Bacillus bogoriensis sp. nov., a novel alkaliphilic, halotolerant bacterium isolated from a Kenyan soda lake

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Strain LBB3^T isolated from Bogoria soda lake in Kenya is an alkaliphilic, Gram-positive, strictly aerobic, non-motile, spore-forming bacterium. It was identified as a member of the genus *Bacillus* on the basis of phenotypic and phylogenetic analyses. The organism grows optimally at 37 °C and pH 10. The G+C content of the genomic DNA is 37·5 mol%. 16S rRNA gene sequence analysis showed 95 and 96 % sequence similarity with *Bacillus pseudofirmus* (DSM 8715^T) and *Bacillus alcalophilus* (DSM 485^T), respectively. Furthermore, DNA–DNA hybridization against these two *Bacillus* species showed 39·0 and 55·5 % similarity, respectively. Based on our observations, strain LBB3^T is proposed to represent a novel species of the genus *Bacillus*, for which the name *Bacillus bogoriensis* sp. nov. is proposed. The type strain of *B. bogoriensis* is LBB3^T (=ATCC BAA-922^T=LMG 22234^T).

The genus *Bacillus* comprises a vast diversity of physiological types. The G+C content (32–69 mol%) of the known *Bacillus* species, as well as DNA–DNA hybridization experiments, has revealed the heterogeneity of the genus. Phenetic diversity of alkaliphilic *Bacillus* strains has been studied, and so far 11 alkaliphilic *Bacillus* strains have been isolated and identified (Fritze *et al.*, 1990; Nielsen *et al.*, 1994, 1995). Soda lakes contain dense populations of aerobic organotrophic and alkaliphilic bacteria. Some of these bacteria are believed to have biotechnological potential as a source of alkali-stable enzymes (Gessesse & Gashe, 1997; Martins *et al.*, 2001).

Strain LBB3^T was recovered from liquid samples collected from Lake Bogoria, Kenya in a screening medium for lipolytic micro-organisms (Vargas *et al.*, 2004), composed of 3 % (v/v) olive oil and basal salts (w/v) [0·08 % K_2HPO_4, 0·06 % KH_2PO_4, 0·1 % $(NH_4)_2SO_4$, 0·02 % $MgSO_4.7H_2O$, 0·005 % $CaCl_2.2H_2O$, 0·3 % NaCl, 0·0001 % $FeCl_3$]. It was grown at 37 °C and pH 10 on an orbital shaker at 200 r.p.m. Cell morphology was examined using a Nikon Optiphot-2 microscope at 1000× magnification. Bacterial size was determined in living cell preparations. Cells were harvested from liquid cultures, washed twice with water and dehydrated through a graded series of ethanol and isopropyl alcohol. Cells were then mounted on 12 mm cover slips, dried in a vacuum desiccator overnight and then gold–palladium (80/20) coated. Biochemical characteristics were screened by using the API 20 E system (bioMérieux) according to Logan & Berkeley (1984). Sugar assimilation was determined by using the API 50 CHB system.

Genomic DNA was extracted and purified according to Marmur (1961), and its purity was assessed from the A_{260}/A_{280} and A_{260}/A_{230} ratios (Johnson, 1994). Universal primers were used to amplify 16S rRNA genes (8-27F, AGAGTTTGATCCTGGCTCAG; 1422R, GGTTACCTTGTTACGACTT) (Weisburg *et al.*, 1991). PCR products were purified using the QIA quick PCR Purification kit (Qiagen) and then resuspended in a final volume of 40 µl. DNA sequencing on both strands was performed by using the dideoxy chain-termination method with an ABI Prism 3100 DNA Analyzer, using an ABI Prism Big Dye Terminator Cycle Sequencing Ready Reaction kit (PE Applied Biosystems) according to the manufacturer's protocol. GenBank and RDP databases were used to search for 16S rRNA gene sequence similarities (Maidak

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of LBB3^T is AY376312.

A phylogenetic tree and electron micrographs are available as supplementary material in IJSEM Online.

