Bacillus shackletonii sp. nov., from volcanic soil on Candlemas Island, South Sandwich archipelago

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A sample of mossy soil taken from the eastern lava flow of northern Candlemas Island, South Sandwich archipelago, yielded six isolates of aerobic, endospore-forming bacteria. Miniaturized routine phenotypic tests and other observations, amplified rDNA restriction analysis and SDS-PAGE analysis suggested that the strains represent a novel taxon. 16S rDNA sequence comparisons support the proposal of a novel species, *Bacillus shackletonii* sp. nov., the type strain of which is LMG 18435^T (= CIP 107762^T).

Logan *et al.* (2000) reported the isolation of aerobic, endospore-forming bacteria from six soil samples collected during the 1996–1997 austral summer from different parts of northern Candlemas Island in the South Sandwich archipelago (see figures and plates in Tomblin, 1979; Fig. 1 in Logan *et al.*, 2000). The northern part of Candlemas Island is actively volcanic and comprises a roughly circular mass of five main lava flows surrounding Lucifer Hill, which is a complex of scoria cones with active fumaroles. The east flow is one of the oldest and bears an ash mantle, while the youngest, north, lava field is very recent (Tomblin, 1979). Samples of mossy soil, whose temperatures ranged from 85 °C at the summit of the hill to 0 °C at its base, were collected by British Antarctic Survey personnel for the purpose of investigating the aerobic, endospore-forming bacterial flora. It was found that two strains (LMG 18422^T and LMG 18435^T) represented unidentifiable species of *Bacillus*, while other samples yielded unidentified members of *Paenibacillus*. Further isolations from the soil sample that yielded LMG 18422^T led to the proposal of the novel species *Bacillus luciferensis* (Logan *et al.*, 2002). The present note describes the isolation and characterization of LMG 18435^T and five other isolates found in a sample of unheated soil taken from the east lava flow, and proposes the novel species *Bacillus shackletonii* sp. nov.

Strains were isolated from trypticase soy broth enrichments at pH 5.5 incubated at 40 °C, but were subsequently cultivated and maintained on trypticase soy agar at pH 6.5, containing 5 mg MnSO₄ l⁻¹ to enhance sporulation, with incubation at 30 °C as described by Logan *et al.* (2000). Strain LMG 18435^T (= B1724^T) and the further strains R-11667, R-11668, R-14112, R-14113 and R-14114 (= B1725, B1726, B1843, B1844 and B1845, respectively) were isolated from a sample of unheated mossy soil collected at the north-western margin of the east lava flow near the foot of Lucifer Hill (temperature range 0–15 °C; altitude 30 m; site 6 in Logan *et al.*, 2000); this flow forms a plain, known as Breakbones Plateau, that is covered by a mantle of stratified ash (Holdgate & Baker, 1979; Tomblin, 1979). Strains were phenotypically characterized as described by Logan & Berkeley (1984) and Logan *et al.* (2000); anaerobic growth was tested using a GasPak jar (Becton Dickinson) with a methylene blue indicator strip, and an aerobic control culture. All six strains were subjected to GC analysis of methylated fatty acids, SDS-PAGE analysis, amplified rDNA restriction analysis and DNA base composition analysis; strain LMG 18435^T was also subjected to 16S rDNA sequencing, as described by Logan *et al.* (2000, 2002). The G+C content of the DNA was determined by HPLC (Mesbah *et al.*, 1989) using further specifications given by Logan *et al.* (2000). Antibiotic sensitivities were measured using Mastrings (MAST Laboratories) on Iso-Sensitest agar (Oxoid).

All of the strains were found to be Gram-negative, aerobic, motile rods that formed ellipsoidal spores lying subterminally, and sometimes paracentrally, in sporangia that were usually slightly swollen (Fig. 1). The strains gave similar patterns of results in the phenotypic analyses, although

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The EMBL accession number for the 16S rRNA gene sequence of *Bacillus shackletonii* LMG 18435^T is AJ250318.

Details of the fatty acid methyl ester compositions of *B. shackletonii* strains are available as supplementary data in IJSEM Online.

Fig. 1. Photomicrograph of sporangia and vegetative cells of the type strain of *B. shackletonii* sp. nov. viewed by phase-contrast microscopy; ellipsoidal spores lie subterminally, and occasionally paracentrally, in sporangia that are usually swollen. Bar, 2 µm.

