**Subtercola vilae** sp. nov., a novel actinobacterium from an extremely high-altitude cold volcano lake in Chile

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**Abstract** A novel actinobacterium, strain DB165\(^\text{T}\), was isolated from cold waters of Llullaillaco Volcano Lake (6170 m asl) in Chile. Phylogenetic analysis based on 16S rRNA gene sequences identified strain DB165\(^\text{T}\) as belonging to the genus *Subtercola* in the family *Microbacteriaceae*, sharing 97.4% of sequence similarity with *Subtercola frigoramans* DSM 13057\(^\text{T}\), 96.7% with *Subtercola lobariae* DSM 103962\(^\text{T}\), and 96.1% with *Subtercola boreus* DSM 13056\(^\text{T}\). The cells were observed to be Gram-positive, form rods with irregular morphology, and to grow best at 10–15 °C, pH 7 and in the absence of NaCl. The cross-linkage between the amino acids in its peptidoglycan is type B2\(\gamma\); 2,4-diaminobutyric acid is the diagnostic diaminoc acid; the major respiratory quinones are MK-9 and MK-10; and the polar lipids consist of phosphatidylglycerol, diphosphatidylglycerol, 5 glycolipids, 2 phospholipids and 5 additional polar lipids. The fatty acid profile of DB165\(^\text{T}\) (5\% >) contains iso-C14:0, iso-C16:0, anteiso-C15:0, anteiso-C17:0, and the dimethylacetal iso-C16:0 DMA. The genomic DNA G+C content of strain DB165\(^\text{T}\) was determined to be 65 mol%. Based on the phylogenetic, phenotypic, and chemotaxonomic analyses presented in this

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study, strain DB165\(^T\) (= DSM 105013\(^T\) = JCM 32044\(^T\)) represents a new species in the genus *Subtercola*, for which the name *Subtercola vilae* sp. nov. is proposed.

**Keywords** Cold environments · Llullaillaco volcano · Microbacteriaceae · New species · *Subtercola vilae*

**Introduction**

Members of the family *Microbacteriaceae* are widely distributed in terrestrial and aquatic environments or associated with macroorganisms (Evtushenko 2012). Some representatives, including species of the genus *Subtercola*, have been found in cold environments such as glacial ice (Christner et al. 2007), boreal groundwater (Männistö et al. 2000), and Antarctic sediments (Li et al. 2010). At present the genus *Subtercola* contains three validly named species, *Subtercola boreus*, *Subtercola frigoramans* and *Subtercola lobariae*; the first two were isolated from Finnish groundwater (Männistö et al. 2000) and the third from the lichen *Lobaria retigera* (Si et al. 2017). Based on the high similarity of 16S rRNA gene sequences (> 96%), other isolates from cold habitats, such as Antarctic and Arctic waters as well as glaciers were found to be affiliated to *Subtercola* (Singh et al. 2014; Zhang et al. 2013; Peeters et al. 2011).

In this study, we characterise strain DB165\(^T\), isolated from a water sample of Llullaillaco Volcano Lake (6170 m) in Chile, one of the highest-elevation lakes on Earth. According to its distinct properties, strain DB165\(^T\) is proposed as the type strain of the new species *Subtercola vilae*.

**Materials and methods**

**Isolation and cell morphology**

Strain DB165\(^T\) was obtained from a water sample collected at the Llullaillaco volcano lake (S24°42.878', W68°33.310') on 18 January 2013, using R2A medium (DIFCO) supplemented with 18 g agar l\(^-1\). Pure cultures were obtained after three successive transfers of single colonies to R2A medium plates. Stock cultures were maintained in SGG medium containing 10 g starch, 10 g glucose, 10 ml glycerol (99.7% v/v), 5 g soy peptone, 2.5 g corn steep solids, 2 g yeast extract, 3 g CaCO\(_3\), 1 g NaCl, and 18 g agar in 1 l deionised water (Goodfellow and Fiedler 2010). DB165\(^T\) was cryopreserved using CRYOBANK (Mast Diagnostica GmbH, Germany) for long term storage at − 80 °C.

