

Brevibacillus levickii sp. nov. and *Aneurinibacillus terranovensis* sp. nov., two novel thermoacidophiles isolated from geothermal soils of northern Victoria Land, Antarctica

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Thirteen strains of endospore-forming bacteria were isolated from geothermal soils at Cryptogam Ridge, the north-west slope of Mt Melbourne, and at the vents and summit of Mt Rittmann in northern Victoria Land, Antarctica. 16S rRNA gene sequencing, SDS-PAGE and routine phenotypic characterization tests indicated that the seven isolates from the north-west slope of Mt Melbourne represent a novel species of *Brevibacillus* and that the six isolates from Cryptogam Ridge and the vents and summit of Mt Rittmann represent a novel species of *Aneurinibacillus*. *Brevibacillus* strains were not isolated from the sites at Mt Rittmann or Cryptogam Ridge and *Aneurinibacillus* strains were not isolated from the north-west slope of Mt Melbourne. Preliminary metabolic studies revealed that L-glutamic acid, although not essential for growth, was utilized by both species. The *Brevibacillus* species possessed an uptake system specific for L-glutamic acid, whereas the *Aneurinibacillus* species possessed a more general uptake system capable of transporting other related amino acids. Both species utilized a K⁺ antiport system and similar energy systems for the uptake of L-glutamic acid. The rate of uptake by the *Brevibacillus* species type strain was 20-fold greater than that shown by the *Aneurinibacillus* species type strain. The names *Brevibacillus levickii* sp. nov. and *Aneurinibacillus terranovensis* sp. nov. are proposed for the novel taxa; the type strains are Logan B-1657^T (= LMG 22481^T = CIP 108307^T) and Logan B-1599^T (LMG 22483^T = CIP 108308^T), respectively.

INTRODUCTION

Areas of geothermal activity in continental and maritime Antarctica are of particular biological interest because their moisture supplies are more regular than in other parts of the continent or in the circumpolar islands that are ice-free.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of *Brevibacillus levickii* LMG 22481^T, R-12317, R-22009, LMG 22482, R-12318, R-22010 and R-22013 and *Aneurinibacillus terranovensis* LMG 22483^T, R-12871, R-12872, R-12873, R-12874 and LMG 22484 are AJ715378–AJ715390, respectively.

Graphs showing the effects of ionophores on glutamate uptake (Fig. A) and tables giving the fatty acid profiles (Table A) and the effects of various competitors (Table B) and energy inhibitors (Table C) on glutamate uptake in strains LMG 22481^T and LMG 22483^T are available as supplementary material in IJSEM Online.

Vegetation at these sites is supported by the moisture created from the condensation of steam emissions of fumaroles and from the melting of ice (Broady, 1993). Two such sites are Mt Melbourne and Mt Rittmann in northern Victoria Land, Antarctica (Fig. 1a). Mt Melbourne (2733 m; 74° 21' S, 164° 42' E) is a stratovolcano situated in the centre of a relatively young (probably 2–3 × 10⁶ years B.P.) volcanic field that has been formed by a large number of small, individual eruptive centres (Broady *et al.*, 1987) and some of the higher parts of the mountain bear large areas of hot ground and fumaroles. Located on the southern rim of the main summit crater of Mt Melbourne is a Specially Protected Area called Cryptogam Ridge (Fig. 1b). This is a deglaciated site with soil temperatures typically reaching 40–50 °C at depths of a few centimetres; it supports a unique community including algal and bryophyte species unknown elsewhere in Antarctica (Nicolaus *et al.*, 1991).

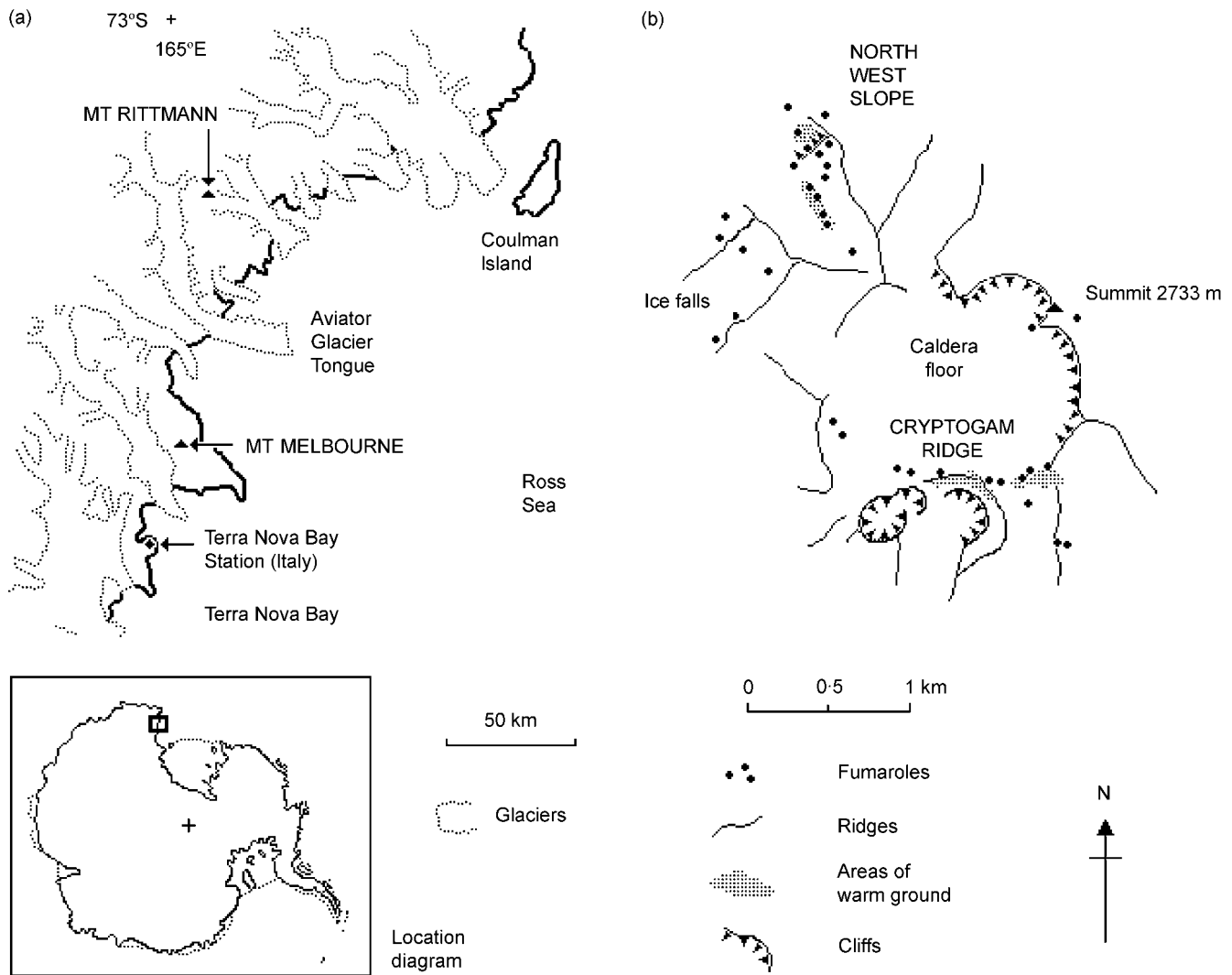


Fig. 1. (a) Map of northern Victoria Land showing the locations of Mt Melbourne and Mt Rittmann, with an inset map of Antarctica showing the location of the region (based on Logan *et al.*, 2000). (b) Map of the caldera region at the summit region of Mt Melbourne and the location of Cryptogam Ridge (based on Broady *et al.*, 1987).