Table 1. Phenotypic characteristics that differentiate *Bacillus bogoriensis* LBB3^T from phylogenetically related species

Species: 1, *Bacillus bogoriensis* LBB3^T; 2, *B. alcalophilus* DSM 485^T; 3, *B. pseudofirmus* DSM 8715^T; 4, *B. agaradhaerens* DSM 8721^T; 5, *B. clarkii* DSM 8720^T; 6, *B. pseudalcaliphilus* DSM 8725^T; 7, *B. halodurans* DSM 497^T. All data are from Nielsen *et al.* (1995) except those for *B. bogoriensis* (this study). All strains are rod-shaped, and negative for erythritol, L-xylose, adonitol, dulcitol, inulin, D-fucose and L-fucose utilization. All strains utilize L-arabinose, D-xylose, fructose, salicin, cellobiose, maltose, trehalose, gentiobiose, starch, glycogen and ribose.

Characteristic	1	2	3	4	5	6	7
Growth at:							
10 °C	+	+	+	+	−	+	−
45 °C	+	−	+	+	+	−	+
55 °C	−	−	−	−	−	−	+
pH optimum	10	9	9	>10	>10	10	9–10
Growth with:							
NaCl (10 %)	+	−	+	+	+	+	+
NaCl (16 %)	−	−	+	+	+	−	−
Colony morphology							
Pigmentation	Cream–yellow	White	Yellow	White	Cream–white	White	White
Margins	Entire	Entire	Irregular	Filamentous	Entire	Undulate	Filamentous
Cell size (μm)	0·3–0·4 × 2·0–3·5	0·5–0·7 × 3·0–5·0	0·6–0·8 × 2·0–4·0	0·5–0·6 × 2·0–5·0	0·6–0·7 × 2·0–5·0	0·5–0·6 × 2·0–4·0	0·5–0·6 × 3·0–4·0
G+C content (mol%)	37·5	36·2–38·4	39·0–40·8	39·3–39·5	42·4–43·0	38·2–39·0	42·1–43·9
Starch hydrolysis	+	+	+	+	−	+	+
Gelatin hydrolysis	−	+	+	+	+	+	+
Nitrate reduction	−	+†	−	+	+	−	+†
Growth on:							
Glucose	−	+	+	+	−*	+	+
Sucrose	−	+	+	+	−*	+	+
Amygdalin	−	+	+	+	−*	+	+
Inositol	−	+	+†	−	−*	−	+
Lactose	+	+	−	+	−*	+	+
Mannose	+	+	+	+	−*	−	+
Rhamnose	+	+	−	+	−*	+	+
Melibiose	−	+	−	+	−*	−	+
Lactose	+	+	−	+	−*	+	+
Raffinose	+	+	−	+	−*	−	+
N-Acetylglucosamine	+	+	+	+	−*	−	+
Methyl β-xyloside	−	+	−	−	−*	+	+
Galactose	−	+	+†	+	−*	+	+
Sorbitol	−	−	−	+	−*	−	−
Methyl α-D-mannoside	−	+	−	+	−*	−	−
Methyl α-D-glucoside	−	+	+	+	−*	+	+
Melezitose	−	+	−	+	−*	−	+
Xylitol	−	−	+†	−	−*	−	+
D-Turanose	−	+	+	+	−*	+	+
D-Lyxose	−	+	+	+	−*	+	+
D-Tagatose	−	+	−	+	−*	+	+†
Gluconate	−	−	+	−	−*	+	+
2-Ketogluconate	−	−	+†	+	−*	+	+
D-Arabitol	−	+	+	+	−*	−	+

*No growth is observed in minimal medium for carbohydrate utilization at pH 10.
†Weakly positive.

et al., 2000). A 16S rRNA gene sequence analysis was performed with the aid of the DNAMAN 4.03 software package by using the neighbour-joining method and the Jukes–Cantor distance correction matrix method (Saitou & Nei, 1987). Determination of peptidoglycan type was carried out as described previously (Schleifer & Kandler,

1972; Schleifer, 1985), with the modification that TLC on cellulose was used instead of paper chromatography.

Genomic G+C content (mol%) and DNA–DNA hybridization were determined by the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ, Braunschweig, Germany). DNA was isolated by chromatography on hydroxyapatite by the procedure of Cashion *et al.* (1997). G+C content was calculated according to the method of Mesbah *et al.* (1989). DNA–DNA hybridization was carried out as described by De Ley *et al.* (1970) with the modifications described by Huss *et al.* (1983) and Escara & Hutton (1980) using a Gilford System model 2600 spectrophotometer equipped with a Gilford model 2527-R thermoprogrammer and plotter. Renaturation rates were computed with the TRANSFER.BAS program described by Jahnke (1992).

