Bacillus acidiceler sp. nov., isolated from a forensic specimen, containing *Bacillus anthracis* pX02 genes

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Research at the Center for Biological Defense identified plasmid-borne forms of *Bacillus anthracis* pXO2 genes in a Gram-positive, endospore-forming rod, isolated from a forensic specimen considered a credible threat of harbouring anthrax. Conventional, commercial and molecular-based methods indicated that the isolate (CBD 119^T) was not *B. anthracis* and considered not to be a member of the *Bacillus cereus* group. Based on the 16S rRNA gene sequence similarities, strain CBD 119^T was most closely related to *Bacillus luciferensis* LMG 18422^T (99.3 %). Phenotyping and fatty acid methyl ester analysis of the isolate were conducted alongside *B. luciferensis* JCM 12212^T. The major cellular fatty acids (anteiso-$C_{15:0}$, iso-$C_{15:0}$, and >7 iso or anteiso forms) supported inclusion of the isolate in the genus *Bacillus*. Strain CBD 119^T was inconsistent with *B. luciferensis* JCM 12212^T for 18 of 96 traits evaluated including motility, degree of endospore-driven swelling and pH optimum; the two were linked by fatty acid methyl ester analysis as separate but closely related species. DNA–DNA relatedness between strain CBD 119^T and *B. luciferensis* JCM 12212^T resulted in less than 20 % hybridization. The results of biochemical and physiological characterization, chemotaxonomic analysis and DNA–DNA hybridization differentiated strain CBD 119^T both phenotypically and genotypically from the only species with validly published name with greater than 97 % 16S rRNA gene sequence similarity. The isolate has an accelerated doubling time when grown in aerated broth at pH 5.9 relative to that at pH 7.1. Therefore, it is proposed that strain CBD 119^T represents a novel species, *Bacillus acidiceler* sp. nov. The type strain is strain CBD 119^T (=NRRL B-41736^T=DSM 18954^T).

Bacillus anthracis requires both pX01 and pX02 plasmids encoding the toxin and capsule genes, respectively, to cause disease. As a result of the anthrax bioterrorism of October 2001, a loose, white powder discovered on a window sill was suspected by law enforcement officers of possibly harbouring *B. anthracis*. The law enforcement officers transported the specimen to the Florida Department of Health (FDOH), Bureau of Laboratories, Tampa Branch, Laboratory Response Network (LRN) for analysis. It is unknown but unlikely that the collection method and container used by the first responders were sterile. A portion of the loose powder was placed on tryptic soy agar (TSA) with 5 % sheep blood (BA) and incubated overnight at 35 °C. Four colonies grew on the plate, a *Bacillus cereus*, a *Bacillus pumilus*, an unidentified Gram-positive rod and an apparent *Bacillus* sp. that tested positive, by PCR, for the pX02 plasmid. The results produced by the FDOH personnel following LRN protocols (Weyant *et al.*, 2001)

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Abbreviations: CBD, Center for Biological Defense; DFA, direct fluorescent antibody; ED, Euclidean distance; FDOH, Florida Department of Health; LRN, Laboratory Response Network; MRVP, Methyl red Voges–Proskauer.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain CBD 119^T is DQ374637.

Supplementary tables are available with the online version of this paper.

are compiled as Supplementary Table S1 (available in IJSEM Online). After the presence of *B. anthracis* was ruled out, the powder and *Bacillus* sp. were released to the Center for Biological Defense (CBD) for further study. Colonies consistent with *Bacillus* sp. CBD 119^T were repeatedly isolated by touching a sterile swab pre-moistened in 1 × PBS to the loose powder then rolling the swab across the surface of a BA plate, incubated as before. Reisolations of strain CBD 119^T consistently tested positive by PCR for the signature chromosomal element BA3 and the pX02 plasmid but negative for the pX01 plasmid. Additionally, the isolates were repeatedly ruled out as *B. anthracis* based on negative results for gamma phage susceptibility and direct fluorescent antibody (DFA) testing for cell wall and capsule. Failure to grow on mannitol egg yolk polymyxin B agar indicated that *Bacillus* sp. CBD 119^T was not a member of the *B. cereus* group (Luna *et al.*, 2005). Luna *et al.* (2006) confirmed the presence of a large plasmid that carries 10 genes (*acp*A, *cap*A, *cap*B, *cap*C, *cap*R, *cap*D, IS1627, ORF 48, ORF 61 and *rep*A) and the capsule-promoter sequence present on pX02 in strain CBD 119^T. Gene sequences from the isolate shared 100 % nucleotide identity to *B. anthracis* plasmid pX02 for nine of the genes and 99.9 and 99.7 % identity to *cap*D and capsule promoter, respectively. Although some of these plasmid genes have been reported in *B. cereus* and *Bacillus thuringiensis* (Pannucci *et al.*, 2002), the CBD was the first to report pX02 capsule genes in *Bacillus* species that are not in the *B. cereus* group.