Gram-staining was prepared using the Color Gram 2 kit (BioMérieux, France), following the manufacturer’s protocol. Endospore staining was performed using the green malaquite method and light microscopy (Schaeffer and Fulton 1933). Cell morphology, shape and size were determined using scanning electron microscopy (SEM) according to Gärtnerr et al. (2008), after cultivation of trypticase soy medium (Trypticase Soy Broth (Becton, Dickinson and company, France) supplemented with 18 g agar l\(^-1\).

The reference strains *S. boreus* DSM 13057\(^T\) and *S. frigoramans* DSM 13057\(^T\) were obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ) and cultured under the same conditions as strain DB165\(^T\) for comparative purposes.

**Physiological characteristics**

Enzyme activities and utilisation of carbon sources for strain DB165\(^T\), *S. boreus* DSM 13057\(^T\) and *S. frigoramans* DSM 13057\(^T\) were examined using API ZYM, API 20E and API 50CH (BioMérieux, France), following the manufacturer’s recommendations. The effect of sodium chloride (0, 0.1, 0.3, 0.6, 0.9, 1, 2.5, 5, 7.5, and 10% w/v) and pH (2, 3, 4, 5, 6, 7, 8, 9 and 10) on the growth was tested according to Kutzner (1981), using ISP2 medium containing 4 g yeast extract, 10 g malt extract, 4 g dextrose, and 18 g agar in 1 l of distilled water. The optimal range of temperature was tested at 5, 10, 15, 20, 28 and 30 °C using SGG medium.

**Chemotaxonomic analyses**

Polar lipids were extracted according to a modified protocol of Bligh and Dyer (1959), and the total lipid material was detected using molybdatophosphoric acid and specific functional groups were detected using spray reagents specific for defined functional groups (Tindall et al. 2007). The lipoquinones were
extracted and identified using the two-stage method described by Tindall (1990a, b). After cultivation at 25 °C, fatty acid methyl esters were obtained by saponification, methylation and extraction using minor modifications of the method of Miller (1982) and Kuykendall et al. (1988). The fatty acid methyl esters mixtures were separated and identified using the Sherlock Microbial Identification System (MIS) (MIDI, Microbial ID, Newark, DE 19711, USA).

The peptidoglycan was obtained from 4 g wet weight cell pellet according to the method of Schleifer (1985). The peptidoglycan analyses were performed according to Schumann (2011).

Analyses of polar lipids, respiratory quinones, whole-cell fatty acids and peptidoglycan analyses were carried out by the Identification Service of the DSMZ—Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (Braunschweig, Germany).

DNA base composition

DNA was extracted using the DNeasy Blood & Tissue kit (QIAGEN). The G+C content was calculated from the genome sequence, which was determined with Nextseq 500 (Illumina). The quality of the sequences was checked and filtrated using Trimmomatic (adapters, > Q30, > 1000 bp) (Bolger et al. 2014). The genome was assembled using SPAdes (Kmer = 121) (Bankevich et al. 2012).

Phylogenetic analyses

DNA was extracted using the DNeasy Blood & Tissue kit (QIAGEN) with modifications. The 16S rRNA gene sequence was amplified by PCR using PureTaq Ready-To-Go PCR beads (GE Healthcare) and sequencing according to Gättner et al. (2008).

The 16S rRNA gene sequence of strain DB165T was aligned with sequences of 22 selected type strains of the family Microbacteriaceae, including members of the genera Subtercola, Frondihabitans and Agreia, and in addition Cellulomonas carbonis KCTC 19824T as outgroup using SINA (Pruesse et al. 2012). Phylogenetic trees were constructed using the neighbour-joining (Saitou and Nei 1987) and maximum-likelihood algorithms using MEGA version 6.0 (Tamura et al. 2013). The tree topologies were evaluated with bootstrap analyses based on 1000 replicates.

Results

Morphological and physiological characteristics

Colonies of strain DB165T were observed to be sticky, golden yellow after growth at the optimal growth temperature of 10–15 °C for 6–7 days (also after 7–10 days at 28 °C), but are pale yellow after growth at 5 °C for 2–3 weeks. Optimum growth is observed at 10–15 °C (range from 5 to 28 °C). No growth occurs at 30 °C. Strain DB165T tolerates only low concentrations (up to 0.9%) of NaCl and grows best in the absence of NaCl. The pH range for growth is from 5 to 8, with an optimum at pH 7. Cells show no motility and form no spores, they are irregular short rods of 0.5 μm width and 1.0–1.2 μm length, Gram-positive and have an irregular shape as seen under SEM. Some of the cells are thicker at the ends. Occasionally, coccoid cells were observed (Fig. 1). Variable cell shapes have also been reported for S. boreus and S. frigoramans (Männistö et al. 2000).