The flora of the geothermally heated area of the north-west slope of the mountain is less well developed than that of Cryptogam Ridge. Mt Rittmann (2600 m; $73^{\circ} 27' S$, $165^{\circ} 30' E$), which was discovered during the 4th Italian Expedition (1988–1989; Armienti & Tripodo, 1991), is located in the Mountaineer Range, about 110 km north of Mt Melbourne. During the 6th Italian Antarctic Expedition (1990–1991), fumaroles and heated ground were discovered in a minor caldera rim of this mountain (Bonaccorso *et al.*, 1991). The soil surface temperature is 34.4 – $41.5^{\circ} C$ and there is patchy development of vegetation. Although the soils of the two mountains are comparable, Bargagli *et al.* (1996) showed that there were differences in the mineral contents of soil samples collected from Mt Melbourne (higher Cu and Zn) and Mt Rittmann (higher Cd and Pb). During the 11th and 14th Italian Antarctic Expeditions (1995–1996 and 1998–1999, respectively) samples of heated soils were collected

from Cryptogam Ridge, the north-west slope of Mt Melbourne and Mt Rittmann, in order to study the aerobic endospore-forming bacterial floras of these sites (Logan *et al.*, 2000). This report describes the characterization of six strains of a novel *Aneurinibacillus* species isolated from soils of Cryptogam Ridge and Mt Rittmann and seven strains of a novel *Brevibacillus* species from the north-west slope of Mt Melbourne. The names *Aneurinibacillus terranovensis* sp. nov. and *Brevibacillus levickii* sp. nov. are proposed for these novel taxa.

Brevibacillus and *Aneurinibacillus* were established as new genera arising from the reclassification of the *Bacillus brevis* and *Bacillus aneurinilyticus* groups of species (Shida *et al.*, 1996). Currently, there are 12 *Brevibacillus* species and four *Aneurinibacillus* species. Given their poor reactivity in conventional identification tests, it has been difficult to

distinguish between species of these genera (Logan *et al.*, 2002); amplified rDNA restriction analysis is also unable to discriminate between all of the species (Logan *et al.*, 2002). However, some techniques have proved successful, e.g. SDS-PAGE (Logan *et al.*, 2002) and, more recently, sequencing of the hypervariable regions that are conserved within species and diverge between species (Goto *et al.*, 2004). The unique environmental conditions from which these species have been isolated, the restriction of the *Brevibacillus* strains to the north-west slope of Mt Melbourne and the absence of *Aneurinibacillus* strains from that site stimulated us to compare their metabolism. The investigation of the metabolic pathways of these organisms could be of value in the characterization of these two species, given their poor reactivities in conventional identification tests. Glutamate uptake and metabolism were studied, given the probable availability of this substrate in these habitats from primary producers such as cyanobacteria and microalgae. This is not only the first metabolic study of this kind for members of these genera, but also for aerobic endospore-forming bacteria from Antarctic geothermal environments.

METHODS

Isolation of strains. Strain isolation details are given by Logan *et al.* (2000). Strains LMG 22482, R-22010, R-12318, R-22009, R-12317, LMG 22481^T and R-22013 (strains Logan B-1598, B-1606, B-1607, B-1608, B-1611, B-1657^T and B-1659, respectively) were isolated from the north-west slope of Mt Melbourne. Strains LMG 22483^T and R-12871 (strains Logan B-1599^T and B-1624, respectively) were isolated from Cryptogam Ridge at Mt Melbourne. Strains R-12872 and R-12873 (strains Logan B-1633 and B-1634, respectively) were isolated from vents of Mt Rittmann and strains R-12874 and LMG 22484 (strains Logan B-1636 and B-1641, respectively) were isolated from the summit of Mt Rittmann.

Cultivation and maintenance of strains. Strains were initially grown on a variety of media in order to identify which medium supported optimal growth. Media tested included full-strength, half-strength (1/2 BFA) and a quarter-strength version (1/4 BFA) of *Bacillus fumarioli* agar (BFA; Logan *et al.*, 2000). BFA contained (l^{-1}) 4 g yeast extract, 2.5 g $(NH_4)_2SO_4$, 3 g KH_2PO_4 , 5 mg $MnSO_4$, 0.2 g $MgSO_4 \cdot 7H_2O$, 0.25 g $CaCl_2 \cdot 2H_2O$ and 18 g agar, adjusted to pH 5.5. A defined medium was created, based on BFA and 1/2 BFA, in which the yeast extract and $(NH_4)_2SO_4$ were omitted and replaced with 1 ml vitamin solution described by Dijkhuizen *et al.* (1988) and 0.06 g each of 13 L- and DL-amino acids (L-alanine, L-asparagine, L-aspartic acid, L-cysteine, L-glutamic acid, L-isoleucine, L-leucine, L-lysine, L-methionine, DL-norleucine, DL-norvaline, L-threonine and L-valine). The amino acids to be included in this defined medium were determined by creating a broth consisting of all 23 amino acids and then creating subsequent broths in which families of these amino acids or single amino acids were omitted. Those amino acids that were omitted from broths that yielded no growth were deemed as being essential for growth and included in the defined medium. This defined medium was also prepared without the vitamin solution. A variation of Davis and Mingioli minimal medium (Cruickshank *et al.*, 1975) was also used; it contained (l^{-1}) 20 ml sterile 10% glucose solution, 3.5 g K_2HPO_4 , 1.5 g KH_2PO_4 , 0.5 g sodium citrate ($Na_3C_6H_5O_7 \cdot 2H_2O$), 0.1 g $MgSO_4 \cdot 7H_2O$, 1 g $(NH_4)_2SO_4$, 20 g agar and 2 g yeast extract. A further variation of this medium lacked both glucose and $(NH_4)_2SO_4$. The most rapid

and profuse growth was achieved through the use of 1/2 BFA at pH 5.5 and 40 °C; this medium was used throughout the remainder of the study along with the broth version, 1/2 BFB (*Bacillus fumarioli* broth), which lacked agar and $MnSO_4$. A broth version of the defined medium, lacking agar and $MnSO_4$, was used for the metabolic studies.

DNA preparation, DNA base composition and 16S rRNA gene sequencing. These were carried out as described by Logan *et al.* (2000).

SDS-PAGE of whole-cell proteins. Cells were obtained after 48 h growth on 1/2 BFA at 28 °C. The SDS protein extracts were prepared and electrophoresed according to Pot *et al.* (1994) and data were collected and interpreted as described by Vauterin & Vauterin (1992).

DNA–DNA relatedness. DNA–DNA hybridization was performed using a modification of the microplate method of Ezaki *et al.* (1989), as described by Willems *et al.* (2001).

GC analysis of methylated fatty acids. Cells were grown for 48 h on 1/2 BFA at 28 °C and subsequently analysed as described by Logan *et al.* (2000).