When the isolate was not recognized by three commercial systems, API (bioMérieux), OmniLog (Biolog) and RiboPrinter (DuPont-Qualicon), conventional biochemical and physiological tests were conducted to identify and characterize strain CBD 119^T. Sporulation was induced on nutrient agar (NA) (Becton Dickinson) containing 5 mg $MnSO_4$ l^{-1}, grown at 30 °C for 48−72 h. Morphology, Gram-stain reaction and growth at pH 5.7 and 6.8 were tested with cells grown at 30 °C in 100 ml nutrient broth (NB) (Becton Dickinson) in 250 ml flasks aerated by shaking at 125 r.p.m. for 6, 24 and 30 h. Cell morphology, endospore characterization and swelling of the sporangium, presence of parasporal bodies, capsule production and motility were observed in wet mounts by using phase-contrast microscopy at × 1000 under oil. Cells observed for motility were grown to the exponential phase in aerated tryptic soy broth (TSB) (Becton Dickinson) for 3–6 h at 30 °C. Growth of cells at varied temperatures was tested in 3 ml TSB in 13 × 100 mm tubes for 72 h in water baths set to 30, 35, 40, 45, 50 and 55 °C and examined for turbidity at 24 h intervals. Growth of cells at varied pH was tested in the same manner in TSB adjusted to pH 4.5, 5.5, 6.0, 6.5, 7.0, 8.0 and 9.0, incubated at 30 °C. The pH range was also evaluated in cells grown in aerated NB, set at 0.5 intervals of pH 4.5–9.0, incubated at 30 °C and examined for turbidity at 24 and 48 h. Growth dynamics of the cells were studied in 100 ml buffered TSB at pH 5.9 (25 mM MES), pH 7.1 (25 mM MOPS) and pH 7.5 (25 mM MOPS) in 250 ml flasks aerated at 125 r.p.m. and incubated at 30 °C for 24 h. Estimates of cell doubling times were made from plots of optical density against time and from viable cell counts for generation times. Salt tolerance was tested on NA plates supplemented with varied concentrations of NaCl incubated at 30 °C for 7 days. Physiological tests performed on cells grown in commercial medium (REMEL) at 30 °C were as follows: casein and starch hydrolysis, incubated 14 days; growth on mannitol egg yolk polymyxin agar, incubated 48 h; growth in Methyl red Voges–Proskauer (MRVP) broth for final pH, incubated 7 days; and growth on Sabouraud's 4 % glucose agar, pH 5.6, incubated 72 h. Gelatin hydrolysis was tested in 12 % nutrient gelatin (REMEL), grown at 30 °C for 2 weeks. Oxidase reaction was tested with Kovács' phenylenediamine redox dye reagent (Becton Dickinson). Anaerobic growth of the cells was studied for 1 week at 30 °C in the Mitsubishi Pack-Anaero anaerobic gas generating system with the following pre-reduced media: fluid thioglycollate medium with glucose and indicator (REMEL), marine agar plates (Becton Dickinson) and anaerobic agar (Claus & Berkeley, 1986), inoculated in the molten state. Acid production from 49 carbohydrates or carbohydrate derivatives was tested using the API 50CH panel with API CHB/E medium in combination with 11 biochemical tests from the API 20E kit. Following the manufacturer's instructions, the API panels were incubated at 30 °C for 48 h and evaluated by using the API LAB Plus Identification Program V.3.3.3/3.0 (bioMérieux).