An almost-complete 16S rRNA gene sequence was determined for strain LBB3^T and compared with other available sequences in the GenBank and RDP databases (Fig. A, available as supplementary material in IJSEM Online). This analysis placed the novel strain in the family *Bacillaceae*, with the closest related micro-organisms being *Bacillus pseudofirmus* (DSM 8715^T) and *Bacillus alcalophilus* (DSM 485^T), bearing 95 and 96 % sequence similarity, respectively.

A comparison of the morphological and taxonomic characteristics of strain LBB3^T with those of phylogenetically related species is shown in Table 1. Strain LBB3^T produces cream–yellow, circular, convex, smooth, entire, opaque colonies that are 5–6 mm in diameter after 48 h incubation at 37 °C. It is Gram-positive. Microscopic examination showed the cells to be spore-forming and non-motile rods that are 0·3–0·4 μm wide and 2·0–3·5 μm long. In unstained preparations, cells occur singly, in pairs and in irregular clumps (Fig. B, available as supplementary material in IJSEM Online).

Strain LBB3^T revealed uniform growth in the liquid medium, which led to a turbid culture broth and no formation of pellicles. It was strictly aerobic and mesophilic, exhibiting growth between 10 and 40 °C, with an optimum at 37 °C. Growth was supported between pH 8 and 11, with an optimum at pH 10, and with up to 2 M NaCl in the medium. Analysis of the cell wall showed the presence of peptidoglycan type A4β L-Orn–D-Asp. This pattern is typical for members of the genus *Bacillus* and related genera.

As shown in Table 1, the G+C content of strain LBB3^T is 37·5 mol%. This value lies within the range for low G+C content Gram-positive bacteria. DNA–DNA hybridization analysis revealed 39·0 % similarity to *B. pseudofirmus* DSM 8715^T and 55·5 % similarity to *B. alcalophilus* DSM 485^T. These values for hybridization are significantly lower than the recommended threshold value of at least 70 % accepted as the definition of a novel species (Wayne *et al.*, 1987), hence supporting the distinct position of strain LBB3^T in the genus *Bacillus*. Differences in 16S rRNA gene sequence identity of 3 % and the G+C content justify the description of a novel *Bacillus* species to accommodate strain LBB3^T.

Based on the analyses of morphological, physiological and phylogenetic traits, we propose strain LBB3^T to be a novel alkaliphilic, halotolerant species of the genus *Bacillus*, for which the name *Bacillus bogoriensis* sp. nov. is proposed.

Description of *Bacillus bogoriensis* sp. nov.

Bacillus bogoriensis (bo.gor.i.en′sis. N.L. adj. *bogoriensis* pertaining to Lake Bogoria, a soda lake in Kenya).

Cells are rod-shaped (0·3–0·4 × 2·0–3·5 μm), Gram-positive, spore-forming and non-motile. Catalase-positive and urease-negative. Nitrate is not reduced to nitrite. Strictly aerobic. Optimal growth occurs aerobically at 37 °C (range 10–40 °C), pH 10 (range 8–11) and maximum salt tolerance of 2 M NaCl. Heterotrophic; utilizes rhamnose, sucrose, L-arabinose, D-xylose, fructose, mannose, amygdalin, salicin, cellobiose, maltose, arbutin, aesculin, lactose, trehalose, gentiobiose, starch, glycogen, glycerol, ribose, raffinose, *N*-acetylglucosamine and 5-ketogluconate. Does not grow on mannitol, erythritol, D-arabinose, L-xylose, adonitol, methyl β-xyloside, D-glucose, galactose, melibiose, L-sorbose, dulcitol, inositol, sorbitol, methyl α-D-mannoside, methyl α-D-glucoside, inulin, melezitose, xylitol, D-turanose, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, gluconate or 2-ketogluconate. DNA G+C content is 37·5 mol% (determined by HPLC).

The type strain is LBB3^T (=ATCC BAA-922^T=LMG 22234^T).

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