Phenotypic analysis. Isolates were grown on 1/2 BFA at pH 5.5 and 40 °C for 24–72 h and vegetative cells and sporangial morphologies were observed as described by Logan *et al.* (2000). As colonies of some strains on agar media were tenacious and cohesive, capsule production was investigated using an adaptation of the India ink wet-film method described by Duguid (1951); initially the cells were stained with 1% rose Bengal and India ink was substituted with 1% nigrosin. Temperature ranges for growth were determined by incubating the organisms in 10 ml 1/2 BFB in water baths set at 15, 20, 25, 30, 37, 40, 45, 50 and 55 °C. The pH ranges for growth were determined by growing the organisms at 40 °C in 10 ml 1/2 BFB adjusted to pH 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5 and 7.0 using HCl and NaOH. Turbidities of both series were determined using a spectrophotometer (Cecil CE1010) set at 680 nm, at 24 h intervals. The isolates were tested for anaerobic and microaerobic growth in GasPak jars (BBL) using aerobically grown isolates as controls; a *Campylobacter* sachet (BBL) was used to create microaerobic conditions. Haemolysis was tested using 5% horse blood with Columbia Blood Agar base (Oxoid) and 1/2 BFA as a base. Egg-yolk reaction, starch hydrolysis and casein hydrolysis were tested using the methods of Gordon *et al.* (1973) with 1/2 BFA as the base. Gelatin hydrolysis was determined by aseptically removing the charcoal gelatin from API 20E strips (bioMérieux) and adding them to 1 ml 1/2 BFB before inoculation. Strains were also characterized using the API 20E kit as described by Logan & Berkeley (1984). All isolates were tested for carbon source utilization and for acid or alkali production from the same substrates using the Biotype 100 gallery (bioMérieux), which comprises 99 carbon sources, such as carbohydrates, amino acids and organic acids, and one control tube. 1/2 BFB (200 ml) was inoculated and incubated for 24 h at 40 °C. The cells were then centrifuged at 2000 g for 15 min, the supernatant was discarded, the pellet was resuspended in 10 ml of 10 mM phosphate buffer and re-centrifuged (2000 g for 15 min). The resulting supernatant was discarded and the pellet was resuspended in 2 ml phosphate buffer. Drops of this bacterial suspension were added to sterile 0.45% sodium chloride solution until a turbidity equivalent to 3 on the McFarland scale was achieved. For carbon source utilization testing, this suspension (2 ml) was homogenized in 60 ml Biotype medium 2 (bioMérieux) and for acid or alkaline reactions, 2 ml suspension was homogenized in 60 ml 1/2 BFB prepared without yeast extract or $(NH_4)_2SO_4$, but containing phenol red at a final concentration of 0.006%. In order to allow reading of acid and alkaline reactions, and direct comparison with other *Aneurinibacillus*

and *Brevibacillus* species, the medium was adjusted to pH 7.0, although this lay outside the optimal pH range for the Antarctic isolates. These suspensions were used to inoculate both the tubes and cupules of the Biotype 100 strips. For utilization tests, results were read at 24 h intervals according to the manufacturer's instructions; for most substrates, utilization was indicated by turbidity. For acid or alkali production, results were read at 24 h intervals for 96 h with a yellow colour reaction scored as positive for acid reactions and a violet colour scored as positive for alkaline reactions. It was noted, however, that several tubes showed reactions immediately upon inoculation – two tubes produced alkaline reactions and several other tubes, mostly containing organic acids, gave acid reactions; these were not regarded as evidence of substrate utilization.

Metabolic studies. A standard glutamate transport assay was performed on strains LMG 22483^T and LMG 22481^T to determine the uptake of glutamate under normal conditions. 1/2 BFB (200 ml) was inoculated and incubated for 24 h at 40 °C in an orbital incubator. Cells were then centrifuged at 2000 g for 15 min, the supernatant was discarded, and the pellet was resuspended in 10 ml of 10 mM phosphate buffer (KH₂PO₄/K₂HPO₄, pH 7) and recentrifuged (2000 g for 15 min). The resulting supernatant was discarded and the pellet was resuspended in 2 ml phosphate buffer. Defined medium (200 ml) was inoculated with this suspension (0.3 ml) and incubated at 40 °C for 17 h. Cells were harvested as before and resuspended in 2 ml phosphate buffer. Aliquots (800 µl) of the cell suspension were incubated with 195 µl phosphate buffer for 5 min at 40 °C and 5 µl of 0.05 µCi (1.85 kBq) [¹⁴C]L-glutamic acid (10 mM) was introduced to initiate transport measurement. Samples (150 µl) were taken from the 1 ml assay mixture at 30 s intervals for 150 s and cells were immediately filtered on glass microfibre GF/B discs (Whatman) and washed in 2 × 10 ml cold phosphate buffer. The filters were then dried and the ¹⁴C in the samples was assayed by liquid scintillation counting using 4 ml scintillation fluid (Optiscint HiSafe; Perkin Elmer).

Competition experiments were carried out using unlabelled amino acids (L-glutamic acid, D-glutamic acid, L-proline, L-arginine and DL-ornithine) to determine whether the uptake system used by the strains was specific for L-glutamate or was also involved in the uptake of other, metabolically related amino acids. To create a 50-fold molar excess of each amino acid, in comparison to [¹⁴C]L-glutamic acid, 100 µl of each amino acid (25 mM) with 95 µl phosphate buffer was introduced to the cell suspension and incubated for 5 min before adding [¹⁴C]L-glutamic acid. Secondary competition experiments involved testing the uptake of [¹⁴C]arginine and [¹⁴C]proline (0.05 µCi) in the presence of a 50-fold molar excess of unlabelled L-glutamic acid. The same volumes of amino acids, radiolabelled substrate and phosphate buffer were used as before and the cells were pre-grown in the presence of L-glutamic acid (to induce uptake).

To determine whether Na⁺ or K⁺ channels were involved in glutamate uptake, 1 ml assay mixtures were prepared with valinomycin (K⁺ ionophore), monensin (Na⁺ ionophore) or nigericin (K⁺/Na⁺ ionophore) (final concentrations of 10, 0.1 and 10 µM, respectively). The ionophores, 10 µl of 1 mM valinomycin (dissolved in DMSO), 10 µl of 10 µM monensin (dissolved in DMSO) or 10 µl of 1 mM nigericin (dissolved in ethanol), respectively, were introduced and incubated for 5 min before the addition of [¹⁴C]L-glutamic acid. Controls containing only 10 µl DMSO or 10 µl ethanol were used to ensure that the ionophores were the only differing factors in the experiments.

To create artificial Na⁺, K⁺ and H⁺ ion gradients, NaCl, KCl or HCl, respectively, were introduced to create final concentrations of 50 or 100 mM in the 1 ml assay mixtures, 5 min prior to the addition of [¹⁴C]L-glutamic acid. To determine whether glutamate uptake is

linked to an osmotic effect, sucrose was introduced to create final concentrations of 50 and 100 mM in the 1 ml working suspensions; the resulting mixtures were incubated for 5 min before adding [¹⁴C]L-glutamic acid.

Energy inhibitors were employed to identify the system used by the micro-organisms for glutamate uptake. Sodium fluoride (glycolysis inhibitor), dinitrophenol (respiration uncoupler) or sodium arsenate (ATP synthesis inhibitor) were introduced to the 1 ml working stock to give 10 mM final concentrations and incubated for 5 min before adding [¹⁴C]L-glutamic acid. Iodoacetic acid (glycolysis inhibitor) or *N*-ethylmaleimide *p*-chloromercuribenzoate (S-H reagent, in ethanol) were introduced to the cell suspension to give 5 mM final concentrations and incubated for 5 min before adding [¹⁴C]L-glutamic acid. Carbonyl cyanide *m*-chlorophenyl hydrazone [respiration uncoupler (collapses ion gradients), in ethanol] or *N*-*N*-dicyclohexylcarbodiimide (ATPase inhibitor, in ethanol) were introduced to the cell suspension to give 0.1 mM final concentrations and incubated for 5 min before adding [¹⁴C]L-glutamic acid. An ethanol control was used to ensure that the energy inhibitors were the only differing factors in the experiment.

RESULTS AND DISCUSSION

Cultivation of strains

All of the Antarctic isolates demonstrated poor outgrowth on 1/2 BFA at pH 5.5 at 40 °C, but restreaking the few initial colonies on the same plate and reincubating resulted in better growth. On BFA with and without (NH₄)₂SO₄, the seven isolates from the north-west slope of Mt Melbourne demonstrated growth equivalent to that on 1/2 BFA, whereas the other six isolates generated less growth. This suggested that the Cryptogam Ridge and Mt Rittmann isolates, in comparison with the Mt Melbourne north-west slope isolates, have a preference for nutritionally weaker media. This inference was supported by the observation that only north-west slope isolates grew on egg-yolk agar and blood agar (both prepared with a blood agar base), and on both variants of Davis and Mingioli minimal medium, all of which are comparatively rich media in comparison with 1/2 BFA. It is also consistent with the observation that the non-slope isolates, in comparison with the north-west slope isolates, grew better in the nutritionally weaker unsolidified defined medium. All of the isolates demonstrated less growth on 1/4 BFA. The best growth was achieved using liquid rather than solid media, especially 1/2 BFB and unsolidified defined medium, which is consistent with a preference for microaerobic conditions.