Susceptibility studies were performed using the Sensititre (TREK Diagnostics) instrument-based system for MIC determination with ampicillin, chloramphenicol, ciprofloxacin, clarithromycin, clindamycin, erythromycin, gatifloxicin, gentamicin, levofloxacin, moxifloxacin, oxacillin, penicillin, quinopristin/dalfopristin, rifampicin, streptomycin, tetracycline and vancomycin. The Sensititre platform uses a 96-well format with a panel of precision-dosed antimicrobial dilutions to automate the classical microbroth dilution method. The microtitre plates were incubated at 30 °C in the Sensititre ARIS component. The MIC value for each antimicrobial was determined by the instrument and confirmed manually. Amoxicillin and ceftriaxone were tested by Etest (AB Biodisk) following the standardized protocol (CLSI, 2005); the MIC values were evaluated following the manufacturer's instructions. The breakpoints for the *Bacillus* species (CLSI, 2006b) were used for all antimicrobials except clarithromycin, gatifloxacin, moxifloxacin, oxacillin and quinopristin/dalfopristin for which the breakpoints for the *Staphylococcus* species were used (CLSI, 2006a). Results are given in the species description.

Amplification of 16S rRNA gene sequence used universal eubacterial primers 27F and 1492R (Herrick *et al.*, 1993) to give a product of approximately 1500 bp in length. The amplified product was purified with Montage PCR Centrifugal Filter Devices (Millipore). Sequencing was

performed by the Oklahoma Medical Research Foundation DNA Sequencing facility by using an ABI capillary sequencer with primers 27F, 907R, 926F and 704F (Johnson, 1994). Sequencher software (Windows V.4.2, Gene Codes) was used to assemble the fragments. The sequencing data was analysed by comparison of the consensus sequences with sequences available in the GenBank database using the Basic Local Alignment Search Tool in a nucleotide-to-nucleotide (BLASTN 2.2.16) search. 16S rRNA gene sequences from type strains of selected *Bacillus* species were obtained from GenBank and aligned using CLUSTAL_X (Thompson *et al.*, 1997).

When 1495 bp of 16S rRNA gene sequences were compared to sequences available in the GenBank database, strains having greater than 98.5 % sequence similarity (Keswani & Whitman, 2001) to strain CBD 119^T were *Bacillus luciferensis* LMG 18422^T (AJ419629) (99.3 %; gaps=1/1488) and three unpublished *Bacillus* spp., KJ2C12 (AY514023) (99.3 %; gaps=3/1465), GPTSA100-1 (DQ854980) (99.1 %; gaps=1/1447) and L105 (DQ248043) (98.9 %; gaps=1/1435). Unpublished strains *Bacillus* spp. KJ2C12, GPTSA100-1 and L105 are cited in GenBank as an antagonist to *Phytophthora* blight of pepper, an isolate from a warm spring in Assam, India and an isolate from human impact zones in Kartchner Caverns, Arizona, respectively. Strain CBD 119^T had only 95 % sequence similarity to each of the three facultative or obligate alkaliphiles closely related to *B. luciferensis* (Logan *et al.*, 2002), *Bacillus halmapalus* (X76447), *Bacillus horikoshii* (AB043865) and *Bacillus cohnii* (X76437).

The hypervariable regions, V1–V3, in the 16S rRNA gene sequence of the *Bacillus* species correspond to nt 70–344 of *rrnE* (NC_000964.2, GeneID: 2914197) from *B. subtilis* subsp. *subtilis* strain 168 (Blackwood *et al.*, 2004; Goto *et al.*, 2000). The nucleotides of V1–V3 were identified in the sequence of strain CBD 119^T by alignment with conserved regions at positions 47–69, 345–365 and 491–510 of the *B. subtilis* subsp. *subtilis rrnE*. GenBank BL2SEQ program was used to make the pairwise alignments of 460 bp in the hypervariable region of strain CBD 119^T to those of closely related strains, resulting in 99.3 % similarity to *Bacillus* sp. KJ2C12 with 3 nt differences, 99.1 % to *Bacillus* sp. GTPSA100-1 with 4 nt differences and 98.2 % to *B. luciferensis* LMG 18422^T with 8 nt differences. Thus, both *Bacillus* spp. KJ2C12 and GTPSA100-1 ranked above the *B. luciferensis* type strain as the best match to strain CBD 119^T when the comparison was limited to 460 bp of the V1–V3 hypervariable regions.

