda lake in Hungary) [2], and *Bacillus polygoni* (from indigo balls in Japan) [1].

In the present study, we report the discovery of a novel moderately halophilic, alkaliphilic *Bacillus* species during a study of bacterial diversity in hypersaline habitats using a culture-dependent approach. Seven bacterial strains were isolated from water and sediment samples from hypersaline and alkaline lakes located in three different countries: China, Kenya and Tanzania. The taxonomic status of the isolates was determined using a polyphasic approach.

Strains 103NT4 and WE1 were isolated in 1988 following the methodology described by Duckworth et al. [8]. Strain 103NT4 was isolated from orange-coloured soda crusts surrounding a warm soda seep brine (35 °C) located on the northern shore of Lake Natron (Tanzania) (2°08′S, 36°00′E, pH 10.5, conductivity 35 mS cm^{-1}), while strain WE1 was isolated from a sediment sample from the eastern shore of Lake Elmenteita, in the Kenyan section of the East African Rift Valley (0°25′S, 36°15′E, pH 10.5, conductivity 12.7 mS cm^{-1}) [8]. The other five strains were isolated from water (CG1^T and CG2) and sediment (CG4, CG6 and CG7) samples taken from Lake Chagannor, during an expedition in September 2003. This lake is situated near a soda work, 120 km south of Mandulatu (43°16′N 112°55′E, pH 10.5, conductivity 202 mS cm^{-1}), on the Inner Mongolian steppe, northwest of Beijing, China. The water samples were diluted in sterile 10% (w/v) marine salts (g l^{-1}): NaCl, 78; $MgCl_2 \times 6H_2O$, 13; $MgSO_4 \times 7H_2O$, 20.3; $CaCl_2$, 0.33; KCl, 2; $NaHCO_3$, 0.07; NaBr, 0.23 [27], planted on alkaline saline medium and incubated at 37 °C aerobically. The alkaline saline isolation medium contained (g l^{-1}): glucose, 10.0; peptone (Difco), 5.0; yeast

☆ The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strain CG1^T, CG2, CG4, CG6, CG7, 103NT4 and WE1 are FR714930, FR714931, FR714932, FR714933, FR714934, X92163 and X92164, respectively.

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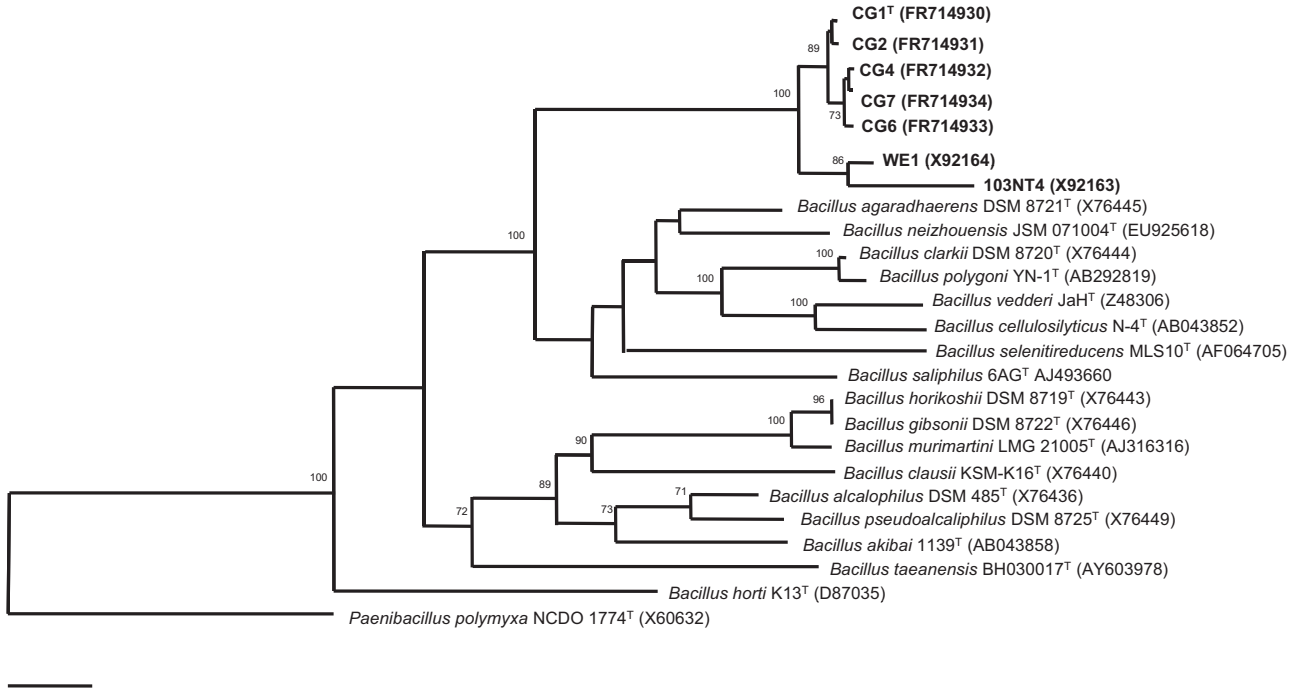