16S rRNA gene sequencing

The nearly complete 16S rRNA gene sequences of all 13 strains were analysed. Clustering of the obtained sequences revealed two groups (Fig. 2): one containing strains isolated from the north-west slope of Mt Melbourne and one containing strains isolated from Cryptogam Ridge at Mt Melbourne, as well as the Mt Rittmann isolates. Both groups show high internal similarity (pairwise similarities of 99.7–99.9 and 99.9–100.0%, respectively). According to a FASTA search (Pearson & Lipman, 1988), strains

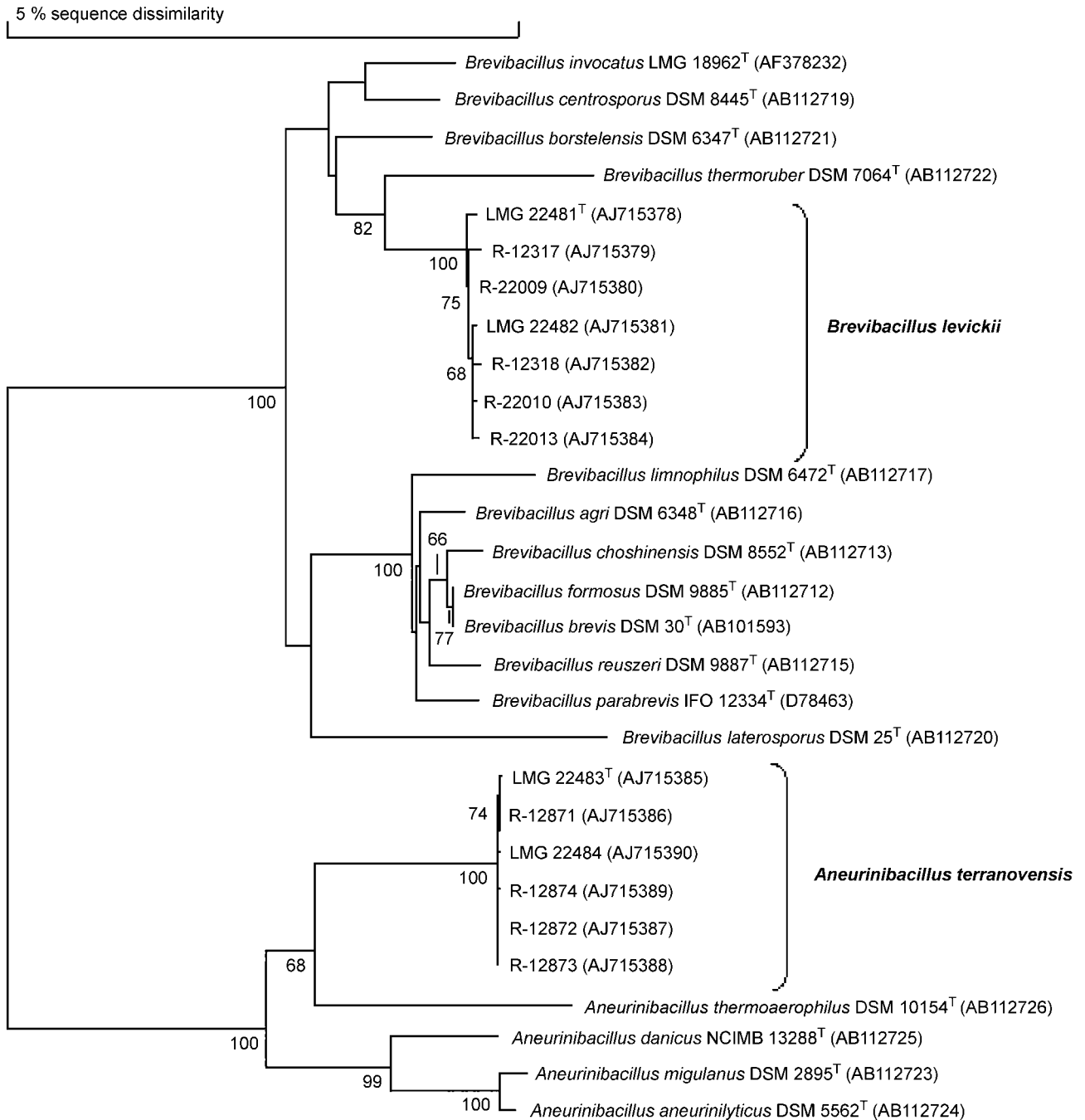


Fig. 2. Phylogenetic positions based on neighbour-joining of the 16S rRNA gene sequences of the Antarctic isolates among the type strains of *Brevibacillus* and *Aneurinibacillus*. Bootstrap values (expressed as percentages of 1000 replications) greater than 60% are shown at branch points.

belonging to the first group show highest sequence similarity to the *Brevibacillus borstelensis* type strain (97.0–97.2% similarity), but are distinct from it in SDS-PAGE analysis (see below). The sequence similarity to all other *Brevibacillus* species is well below 97.0%. These low sequence similarities, on the borderline of species delineation (Stackebrandt & Goebel, 1994; Stackebrandt *et al.*,

2002), together with the specific growth conditions of the strains suggest that the strains possibly represent a novel *Brevibacillus* species. DNA–DNA relatedness measurements confirmed this suggestion, with Antarctic strain LMG 22841^T and *Brevibacillus borstelensis* LMG 16009^T showing reciprocal DNA–DNA association values of 18.3 and 18.9%. These strains are subsequently referred to as *Brevibacillus*

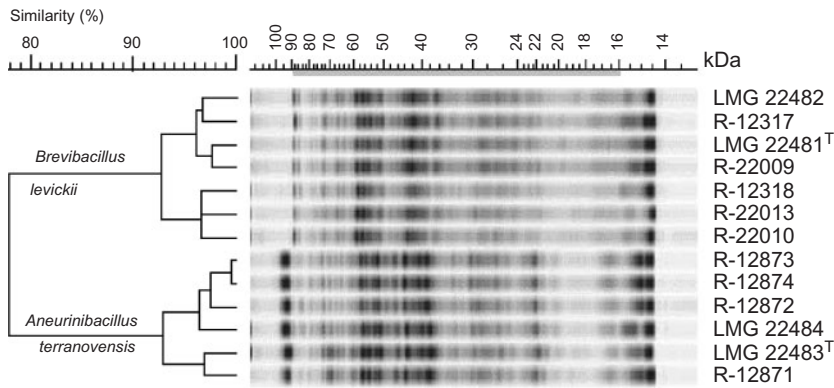


Fig. 3. Normalized computer profiles from SDS-PAGE analyses of whole-cell proteins of *Brevibacillus levickii* and *Aneurinibacillus terranovensis* strains. The dendrogram is based on UPGMA clustering of the correlation coefficient (r) of the total protein profiles. The profiles used for clustering are marked by a grey bar alongside the kDa scale.

levickii sp. nov. (Fig. 2). Based on 16S rRNA gene sequences, the second group of strains is most closely related to members of the genus *Aneurinibacillus* (sequence similarities of 95–96% according to a FASTA search) and forms a tight cluster with them (Fig. 2). According to these sequence similarities (<97%), the strains can be attributed to a novel *Aneurinibacillus* genospecies (Stackebrandt *et al.*, 2002); these strains are subsequently referred to as *Aneurinibacillus terranovensis* sp. nov. (Fig. 2).