Phylogenetic trees were constructed using neighbour-joining (NJ), maximum-parsimony (MP) and maximum-likelihood (ML) (PAUP v. 4.0) methods (data not all shown). The data were consistent in essential aspects whether based on 1340 bp of 16S rRNA gene sequence or 414 bp from the hypervariable region. Strain CBD 119^T was consistently in the same clade as *B. luciferensis* LMG 18422^T in MP and ML trees. In an NJ tree (Fig. 1) based on

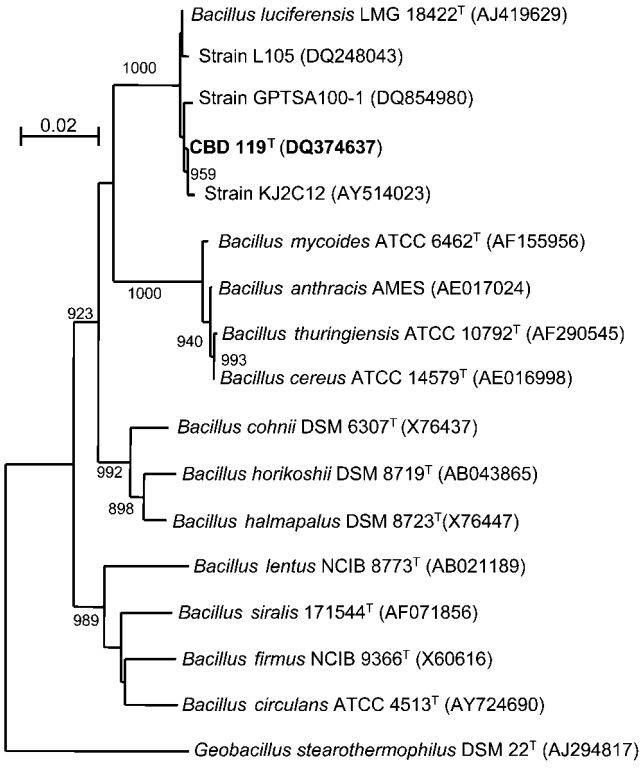

Fig. 1. Phylogenetic relationship of strain CBD 119^T with type strains of other *Bacillus* species and strains from GenBank with highest similarities (accession numbers are given in parentheses). The phylogenetic tree is based on partial 16S rRNA gene sequences (approx. 1340 bp) and was constructed using the NJ algorithm (Swofford, 2002). Numbers show the level of bootstrap support out of 1000 resamplings (values less than 750 are not shown). Bar, 0.02 nucleotide substitutions per nucleotide.

1340 bp comparison, the *B. luciferensis* cluster, as defined by 100 % bootstrap support (Felsenstein, 1985), was divided into two monophyletic branches; strain CBD 119^T groups with *Bacillus* spp. KJ2C12 and GPTSA100-1, while *B. luciferensis* LMG 18422^T groups with *Bacillus* sp. L105. The NJ tree (not shown) based on the comparison of 414 bp also supported *Bacillus* sp. KJ2C12 as the closest relative to strain CBD 119^T. An abbreviated DNA distance matrix (DNADIST, PHYLIP v. 3.6; Felsenstein, 2004) is presented in Supplementary Table S2 (available in IJSEM Online). The matrix shows the nearest neighbours based on the number of substitutions per nucleotide over a sequence length of approximately 1340 bp for all strains. By this measurement, strain CBD 119^T had equal relatedness to both *B. luciferensis* LMG 18422^T and *Bacillus* sp. KJ2C12. The DNA distance matrix, like the MP and ML trees, did not support a closer association of strain CBD 119^T with *Bacillus* sp. KJ2C12 than with *B. luciferensis* LMG 18422^T. Keswani & Whitman (2001) studied the relationship of 16S rRNA gene sequence similarity (*S*) to DNA–DNA hybridization (*D*). Among 40 *Bacillus* species, for an *S* of 0.991, *D*

could be expected to be <0.70 about 99 % of the time. Thus, having an *S* of 99.3 % does not present a high probability that strain CBD 119^T belongs to the same species as either *Bacillus* sp. KJ2C12 or *B. luciferensis* LMG 18422^T.