many reactions were weak, and they clustered together at 95 % similarity in a UPGMA cluster analysis (not shown) based upon these characteristics; this cluster merged with strains of *Bacillus firmus* at only 85 % similarity and with strains of *B. luciferensis*, *Bacillus oleronius* and *Bacillus sporothermodurans* at 82.5 % similarity, indicating that the novel strains form a group that is phenotypically distinct. The characteristics that differentiate this group of strains from phenotypically similar species are shown in Table 1. Although the six strains were isolated from the same sample of soil, they showed sufficient phenotypic variation to suggest that they are not merely repeated isolations of the same strain. Comparison of the amplified rDNA restriction analysis pattern of strain LMG 18435^T with a database of over 1000 authentic strains of species of aerobic endospore-forming bacteria did not yield a clear-cut identification; the highest similarities were only 84 % to the type strain of *Bacillus smithii* and 82 % to the type strain of *B. sporothermodurans*. The six strains subjected to SDS-PAGE analysis showed at least 86 % similarity, reflecting limited intraspecies variation (Fig. 2), which, given the high similarity between these strains in terms of the other phenotypic characteristics, remains consistent with the view that these strains belong to the same species (strain R-11668 is not included in Fig. 2 as it gave a weak pattern). The six strains also showed very similar profiles for major cellular

Table 1. Characteristics that distinguish between *B. shackletonii* sp. nov. and some phenotypically similar and phylogenetically related *Bacillus* species

Species: 1, *B. shackletonii* sp. nov.; 2, *B. sporothermodurans*; 3, *B. oleronius*; 4, *B. luciferensis*; 5, *B. firmus*; 6, *B. cereus*; 7, *B. subtilis*. With the exception of microscopic observations, anaerobic growth and casein hydrolysis, all characteristics were determined using tests in the API 20E and 50 CHB systems (bioMérieux). +, >85 % Positive; (+), 75–84 % positive; V, variable (26–74 % positive); –, 0–15 % positive; w, weak positive reaction. All species are positive for aesculin hydrolysis.

Characteristic	1	2	3	4	5	6	7
Spore formation:							
Spore position*	S (C)	T	S, C	S (T)	S, C	S (C)	S
Sporangia swollen	+	–	+	V	–	–	–
Anaerobic growth	–	–	–	+	+	+	–
Gelatin hydrolysis	–†	–	–	+	+	+	+
Casein hydrolysis	w‡	–	–	+	+	+	+
Nitrate reduction	–	–	+	–	(+)	V	+
Voges–Proskauer reaction	–†	w	V	+	+	+	+
Acid from:							
Amygdalin	+	–	–	+	–	–	+
Galactose	w	–	–	–	–	–	V
β-Gentiobiose	w	–	–	+	–	–	+
Glycerol	–	–	V	–	+	+	+
Lactose	w	–	–	–	–	–	V
D-Mannose	w	–	V	–	(+)	–	+
D-Melezitose	–	–	–	+	–	–	–
Ribose	w	–	+	–	V	+	+
Salicin	+	w	V	+	–	+	+
Starch	–	–	+	w	+	+	+
Sucrose	–	+	V	+	+	V	+
D-Tagatose	w	+	+	–	–	–	–

*C, Central/paracentral; S, subterminal; T, terminal. Positions that are infrequently observed are shown in parentheses.

†Gelatin hydrolysis and Voges–Proskauer reactions may become positive slowly at 40 °C.

‡Grows poorly and gives weak reactions, or does not grow at all, on casein agar.

Fig. 2. Normalized computer profiles from SDS-PAGE analyses of whole-cell proteins of five *B. shackletonii* strains. The dendrogram is based on UPGMA clustering of the correlation coefficient (*r*) of the total protein profiles. The zone used for clustering was between point 50 and point 340 (a complete lane contains 400 points).

Fig. 3. Neighbour-joining clustering of 16S rDNA sequences (rooted with *Filobacillus milosensis*) based on a selection of 16S rDNA sequences from the EMBL database (accession numbers are given) and *B. shackletonii* sp. nov. LMG 18435^T.

entries in the EMBL database, the closest matches achieved for LMG 18435^T were with *B. oleronius* (96.6 % similarity) and *B. sporothermodurans* (97.2 % similarity) (Fig. 3). Our failure to identify the Candlemas Island strains by means of the genotypic and phenotypic methods tried, and the strong phenotypic similarities among the strains, support the proposal of a novel species, *Bacillus shackletonii* sp. nov., with LMG 18435^T as the type strain.

Description of *Bacillus shackletonii* sp. nov.

Bacillus shackletonii (sha.ckle.ton'i.i. N.L. gen. n. *shackletonii* of Shackleton, referring to RRS *Shackleton*, the ship used by the first British scientific expedition to visit Candlemas Island, the vessel being named in honour of the celebrated Anglo-Irish Antarctic explorer Sir Ernest Shackleton).