SDS-PAGE

Numerical analysis of SDS-PAGE patterns of whole-cell proteins (Fig. 3) also enabled discrimination of the 13 isolates in the two groups revealed by 16S rRNA gene sequence analysis. The *Brevibacillus levickii* strains grouped at 93–98% similarity and the patterns represent members of a distinct and homogeneous taxon. Furthermore, the SDS-PAGE profile of *Brevibacillus levickii* was clearly different from that of the type strain of *Brevibacillus borstelensis* (below 65% similarity; data not shown), the species with which the strains share 97.0–97.2% 16S rRNA gene sequence similarity. The *A. terranovensis* strains grouped at 93–99% similarity and the patterns represent members of a distinct and homogeneous taxon. The two clusters merged at a low and insignificant similarity, indicating that the taxa are not closely related.

Fatty acid analysis

The existing *Brevibacillus* species show a dominance of the fatty acids anteiso- $C_{15:0}$ and iso- $C_{15:0}$, whereas the existing species of *Aneurinibacillus* have iso- $C_{15:0}$ as the dominant fatty acid (other fatty acids account for less than 20% of the total; Supplementary Table A in IJSEM Online). The fatty acid profiles of the Antarctic strains are difficult to compare with those of the other species since the strains failed to grow under standard conditions (trypticase soy agar, 24 h, 28 °C). Instead, the fatty acid profile of the Antarctic strains was determined after 48 h growth on 1/2 BFA medium at 28 °C. It has been reported that the fatty acid content, especially branched-chain fatty acids, can be greatly affected by the cultivation medium (Suzuki *et al.*, 1993) and thus the medium could be responsible for the

observed differences. The resulting profiles of the *Brevibacillus levickii* strains showed high amounts of anteiso- $C_{15:0}$ (mean of 74.5%), whereas *A. terranovensis* has high amounts of iso- $C_{15:0}$ and anteiso- $C_{15:0}$ (Supplementary Table A).

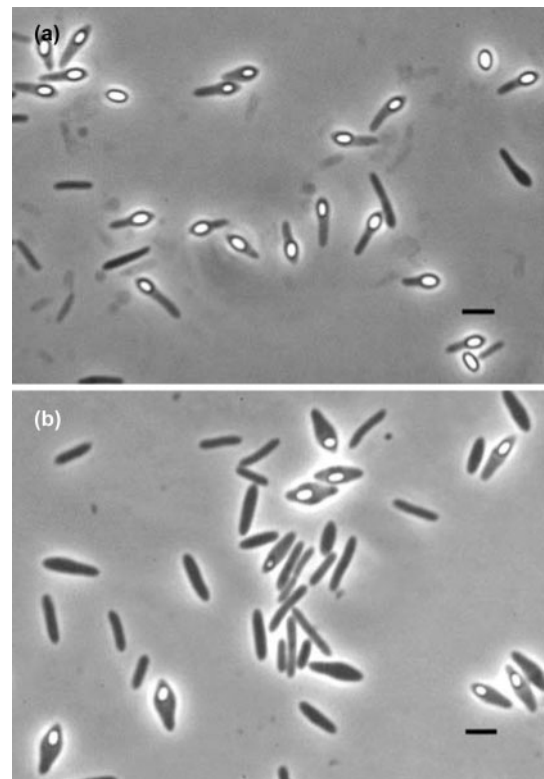


Fig. 4. (a) Photomicrograph of sporangia and vegetative cells of *Brevibacillus levickii* strain R-12317 viewed by phase-contrast microscopy; ellipsoidal spores lie subterminally and terminally in swollen sporangia. Bar, 2 µm. (b) Photomicrograph of sporangia and vegetative cells of *Aneurinibacillus terranovensis* strain R-12871 viewed by phase-contrast microscopy; ellipsoidal spores lie centrally, paracentrally and subterminally in swollen sporangia. Bar, 2 µm.

Phenotypic characterization

The strains were Gram-positive motile rods that lost the positive reaction of the Gram stain in 24–48 h and formed ellipsoidal spores in swollen sporangia (Fig. 4a, b). On 1/2 BFA plates, the *Brevibacillus* strains formed tough, adherent colonies, whereas colonies of the *Aneurinibacillus* strains were butyrous. The *A. terranovens* and *Brevibacillus levickii* strains showed many phenotypic similarities and few biochemical characters useful for differentiation. Their reactions in the various tests are summarized in the species descriptions below and in Table 1 and Table 2. Sporangial morphologies were notably different between the species: sporangia of *A. terranovens* were greatly swollen by central to subterminal spores, whereas those of *Brevibacillus levickii* were less swollen with subterminal and terminal spores (Fig. 4a, b). Strains of both species appeared pleomorphic

and filamentous when grown in 1/2 BFB, whereas cultivation in defined medium generated cells of regular appearance. All strains were weakly positive for catalase and grew well in aerobic conditions but, as explained above, outgrowth was poor so restreaking on the same plate was necessary in order to obtain satisfactory growth; such outgrowth problems were not seen with plates incubated in microaerobic conditions. This is consistent with the observation that growth in broth media was always more abundant than growth on their solid counterparts. Although agar colonies of *Brevibacillus* were adherent and difficult to emulsify in liquid media, capsule production could not be demonstrated. Strains of both species showed negative or weak reactions in many of the phenotypic tests; for example, no hydrolysis was observed around colonies on starch agar and weak reactions only became apparent after the colonies had been scraped from the surface of the medium. In the

Table 1. Phenotypic characters that can be used to differentiate *Brevibacillus levickii* from other *Brevibacillus* species

Species: 1, *Brevibacillus levickii* (7 strains); 2, *B. agri*; 3, *B. borstelensis*; 4, *B. brevis*; 5, *B. centrosporus*; 6, *B. choshinensis*; 7, *B. formosus*; 8, *B. invocatus*; 9, *B. laterosporus*; 10, *B. parabravis*; 11, *B. reuszeri*. +, >85% positive; (+), 75–84% positive; v, variable (16–74% positive); –, 0–15% positive; w, weak positive reaction; NA, no data available.

Character	1*	2	3	4	5	6	7	8	9	10	11
Growth temperature (°C)											
Range	15–55	20–50	20–50	20–45	10–40	NA	20–45	15–35	15–50	20–45	20–45
Optimum	40–45	30–35	30	35–40	28	NA	35–40	NA	30	35–40	35–40
pH range	4.5–6.5	6–9	NA	6–9	NA	NA	6–9	6.0–8.5	NA	6–9	6–9
Optimum pH	5.0–5.5	7.0–7.5	NA	7.0–7.5	NA	NA	7.0–7.5	NA	NA	7.0–7.5	6.5–7.0
Tough, adherent colony consistency	+	–	–	–	–	–	–	–	–	–	–
Nitrate reduction	v	w	+	+	v	–	+	–	+	+	–
Hydrolysis of:											
Casein	v/w	+	+	+	–	–	+	–	+	+	–
Gelatin	+	+	+	+	–	–	+	–	+	+	+
Starch	w	w	–	+	–	–	–	–	–	–	+
Assimilation of:											
D-Alanine	(+)	v	+	+	–	–	+	–	–	+	+
D-Fructose	(+)	+	+	+	–	v	+	–	+	–	v
Fumarate	+	+	+	v	+	v	+	–	+	–	–
D-Gluconate	+	+	+	+	–	v	+	–	–	+	+
Glutamate	+	+	+	+	v	–	+	–	v	+	+
DL-Glycerate	+	–	+	–	–	–	–	–	+	–	+
Glycerol	(+)	+	–	+	–	–	+	–	+	+	–
DL-Lactate	+	+	–	–	+	–	+	–	–	–	+
Lactulose	–	–	–	–	–	–	–	–	–	–	+
Maltose	(+)	+	–	+	–	–	+	–	+	+	–
D-Mannitol	(+)	+	–	+	+	v	+	–	+	+	+
N-Acetyl-D-glucosamine	+	+	+	+	+	v	+	–	+	+	+
Phenylacetate	–	–	+	+	+	v	+	+	–	+	+
Quinate	(+)	–	–	–	–	–	–	+	–	–	–
Sucrose	+	+	–	+	–	–	+	–	–	+	–
D-Trehalose	v†	+	–	+	–	–	+	–	+	+	–
D-Turanose	–	+	–	+	–	+	+	–	–	+	–

**B. levickii* strains were characterized at pH 5.5; other species were tested at pH 6.5–7.0.

†Type strain gives a positive reaction.