Being the only named species with a high percentage 16S rRNA gene sequence similarity to strain CBD 119^T, the type strain of *B. luciferensis*, JCM 12212^T (=LMG 18422^T) was acquired from the Japanese Collection of Microorganisms, RIKEN Bioresource Center, for direct polyphasic taxonomic comparisons. *B. cereus* ATCC 14579^T was obtained from the American Type Culture Collection.

Cellular fatty acid composition of strain CBD 119^T and *B. luciferensis* JCM 12212^T was determined by Microbial ID system (MIDI) using the Sherlock Microbial Identification System library RTSB50 5.00. Strains were grown on TSA at 28 °C for 24 h. The major cellular fatty acids measured in strain CBD 119^T and *B. luciferensis* JCM 12212^T were anteiso-$C_{15:0}$ and iso-$C_{15:0}$, and each had ⩾7 iso or anteiso forms, consistent with the *Bacillus* species (Kaneda, 1967; Welch, 1991). Total cellular fatty acids are compared (see Supplementary Table S3 available in IJSEM Online). The isolate and type strain were misidentified as *Brevibacillus*, SI of 0.655 and 0.706, respectively. In proprietary dendrograms scaled to Euclidean distance (ED), an ED of ⩽10 links strains of the same species, an ED of approximately six links subspecies, and *Bacillus* isolates of the same strain link at approximately three ED (MIDI). Strain CBD 119^T linked with *B. luciferensis* JCM 12212^T at ⩾10.5 ED, indicating the two are likely to be different but closely related species.

Capsules were observed in India ink mounts by phase-contrast microscopy at ×1000 under oil (Luna *et al.*, 2006). Strain CBD 119^T produced thin but omnipresent capsules on TSA grown overnight at 35 °C with or without 5 % CO_2 atmosphere. *B. luciferensis* JCM 12212^T failed to produce capsules under all conditions tested. Strain CBD 119^T was persistently negative in DFA capsule tests, suggesting that while the complete operon is present (Luna *et al.*, 2006) the capsule does not manifest the epitope recognized by the test antibody. Alternatively, the observed capsule may be the product of genes other than those on the pX02-like plasmid. The chemical nature of the CBD 119^T capsule has not been determined.

Phenotypically, strain CBD 119^T was inconsistent with *B. luciferensis* JCM 12212^T for 18 of 96 phenotypic traits evaluated (data not all shown), including motility, degree of chain formation and endospore-driven swelling. Strain CBD 119^T had a more narrow temperature range for robust growth and an acidic pH optimum, suggesting that the isolate and *B. luciferensis* occupy different niches. Differential traits for strain CBD 119^T and the type strain are compared in Table 1. The G+C DNA contents of strain CBD 119^T and *B. luciferensis* JCM 12212^T were determined by the Identification Service of the DSMZ following the methods of Cashion *et al.* (1977), Mesbah *et al.* (1989) and Tamaoka & Komagata (1984); for the percentage of DNA–DNA hybridization, DSMZ followed Cashion *et al.* (1977) and De Ley *et al.* (1970). DNA base composition of *B. luciferensis* JCM 12212^T (=LMG 18422^T) was 36.8 mol% G+C, whereas strain CBD 119^T was 37.3 mol%. Logan *et al.* (2002) reported 33.0 mol% DNA G+C content of LMG 18422^T by using a different HPLC system, column, running conditions and solvent system. Two measurements of DNA–DNA relatedness indicated very low-level affinity of 12.9 and 17.9 % between strain CBD 119^T and *B. luciferensis* JCM 12212^T. Based on

Table 1. Differential characteristics of the type strains of *Bacillus acidiceler*, *B. luciferensis* and *B. cereus*