The metabolic properties of strain DB165T, in comparison with the type strains of S. boreus and S. frigoramans are shown in Table 1. Strain DB165T was found to metabolise inositol, D-sorbitol, D-sucrose, D-melibiose, glycerol, L-arabinose, D-xylose, methyl-β-D-xylopyranoside, D-galactose, D-glucose, D-fructose, D-mannose, L-rhamnose, D-mannitol, amygdalin.
The polar lipids of the strain DB165T were found to consist of phosphatidylglycerol, diphosphatidylglycerol, 5 unidentified glycolipids, 2 unidentified phospholipids and unidentified 5 lipids. The diamino acid in the peptidoglycan was identified as 2,4-diaminobutyric acid (DAB). The molar ratio of alanine:glycine:glutamic acid:DAB was 1.1:1.0:0.04:1.7. Instead of glutamic acid, high amounts of 3-hydroxyl-glutamic acid were found. Assuming that much of the glutamic acid is replaced by 3-hydroxyl-glutamic acid, and despite this

### Table 1  Physiological characteristics of strain DB165T compared to the type strains of *S. frigoramans* and *S. boreus*

|                      | S. vilae DB165T | S. frigoramans DSM 13057T | S. boreus DSM 13056T |
|----------------------|-----------------|--------------------------|----------------------|
| **APIZYM**           |                 |                          |                      |
| Alkaline phosphatase | –               | (+)                      | +                    |
| Esterase (C4)        | +               | (+)                      | +                    |
| Valine arylamidase   | (+)             | (+)                      | +                    |
| Cysteine arylamidase | (+)             | –                        | –                    |
| Trypsin              | (+)             | –                        | –                    |
| α-Galactosidase      | (+)             | –                        | –                    |
| β-Glucuronidase      | –               | –                        | +                    |
| n-Acetyl-β-glucosaminidase | –            | (+)                      | –                    |
| α-Mannosidase        | –               | (+)                      | (+)                  |
| **API20E**           |                 |                          |                      |
| Arginine dihydrolase | –               | (+)                      | –                    |
| Citrate as unique carbon source | –           | (+)                      | (+)                  |
| Tryptophan deaminase | +               | –                        | –                    |
| Tryptophanase        | +               | –                        | –                    |
| Voges–Proskauer test (production of acetoin) | +          | –                        | –                    |
| **API 50CH**         |                 |                          |                      |
| d-Arabinose          | –               | –                        | +                    |
| d-Ribose             | –               | –                        | +                    |
| Methyl-β-d-xylopyranoside | +           | –                        | –                    |
| d-Mannose            | +               | –                        | +                    |
| Arbutin              | +               | –                        | –                    |
| Salicin              | +               | –                        | –                    |
| d-Lactose (bovine origin) | –           | –                        | +                    |
| d-Melezitose         | +               | –                        | –                    |
| Gentiobiose          | +               | –                        | –                    |

In the present study, all *Subtercola* strains showed positive activity in the tests for esterase lipase (C8), leucine arylamidase, acid phosphatase, Naphthol-AS-BI-phosphohydrolase, α-glucosidase, β-glucosidase, β-galactosidase (weak in API ZYM test), d-glucose, d-mannitol, inositol, d-sorbitol, l-rhamnose, d-sucrose, d-melibiose, amygdalin, l-arabinose, glycerol, d-xylene, d-galactose, d-fructose, esculin, d-cellobiose, d-maltose, d-trehalose and d-turanose; and negative activity for lipase (C14), α-chymotrypsin, α-fucosidase, lysine decarboxylase, ornithine decarboxylase, sulfide production, urease, gelatinase, erythritol, l-xylene, l-sorbose, dulcitol, methyl-α-d-mannopyranoside, methyl-α-d-glucopyranoside, N-acetylgalactosamine, inulin, d-raffinose, starch, glycogen, xylitol, d-lyxose, d-tagatose, d-fucose, d-arabitol, l-arabinose, potassium gluconate, potassium 2-ketogluconate and potassium 5-ketogluconate