Table 2. Phenotypic characters that can be used to differentiate *Aneurinibacillus terranovensis* from other *Aneurinibacillus* species

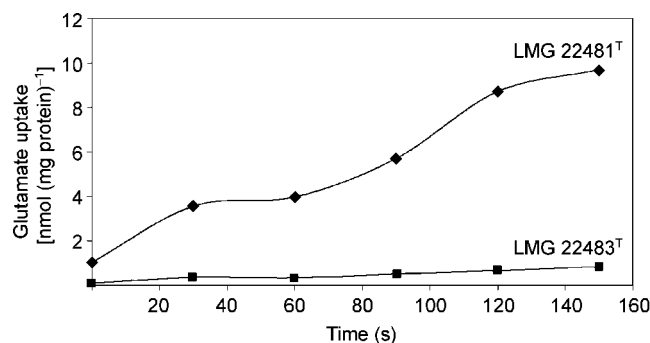
Species: 1, *A. terranovensis* (6 strains); 2, *A. aneurinilyticus*; 3, *A. migulanus*; 4, *A. thermoaerophilus*. +, >85% positive; (+), 75–84% positive; v, variable (16–74% positive); –, 0–15% positive; w, weak positive reaction; NG, no growth.

Character	1*	2	3	4
Growth temperature (°C)				
Range	20–55	20–50	20–45	35–65
Optimum	37–45	35–40	35–40	50–55
pH range	3.5–6.0	5–9	6–9	6–9
Optimum pH	5.0–5.5	6.5–7.0	6.5–7.0	6.5–7.0
Tough, adherent colony consistency	–	–	–	–
Nitrate reduction	(+)	+	+	–
Hydrolysis of:				
Casein	NG	–	–	+
Gelatin	+	–	+	+
Starch	w	+	+	–
Assimilation of:				
D-Alanine	–	+	+	+
D-Fructose	(+)	–	+	v
Fumarate	v†	+	+	+
D-Gluconate	(+)	v	–	–
Glutamate	(+)	+	+	+
DL-Glycerate	v	–	–	v
Glycerol	(+)	+	+	+
DL-Lactate	(+)	+	+	+
Lactulose	–	–	–	–
Maltose	–	–	–	–
D-Mannitol	v	–	–	v
N-Acetyl-D-glucosamine	v	–	–	+
Phenylacetate	–	+	+	+
Quinate	v†	–	–	+
Sucrose	v	–	–	–
D-Trehalose	v	–	–	–
D-Turanose	–	–	–	v

**A. terranovensis* strains were characterized at pH 5.5; other species were tested at pH 6.5–7.0.

†Type strain gives a positive reaction.

API 20E tests, the two species could be distinguished from each other by the gelatin reaction; a reaction was evident after 48 h for *Brevibacillus levickii*, whereas no reaction was observed in *A. terranovensis*. However, when the charcoal gelatin was aseptically removed from the API 20E tube and introduced into 1/2 BFB, gelatin was hydrolysed by *Brevibacillus levickii* within 24 h and by *A. terranovensis* after 24 h, but within 48 h. Both species may also be separated on the basis of reaction intensities in the API 20E strip, with stronger reactions for arginine dihydrolase, Voges–Proskauer and nitrate reduction being observed for *A. terranovensis* and for citrate utilization in *Brevibacillus*

**Fig. 5.** Uptake of [¹⁴C]L-glutamic acid in *Brevibacillus levickii* strain LMG 22481^T and *Aneurinibacillus terranovensis* strain LMG 22483^T.

levickii. Positive reactions for arginine dihydrolase in the API 20E strip distinguish these species from other members of both genera that have been tested. In the Biotype 100 utilization and acid/alkali production tests, both species demonstrated rapid performance, with substrate utilization (turbidity or acid/alkaline reaction) becoming evident within 24–48 h incubation. The results produced by the utilization tests were inconsistent within species, especially for the *Aneurinibacillus* strains; this problem has been seen with other groups of aerobic endospore-formers (Heyrman *et al.*, 2003). The results obtained do, however, indicate that *Brevibacillus* strains are capable of utilizing a wide range of carbon sources and that the *Aneurinibacillus* strains utilize a more limited and less consistent range of substrates. The results of the acid/alkali production tests indicated that both species utilize similar kinds of carbon sources, mainly organic acids and amino acids; however, as noted above, these tests were done outside the optimal pH ranges of these organisms.

Metabolic studies

Metabolic studies show that both strains utilize glutamate, which is most probably available from cyanobacteria and microalgae in their natural habitats (Siebert & Hirsch, 1988). Glutamate is non-essential, however, as the organisms grew on a formulation of the defined medium from which it had been omitted. Both species appear to use similar glutamate uptake systems, as experimental outcomes were similar. When grown in the presence of a mixture of amino acids, *Brevibacillus levickii* strain LMG 22481^T demonstrated uptake of [¹⁴C]L-glutamic acid at a mean rate of 0.105 nM s⁻¹ mg⁻¹, whereas *A. terranovensis* strain LMG 22483^T demonstrated uptake at a mean rate of 0.0044 nM s⁻¹ mg⁻¹, over 150 s (Fig. 5). Perhaps *Brevibacillus levickii* has a higher affinity uptake system for glutamate on account of nutritional limitations in its habitat. The rate of uptake in the *Brevibacillus levickii* strain is greater than that of *Bacillus* sp. strain TA2.A1, which was isolated from a thermal spring and had an L-glutamate uptake of 0.0117 nM s⁻¹ mg⁻¹ in an external glutamate

concentration of 50 μM (Peddie *et al.*, 1999). In comparison, the *A. terranovensis* strain had a slower rate of uptake than that of *Bacillus* sp. strain TA2.A1. The uptake system of the *Brevibacillus levickii* strain appears to be specific for the L-glutamate isomer; this is evident from the fact that D-glutamate caused no inhibition of [^{14}C]L-glutamate uptake and that the other amino acids related to L-glutamate (L-proline, L-arginine and DL-ornithine) were fourfold less efficient in inhibiting uptake of [^{14}C]L-glutamate than unlabelled L-glutamate, despite being present in excess. This conclusion is supported by the observation that [^{14}C]L-proline and L-arginine uptakes were not inhibited by an excess of L-glutamate, which implies that there are separate uptake systems for glutamate, proline and arginine (results not shown). The uptake system of the *A. terranovensis* strain appeared not to be specific for the L-glutamate isomer, but could transport most of the other related amino acids; the exceptions were L-proline, which caused partial inhibition, and D-glutamate, which caused no inhibition when present in excess (Supplementary Table B in IJSEM Online). No inhibition of [^{14}C]L-proline or [^{14}C]L-arginine uptake was observed in *A. terranovensis* pre-grown with L-glutamic acid when in the presence of an excess of unlabelled L-glutamic acid, thus implying that separate uptake systems for proline and arginine exist (results not shown). In *Brevibacillus levickii*, the ionophores nigericin and valinomycin decreased [^{14}C]L-glutamic acid uptake two- and tenfold, respectively. The addition of monensin, however, did not cause any decrease in the rate of uptake (Supplementary Fig. Aa in IJSEM Online). In *A. terranovensis*, the addition of nigericin resulted in a 40-fold decrease in the rate of [^{14}C]L-glutamic acid uptake and valinomycin caused complete inhibition. Very little inhibition (less than twofold) was observed in the presence of monensin (Supplementary Fig. Ab). Glutamate uptake inhibition by the ionophores valinomycin and nigericin implies that a K^+ gradient is involved. However, slight inhibition by monensin in the *A. terranovensis* strain suggests that a charge gradient may also be involved in the uptake of glutamate in this species. In both strains, the rate of [^{14}C]L-glutamic acid uptake in pre-grown cells decreased twofold in the presence of NaCl at 50 mM. In the presence of KCl at 50 mM, the rate of uptake in *Brevibacillus levickii* decreased fourfold and in *A. terranovensis* decreased eightfold. In the presence of HCl at 50 mM, cell death occurred in both strains (results not shown). The creation of an artificial K^+ gradient resulted in a decrease in the rate of glutamate uptake in both species, thus suggesting that the uptake mechanism is a K^+ antiport system, with the increased K^+ concentration outside the cell inhibiting K^+ export from inside the cell, hence resulting in decreased glutamate uptake. This is of particular interest given that previous studies on glutamate uptake metabolism in other thermophilic bacteria have shown it to be driven by Na^+ gradients; *Geobacillus stearothermophilus*, for example, utilizes a sodium/proton symport mechanism (de Vrij *et al.*, 1990). In the presence of 50 mM sucrose, pre-grown cells of both strains showed no decrease in the rate of [^{14}C]L-glutamic