Isolated from unheated volcanic soil taken from the east lava flow of Candlemas Island, South Sandwich archipelago. Cells are motile, round-ended rods (0.7–0.9 × 2.5–4.5 µm) occurring singly. Gram-variable; Gram-positive reactions are only seen in cultures at 18 h or at temperatures below 30 °C. Endospores are ellipsoidal, lie subterminally and occasionally paracentrally, and usually cause the sporangia to swell (Fig. 1). After 2 days on trypticase soy agar, colonies are 2–5 mm in diameter, have a granular appearance and butyrous texture, have opaque, cream-coloured centres and have translucent, irregular margins. The minimum temperature for growth lies between 15 and 20 °C, the optimum temperature for growth is 35–40 °C and the maximum growth temperature is 50–55 °C. The minimum pH for growth lies between 4.5 and 5.0, the optimum pH for growth is 7.0 and the maximum pH for growth lies between 8.5 and 9.0. Organisms are strictly aerobic and catalase-positive. They do not grow readily on casein agar but, when they do grow on it, they may hydrolyse the casein. Starch is not hydrolysed. In the API 20E strip (bioMérieux) incubated at 30 °C, ONPG is hydrolysed slowly; reactions for arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, citrate utilization, hydrogen sulphide production, urease, tryptophan deaminase, indole production, the Voges–Proskauer reaction, gelatin hydrolysis and nitrate reduction are negative (in the API 20E strip incubated at 40 °C, citrate may be utilized slowly, gelatin may be hydrolysed slowly and the Voges–Proskauer reaction may be positive). In the API 50 CH gallery (bioMérieux), hydrolysis of aesculin is positive. Acid without gas is produced from the following carbohydrates in the API 50 CH gallery using the CHB suspension

medium (bioMérieux): amygdalin, cellobiose, D-glucose, *N*-acetylglucosamine and salicin; weak acid reactions were detected for arbutin, D-fructose, galactose, β-gentiobiose, lactose, maltose, D-mannitol, D-mannose, ribose, D-tagatose and D-trehalose. Acid is not produced from the following carbohydrates: adonitol, D- and L-arabinose, D- and L-arabitol, dulcitol, erythritol, D- and L-fucose, gluconate, glycerol, glycogen, *meso*-inositol, inulin, 2-keto-D-gluconate, 5-keto-D-gluconate, D-lyxose, D-melezitose, melibiose, methyl α-D-glucoside, methyl α-D-mannoside, methyl xyloside, D-raffinose, rhamnose, sorbitol, L-sorbose, starch, sucrose, D-turanose, xylitol and D- and L-xylose. Cells are sensitive to filter-paper disks containing the following antibiotics: ampicillin (25 µg), chloramphenicol (50 µg), colistin sulphate (100 µg), kanamycin (30 µg), nalidixic acid (30 µg), nitrofurantoin (50 µg), streptomycin (25 µg) and tetracycline (100 µg). The major cellular fatty acids are anteiso C_{15:0}, iso C_{15:0}, iso C_{16:0} and anteiso C_{17:0} (respectively representing about 35, 31, 6 and 18 % of total fatty acids). The following fatty acids were present in smaller amounts (between about 1 and 3 %): C_{14:0}, iso C_{14:0}, iso C_{16:1ω11c}, summed feature 4 (C_{17:1} iso I and/or C_{17:1} anteiso B) and iso C_{17:1ω10c}. Details of the fatty acid methyl ester composition are available as supplementary data in IJSEM Online. The G + C content of the DNA varies between 35.4 mol% (type strain) and 36.8 mol%.

The type strain is LMG 18435^T (=CIP 107762^T=Logan collection number B1724^T=isolate SSI024^T).

Acknowledgements

We are very grateful to P. Convey of the British Antarctic Survey for collecting soil samples from Candlemas Island. We are most grateful to bioMérieux sa and bioMérieux, Inc. for providing API materials and for supporting G.F. and M.R.-D.; P.D.V. is indebted to the National Fund for Scientific Research, Flanders (FWO, Vlaanderen) for personnel and research grant G.0156.02.

References

- Holdgate, M. W. & Baker, P. E. (1979). The South Sandwich Islands: I. General description. *Br Antarct Surv Sci Rep* **91**, 1–76.
- Logan, N. A. & Berkeley, R. C. W. (1984). Identification of *Bacillus* strains using the API system. *J Gen Microbiol* **130**, 1871–1882.
- Logan, N. A., Lebbe, L., Hoste, B., Goris, J., Forsyth, G., Heyndrickx, M. & De Vos, P. (2000). Aerobic endospore-forming bacteria from geothermal environments in northern Victoria Land, Antarctica, and Candlemas Island, South Sandwich archipelago, with

the proposal of *Bacillus fumarioli* sp. nov. *Int J Syst Evol Microbiol* **50**, 1741–1753.

Logan, N. A., Lebbe, L., Verhelst, A., Goris, J., Forsyth, G., Rodriguez-Diaz, M., Heyndrickx, M. & De Vos, P. (2002). *Bacillus luciferensis* sp. nov., from volcanic soil on Candlemas Island, South Sandwich archipelago. *Int J Syst Evol Microbiol* **52**, 1985–1989.

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.

Tomblin, J. F. (1979). The South Sandwich Islands: II. The geology of Candlemas Island. *Br Antarct Surv Sci Rep* **92**, 1–33.