Strains: 1, *B. acidiceler* CBD 119^T; 2, *B. luciferensis* JCM 12212^T; 3, *B. luciferensis* LMG 18422^T (Logan *et al.*, 2002); 4, *B. cereus* ATCC 14579^T. +, Positive; −, negative; (+), weakly positive; ((+)), very weakly positive; ± positive and negative reactions for strain; NR, not reported. Both CBD 119^T and JCM 12212^T produced acid in API 50CH panels from amygdalin, arbutin, D-cellobiose, D-fructose, gentiobiose, D-glucose, *N*-acetyl-D-glucosamine, maltose, salicin, sucrose and trehalose; did not produce acid from adonitol, D-arabinose, L-arabinose, D-arabitol, L-arabitol, dulcitol, erythritol, D-fucose, L-fucose, D-galactose, 2- or 5-keto-D-gluconate, methyl α-D-glucoside, inositol, inulin, lactose, L-lyxose, methyl α-D-mannoside, D-melezitose, melibiose, D-raffinose, L-raffinose, L-rhamnose, D-ribose, D-sorbitol, sorbose, D-tagatose, xylitol, D-xylose, L-xylose or β-methyl-xyloside.

Characteristic	1	2	3	4
Chain formation pronounced	+	−	−	−
Endospore-driven swelling overt	+	−	−	−
Motility	−	+	+	+
Oxidase reaction	+	±	NR	−
Hydrolysis of casein	+	(+)	+	+
Hydrolysis of gelatin:				
API 20E panel	+	((+))	+	+
Nutrient gelatin	+	−	NR	NR
Nitrate reduction:				
API 20E panel	−	−	−	+
Agar-based test	+	−	NR	NR
pH in MRVP broth, incubated 1 week	~6	~5	NR	NR
pH optimum	5.8–6.3	~7	7	NR
Colony diameter on pH 5.6 agar, 3 days	⩾5 mm	⩽1 mm	NR	NR
Acid production from:				
Gluconate	+	−	−	−
Glycerol	(+)	−	−	+
Glycogen	(+)	−	−	+
D-Mannitol	+	−	−	−
D-Mannose	+	−	−	−
Starch	(+)	−	(+)	+
D-Turanose	−	+	+	−
Anaerobic growth:				
In thioglycollate medium	−	−	NR	+
In anaerobic agar	−	−	NR	+

the recommended threshold value of 70 % DNA–DNA relatedness between strains of the same bacterial species (Wayne *et al.*, 1987; Stackebrandt *et al.*, 2002), strain CBD 119^T does not belong to the species *B. luciferensis*. Having <20 % DNA–DNA relatedness value with *B. luciferensis* JCM 12212^T, the only species with validly published name to which the isolate had >97 % 16S rRNA sequence similarity, a new species designation for CBD 119^T is justified.

Description of *Bacillus acidiceler* sp. nov.

Bacillus acidiceler (a.ci.di.ce′ler. L. neut. n. *acidum* acid, L. masc. adj. *celer* fast, N.L. masc. adj. *acidiceler* fast growing in acid.).