acid uptake (data not shown). This lack of inhibition by sucrose demonstrates that the uptake of glutamate in both species is not osmotically sensitive. For both strains, high levels of inhibition of [^{14}C]L-glutamic acid uptake occurred through the addition of the energy inhibitors dinitrophenol, iodoacetic acid, *N*-ethylmaleimide *p*-chloromercuribenzoate, carbonyl cyanide *m*-chlorophenyl hydrazone and *N*-*N*-dicyclohexylcarbodiimide (Supplementary Table C in IJSEM Online). Inhibition of [^{14}C]L-glutamate uptake by energy inhibitors implies the presence of an active transport system for glutamate. Almost complete inhibition of glutamate uptake in both species by *N*-*N*-dicyclohexylcarbodiimide, an inhibitor of ATP synthesis, and iodoacetic acid, a glycolysis inhibitor, suggests a role for ATP in uptake. However, NaAsO_4 and NaF, both of which directly inhibit ATP generation, cause only 10% inhibition of uptake in *Brevibacillus levickii* strain LMG 22481^T, thus suggesting that the system does not require ATP directly. This would imply that the mechanism of uptake is not a primary uptake system (directly coupled to ATP hydrolysis), but more likely a secondary transport system that utilizes a gradient. A similar system is probably utilized by *A. terranovensis*. However, a significant level of inhibition is apparent in the presence of sodium arsenate (Supplementary Table C). Many bacteria can utilize ATP to generate a transmembrane proton-motive force, directly by respiration, which can be coupled to solute uptake. A role for such a proton-motive force in the *Brevibacillus* and *Aneurinibacillus* strains is also implied by the inhibition of glutamate uptake by the ionophores. Collectively, the metabolic data indicate that the mechanism of glutamate uptake in *Brevibacillus levickii* is an energy-dependent, glutamate-specific K^+ antiport system, whereas in *A. terranovensis* it is an energy-dependent K^+ antiport system capable of transporting glutamate and other related amino acids.

Perhaps the most interesting feature of these two novel species is that they were not found in the same habitat despite repeated soil sampling on separate occasions, and repeated enrichment and isolation at different times in the laboratory. *Brevibacillus levickii* strains were only isolated from the north-west slope of Mt Melbourne, whereas *A. terranovensis* strains were not isolated from this site, but were found in soils from Cryptogam Ridge of Mt Melbourne and in soils from Mt Rittmann. This is reminiscent of the failure of Logan *et al.* (2000) to isolate *Bacillus fumarioli* from the north-west slope of Mt Melbourne, although it could be cultivated from Cryptogam Ridge and Mt Rittmann. Although the soil and environmental conditions at each isolation site are superficially similar, our findings clearly imply that there are some important differences between the soil of the north-west slope of Mt Melbourne and those of other sites. Bargagli *et al.* (1996) found that although mean element concentrations from the fumarolic areas of the two mountains were broadly similar, there were some differences in trace element contents. Soils from Mt Melbourne had higher Cu and Zn contents, whereas soils from Mt Rittmann had higher Cd and Pb; no data for

the north-west slope of Mt Melbourne have yet been reported. A notable feature of the north-west slope of Mt Melbourne is the near-absence of moss (Broady *et al.*, 1987); this may indicate that this soil contains substances inhibitory to the mosses that otherwise grow so abundantly on the nearby Cryptogam Ridge, or else the soil lacks one or more essential nutrients. Such conditions may also affect the aerobic endospore-forming floras in these soils, and the paucity of moss growth on the north-west slope may limit the organic substrates available to heterotrophic bacteria.

Our failure to identify our Antarctic strains by the genotypic and phenotypic methods applied and the similarities of the members of each group of strains to each other in these analyses support the proposal of two novel species; their descriptions are given below.

Description of *Brevibacillus levickii* sp. nov.

Brevibacillus levickii (le.vic.ki'i. N.L. adj. *levickii* of Levick, named after G. Murray Levick, surgeon and biological scientist of Captain R. F. Scott's Northern Party, the first scientific expedition to visit the vicinity of Mt Melbourne in 1912).

Cells are Gram-positive, becoming Gram-negative after 48 h, motile, round-ended rods (0.7–0.8 × 2.0–5.0 μm) occurring singly, in pairs and in chains. Endospores are ellipsoidal, occurring subterminally or terminally in swollen or slightly swollen sporangia (Fig. 4a). After 48 h incubation at 40 °C on 1/2 BFA (pH 5.5), colonies are circular, flat, up to 3.0 mm in diameter and cream-coloured with a matt appearance. Colony consistency becomes tough and difficult to break with a loop. Minimum growth temperature lies between 15 and 20 °C, with optimum growth temperature of 40–45 °C and maximum growth temperature of 50–55 °C. Growth occurs between pH 4.5 and 6.5 and the optimum pH for growth is pH 5.0–5.5. Growth is inhibited by 2–3% NaCl. The organisms are microaerophilic and weakly catalase-positive. Horse blood agar is partially haemolysed. Gelatin is hydrolysed in 24 h and starch hydrolysis is weakly positive. Casein hydrolysis is weakly positive or negative. In the API 20E strip reactions, arginine dihydrolase, citrate utilization and the Voges-Proskauer reaction are positive. Gelatin is hydrolysed and nitrate reduction is variable. Reactions for ONPG hydrolysis, lysine decarboxylase, ornithine decarboxylase, hydrogen sulfide production, urease, tryptophan deaminase and indole production are negative. The following carbon sources are utilized in the API Biotype 100 system: α-D-glucose, adonitol, aesculin, α-lactose, D-alanine, L-alanine, L-arabitol, L-aspartate, β-D-fructose, *cis*-aconitate, citrate, fumarate, D-galacturonate, D-gluconate, D-glucuronate, L-glutamate, DL-glycerate, glycerol, 2-keto-D-gluconate, 5-keto-D-gluconate, DL-lactate, D-malate, L-malate, maltose, maltotriose, D-mannitol, D-mannose, *meso*-tartrate, 1-O-methyl-β-galactopyranoside, 1-O-methyl-β-D-glucopyranoside, *N*-acetyl-D-glucosamine, L-proline, putrescine, quinate, L-serine, D-sorbitol, succinate, sucrose and