Fig. 1. Neighbour-joining tree, based on the 16S rRNA gene sequence comparison, showing the relationship of strain CG1^T with related species. The accession numbers of the sequences used in this study are shown in parentheses after the strain designation. *Paenibacillus polymyxa* NCDO 1774^T was used as an outgroup. Bootstrap values >70% are shown. The scale bar represents 0.01 substitutions per nucleotide position.

extract (Difco), 5.0; KH_2PO_4, 2.0; $MgSO_4 \times 7H_2O$, 0.4; NaCl, 80; Na_2CO_3, 20. The salts NaCl and Na_2CO_3 were autoclaved separately and added to the organic components at 60 °C. The final pH of this medium was about pH 10. When it was necessary, the medium was solidified by adding 2.0% (w/v) agar. The sediments (0.1 g) were suspended in 10% (w/v) marine salts. The suspensions were vortexed for 1 min, allowed to settle, serially diluted in 10% (w/v) marine salts and then spread-plated in duplicate on alkaline saline medium followed by aerobic incubation at 37 °C. The strains were subsequently purified three times by plating on the same medium and maintained on the same alkaline saline medium and at −80 °C on this medium without agar and supplemented with 30% (v/v) glycerol. In addition to the seven isolates, *Bacillus agaradhaerens* DSM 8721^T was obtained from the Deustche Sammlung von Mikroorganismen und Zellkulturen (DSMZ), Braunschweig, Germany, and cultivated at 37 °C on alkaline saline medium. This bacterium was used as reference for comparative phenotypic and chemotaxonomic studies.

The phylogenetic position of the seven isolates was determined by complete 16S rRNA gene sequence analysis. Genomic DNAs were prepared using the method described by Marmur [19]. PCR amplifications of the 16S rRNA gene were carried out with the forward primer 16F27 and the reverse primer 16R1488. Sequencing was performed using an automated DNA sequencer model 3130XL (Applied Biosystems). Identification of phylogenetic neighbours and calculation of pairwise 16S rRNA gene sequence similarities were achieved using the EzTaxon server version 2 (http://www.eztaxon.org/; [5]). The 16S rRNA gene sequences were aligned with the published sequences of closely related bacteria.

Table 1
Cellular fatty acid composition of the seven isolate strains and *B. agaradhaerens* DSM 8721^T grown on alkaline saline medium (pH 10) at 37 °C, for 48 h. Data are percentages of the total fatty acids. –, Values less than 0.5% in all strains; ND, not detected.

Fatty acids	Strains							
	CG1^T	CG2	CG4	CG6	CG7	WE1	103NT4	DSM 8721^T
Straight chain								
$C_{12:0}$	–	–	–	–	–	0.7	0.8	0.5
$C_{14:0}$	1.1	1.0	0.7	0.7	0.7	1.4	1.3	0.7
$C_{16:0}$	4.3	3.9	5.3	3.9	5.6	2.1	2.4	6.0
$C_{18:0}$	1.0	–	0.7	0.5	0.8	0.5	ND	–
Branched								
Iso-$C_{14:0}$	3.6	3.1	4.5	3.9	4.6	9.0	5.3	1.0
Iso-$C_{15:0}$	11.4	11.4	11.5	11.1	10.3	15.9	11.5	23.3
Anteiso $C_{15:0}$	54.1	55.9	42.0	44.2	39.4	46.2	61.5	40.9
Iso-$C_{16:0}$	3.8	3.4	6.1	5.3	6.7	4.9	3.7	3.3
Iso-$C_{17:0}$	3.9	3.6	6.7	5.6	7.2	0.9	1.5	7.0
Anteiso $C_{17:0}$	11.5	11.6	13.9	13.9	15.0	4.4	6.2	11.9
Iso-$C_{18:0}$	ND	ND	–	–	0.7	ND	ND	ND
Unsaturated								
$C_{16:1}$ $\omega 7c$ alcohol	ND	ND	ND	ND	ND	2.0	ND	ND
$C_{16:1}$ $\omega 11c$	1.3	1.5	2.1	3.0	2.8	4.3	1.7	1.2
$C_{17:1}$ iso $\omega 10c$	1.7	2.0	3.7	4.9	4.0	3.8	1.7	1.5
$C_{18:1}$ $\omega 9c$	1.2	1.0	1.1	1.0	1.0	2.0	1.6	1.4