Colonies grown at 30 °C for 24 h on TSA with 5 % sheep blood are approximately 3 mm in diameter, cream to pearly grey, non-haemolytic, like sintered glass in opacity, smooth, shiny, becoming dull with a fine crepe texture with age, round, entire, raised to low convex and becoming crater form with age. Cells grown in NB, pH 5.8, 30 °C, aerated, are non-motile, round-ended rods (0.8–1 × 2–12 μm) with rudimentary branching and pronounced chaining; are initially long and flexed (3 h), becoming grossly pleomorphic with distended forms in torturous whorls flanked by ghost cells (23 h); stain Gram-positive to Gram-variable at 6 h. Cells in TSB, pH 5.9, 30 °C, aerated, at 6 h are pairs or short chains of bifurcating rods (1–1.2 × 7–15 μm) with branches of 2–15 μm in length (Fig. 2a, b); branches originate at one or less often, both poles and occasionally from the sidewalls. Colonies grown at 30 °C for 24 h on NA, pH 6.8, and TSA, pH 6.0 and 7.2, produced only rare rudimentary branching. Endospores are ellipsoidal and usually subterminal in unswollen to overtly swollen cells (Fig. 2c). No parasporal bodies or crystals were observed. Thin, omnipresent capsule is produced; genes (*acp*A, *cap*A, *cap*B, *cap*C, *cap*R, *cap*D, IS*1627*, ORF 48, ORF 61 and *rep*A) and capsule promoter with ⩾99.7 % sequence similarity to the capsule operon of *B. anthracis* are carried on a large plasmid. Catalase- and oxidase-positive, does not grow anaerobically in the presence of glucose. Reduces nitrate to nitrite, weakly Voges–Proskauer positive. β-Galactosidase, arginine dihydrolase, indole, ornithine, lysine decarboxylase and citrate are not utilized. Hydrolyses casein, gelatin and aesculin, but not starch or urea. Optimum pH is near 6; grows well on Sabouraud's agar at pH 5.6 but not at 4.5; alkaline range is to 8.5. Respective doubling times (optical density against time) and generation times at 30 °C in aerated, buffered TSB are 30 and 32 min at pH 5.9, 46 and 38–49 min at pH 7.1, and 52 and 48 min at pH 7.5. Autolysis is incipient as early as 4 h and extensive at 24 h in TSB at pH 7.1 and 7.5. Does not grow with 5 % NaCl or at 45 °C. Fatty acids anteiso-$C_{15:0}$ (46.66 %), iso-$C_{15:0}$ (28.11 %), iso-$C_{16:0}$ (8.21 %) and iso-$C_{14:0}$ (4.47 %) predominate. The DNA G+C content is 37.3 mol%. Acid produced in API 50CH panels from amygdalin, arbutin, D-cellobiose, D-fructose, gentiobiose, gluconate, D-glucose, *N*-acetyl-D-glucosamine, glycerol, glycogen, maltose, D-mannitol, D-mannose, salicin, starch, sucrose, trehalose. Acid not produced from adonitol, D-arabinose, L-arabinose, D-arabitol, L-arabitol, dulcitol, erythritol, D-fucose, L-fucose, D-galactose, 2- or 5-keto-D-gluconate, methyl α-D-glucoside, inositol, inulin, lactose, L-lyxose, methyl α-D-mannoside, D-melezitose, melibiose, D-raffinose, L-raffinose, L-rhamnose, D-ribose, D-sorbitol, sorbose, D-tagatose, D-turanose, xylitol, D-xylose, L-xylose or β-methyl-xyloside. Resistant to penicillin, oxacillin, ampicillin, amoxicillin and ceftriaxone; susceptible to chloramphenicol, ciprofloxacin, clarithromycin, clindamycin, erythromycin, gatifloxicin, gentamicin, levofloxacin, moxifloxacin, quinopristin/dalfopristin, rifampicin, streptomycin, tetracycline and vancomycin.

The type strain CBD 119^T (=NRRL B-41736^T=DSM 18954^T) was isolated in Tampa, FL from a white powder collected on a window sill and suspected of harbouring *B. anthracis* during the aftermath of the anthrax bioterrorism attacks of October 2001.

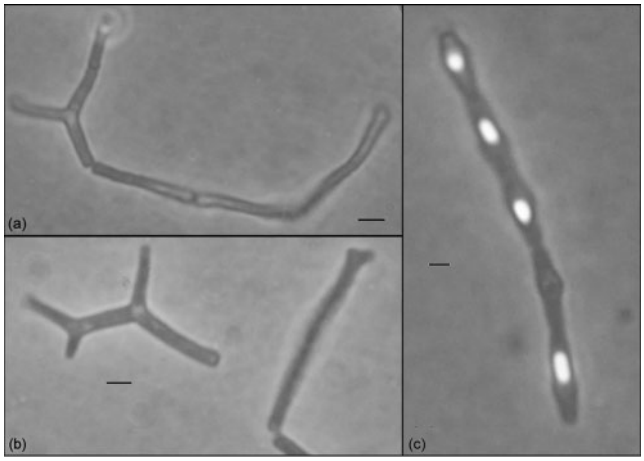

Fig. 2. Photomicrographs of vegetative cells and sporangia of proposed *Bacillus acidiceler* sp. nov. CBD 119^T viewed by phase-contrast microscopy. (a, b) Branched vegetative cells grown at 30 °C for 6 h in aerated TSB, pH 5.9. (c) Ellipsoidal endospores lie subterminally in unswollen or swollen sporangia. Bars, 2 μm.

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