trans-aconitate. Utilization of the following substrates is variable: DL-α-amino-n-butyrate, 2-oxoglutarate, D-galactose, β-gentobiose, mucate, D-ribose, D-saccharate, D-trehalose and L-tryptophan. The following carbon sources are not utilized: i-erythritol, DL-α-amino-n-valerate, α-D-melibiose, L-arabinose, D-arabitol, benzoate, betaine, DL-β-hydroxybutyrate, caprate, caprylate, D-cellobiose, dulcitol, ethanolamine, α-L-fucose, gentisate, D-glucosamine, glutarate, histamine, L-histidine, hydroxyquinoline-β-glucuronide, itaconate, lactulose, D-lyxose, malonate, maltitol, *m*-coumarate, D-melezitose, 1-O-methyl-α-D-glucopyranoside, 3-O-methyl-D-glucopyranose, 1-O-methyl-α-galactopyranoside, *m*-hydroxybenzoate, *myo*-inositol, palatinose, phenylacetate, 3-phenylpropionate, *p*-hydroxybenzoate, propionate, protocatechuate, D-raffinose, α-L-rhamnose, L-sorbose, D-tagatose, D-tartrate, L-tartrate, tricarballyate, trigonelline, tryptamine, D-turanose, L-tyrosine, xylitol and D-xylose. The following substrates in API Biotype 100 produce alkaline reactions when inoculated with a suspension in 1/2 BFB prepared without yeast extract or (NH₄)₂SO₄, but containing 0.006% phenol red: 2-oxoglutarate, L-aspartate, fumarate, D-galacturonate, D-gluconate, L-glutamate, D-malate, L-malate, malonate, mucate, quinate, D-saccharate, succinate, D-tartrate, L-tartrate and tricarballyate. Alkaline reactions for the following substrates are variable: 2-keto-D-gluconate, L-alanine, caprylate, *cis*-aconitate, citrate, D-glucuronate, glutarate, DL-glycerate, L-histidine, itaconate, DL-lactate, *meso*-tartrate, propionate, L-serine, *trans*-aconitate and L-tryptophan.

The major cellular fatty acid is anteiso-C_{15:0}, accounting for approximately 74% total fatty acid content. The following fatty acids are present in smaller amounts (at least 1%): iso-C_{14:0}, iso-C_{15:0}, C_{16:0}, iso-C_{16:0}, summed feature 4 (iso-C_{17:1} and/or anteiso-C_{17:1}) and anteiso-C_{17:0} (detailed fatty acid data are given in Supplementary Table A).

The DNA G + C content is 48.3–50.3 mol% and the G + C content of the type strain (Logan B-1657^T = LMG 22481^T = CIP 108307^T) is 50.3 mol%. In the variable reactions listed above, the type strain is weakly positive for casein hydrolysis and it utilizes DL-α-amino-n-butyrate, 2-oxoglutarate, D-galactose, D-ribose, D-trehalose and L-tryptophan; alkaline reactions are observed for D-glucuronate, itaconate, 2-keto-D-gluconate, *meso*-tartrate and L-tryptophan.

Description of *Aneurinibacillus terranovensis* sp. nov.

Aneurinibacillus terranovensis [terr.a.no.ven'sis. N.L. adj. *terranovensis* referring to Terra Nova Bay Station (Italy), northern Victoria Land, Antarctica, where the strains were first isolated].

Cells are Gram-positive, becoming Gram-negative after 48 h, motile, round-ended rods (0.8–1.0 × 2.0–8.0 μm), occurring singly, in pairs and in chains. Endospores are ellipsoidal, occurring paracentrally and subterminally in

very swollen sporangia (Fig. 4b). After 48 h incubation on 1/2 BFA (pH 5.5) at 40 °C, colonies are circular, flat, up to 1.5 mm in diameter and cream-coloured with a slightly glossy appearance and butyrous consistency. Minimum growth temperature is 20–25 °C, with optimum growth at 37–45 °C and maximum growth temperature of 50–55 °C. Growth occurs between pH 3.5 and 6.0 and the optimum pH for growth is pH 5.0–5.5. Growth is inhibited by 2–3 % NaCl. Microaerophilic and weakly catalase-positive. Gelatin is hydrolysed after 48 h. Starch is weakly hydrolysed. No growth occurs on casein agar. In the API 20E strip reactions, arginine dihydrolase, the Voges–Proskauer reaction and nitrate reduction are positive. Citrate utilization is variable. Reactions for ONPG hydrolysis, lysine decarboxylase, ornithine decarboxylase, hydrogen sulfide production, urease, tryptophan deaminase, indole production and gelatin hydrolysis are negative. The following carbon sources are utilized in the Biotype 100 system: aesculin, β -D-fructose, D-gluconate, α -D-glucose, L-glutamate, glycerol, DL-lactate, D-mannose, 1-O-methyl- β -D-glucopyranoside and *myo*-inositol. Utilization of the following substrates is variable: adonitol, 2-oxoglutarate, α -lactose, L-alanine, D-arabitol, L-arabitol, L-aspartate, DL- β -hydroxybutyrate, *cis*-aconitate, citrate, fumarate, D-galactose, D-galacturonate, D-glucosamine, DL-glycerate, 2-keto-D-gluconate, 5-keto-D-gluconate, D-malate, L-malate, D-mannitol, *meso*-tartrate, 1-O-methyl- β -galactopyranoside, *N*-acetyl-D-glucosamine, L-proline, putrescine, quinate, D-ribose, D-sorbitol, succinate, sucrose, *trans*-aconitate and D-trehalose. The following carbon sources are not utilized: 3-phenylpropionate, DL- α -amino-n-butyrate, DL- α -amino-n-valerate, α -D-melibiose, D-alanine, α -L-fucose, α -L-rhamnose, L-arabinose, benzoate, betaine, β -gentobiose, caprate, caprylate, D-cellobiose, dulcitol, ethanolamine, gentisate, D-glucuronate, glutarate, histamine, L-histidine, hydroxyquinoline- β -glucuronide, i-erythritol, itaconate, lactulose, D-lyxose, malonate, maltitol, maltose, maltotriose, *m*-coumarate, D-melezitose, 1-O-methyl- α -D-glucopyranoside, 1-O-methyl- α -galactopyranoside, 3-O-methyl-D-glucopyranose, *m*-hydroxybenzoate, mucate, phenylacetate, *p*-hydroxybenzoate, palatinose, propionate, protocatechuic acid, D-raffinose, D-saccharate, L-serine, L-sorbose, D-tagatose, D-tartrate, L-tartrate, tricarballic acid, trigonelline, tryptamine, L-tryptophan, D-turanose, L-tyrosine, xylitol and D-xylose. The following substrates in API Biotype 100 produce alkaline reactions when inoculated with a suspension in 1/2 BFB prepared without yeast extract or (NH₄)₂SO₄, but containing 0.006 % phenol red: 2-oxoglutarate, L-aspartate, *cis*-aconitate, citrate, fumarate, D-galacturonate, D-gluconate, L-glutamate, glutarate, DL-glycerate, malonate, mucate, quinate, D-saccharate, succinate, L-tartrate and D-tartrate. Alkaline reactions for the following substrates are variable: D-alanine, L-alanine, DL- β -hydroxybutyrate, caprylate, D-glucuronate, L-histidine, DL-lactate, D-malate, L-malate, *meso*-tartrate, L-proline, propionate, L-serine, *trans*-aconitate, tricarballic acid and L-tryptophan.

The major cellular fatty acids are anteiso-C_{15:0} and iso-C_{15:0}, accounting for approximately 41 and 46 % total fatty acid content, respectively. The following fatty acids are present in smaller amounts (at least 1.0 %): C_{14:0}, iso-C_{14:0}, C_{16:0}, iso-C_{16:0} and C_{16:1} ω 7c alcohol (detailed fatty acid are given in Supplementary Table A).

The G + C content is 43.2–44.6 mol% and the G + C content of the type strain (Logan B-1599^T = LMG 22483^T = CIP 108308^T) is 43.2 mol%. In the variable reactions listed above, the type strain utilizes citrate, fumarate, D-galactose, D-glucosamine, α -lactose, L-malate, quinate and *trans*-aconitate; alkaline reactions are observed in DL-lactate, D-malate, L-malate and *trans*-aconitate.

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