+ positive activity, (+) weak activity, – no activity
replacement, the amino acid composition is consistent with peptidoglycan type B2γ. The major isoprenoid quinones of strain DB165T were identified as MK-9 (47%) and MK-10 (39%). Minor amounts of MK-11 (6%) and MK-8 (4%) were also found to be present. The G+C content of the genomic DNA of the strain DB165T was determined to be 65.0 mol%.

The major fatty acids of the strain DB165T were identified as iso- and anteiso-saturated C15 and C16 fatty acids with anteiso-C15:0 (50%), iso-C16:0 (17%) and iso-C16:0 DMA (17%) as major components (Table 2).

16S rRNA gene sequence analyses

The 16S rRNA gene sequence of strain DB165T (1432 bp, deposited under Genbank accession number MF276890) matches to different Subtercola species in a range of 96.1–97.4% of similarity, i.e., S. frigoramans DSM 13057T (97.4% similarity), S. lobariae DSM 103962T (96.7% similarity) and S. boreus DSM 13056T (96.1% similarity). However, it also showed high similarity to Frondihabitans species, including Frondihabitans peucedani DSM 22180T (96.8% similarity) and Frondihabitans australicus DSM 17894T (96.6% similarity). The phylogenetic analysis (Fig. 2) based on the consensus 16S rRNA gene sequences (1416 bp) showed that strain DB165T forms a cluster with Subtercola species and Agreia with a strong bootstrap support, while Frondihabitans species cluster together, but the separation with the Subtercola Agreia cluster is not well supported by bootstrap analysis. In the Subtercola clade, strain DB165T is found in a distinct cluster with S. frigoramans DSM 13057T with strong bootstrap support.

Genome characteristics

The genome of strain DB165T has a size of approx. 4 Mb and give some hints on the strains’ potential to adapt to the harsh conditions that are found at Llullaillaco volcano lake. These include mechanisms concerning membrane fluidity, biosynthesis of cryoprotectants, and ice-interacting proteins (data not shown).

Discussion

Subtercola species are characterised by a peptidoglycan type B2γ with DAB as diamino acid, MK-9 and MK-10 as major respiratory quinones, and a similar polar lipids profile (Table 3).

Strain DB165T has a peptidoglycan type B2γ in which the glutamic acid is almost completely replaced by 3-hydroxyl-glutamic acid, as is found in other

### Table 2  Fatty acid profiles of strain DB165T, S. frigoramans DSM 13057T, S. boreus DSM 13056T and S. lobariae DSM 103962T

| Percent of total peak area of ion chromatograms is indicated | S. vilae DB165T | S. frigoramans DSM 13057T | S. boreus DSM 13056T | S. lobariae DSM 103962T |
|-------------------------------------------------------------|-----------------|--------------------------|----------------------|------------------------|
| Cells for fatty acid and dimethyl acetals were grown at 25°C, except for S. lobariae where DMAs were obtained from cells cultivated at 20°C | 14:0 | Tr | – | – |
| 14:0 2-OH | – | – | – | 10.3 |
| 16:0 | Tr | – | – | Tr |
| i-14:0 | 5.5 | 6.7 | Tr | 2.3 |
| i-15:0 | Tr | Tr | 4.3 | 1.4 |
| i-16:0 | 17.2 | 10.2 | 4.2 | 6.7 |
| i-17:0 | – | – | – | – |
| a-15:0 | 50.0 | 46.1 | 51.6 | 68.8 |
| a-17:0 | 6.7 | 6.8 | 3.5 | 4.2 |
| a-15:1 | – | – | Tr | – |
| 16:0 DMA | Tr | – | Tr | – |
| i-15:0 DMA | Tr | – | 1.7 | 1.5 |
| i-16:0 DMA | 17.0 | 13.3 | 11.9 | 6.9 |
| a-15:0 DMA | 3.2 | 10.3 | 11.0 | 9.7 |
| a-17:0 DMA | 1.9 | 2.9 | 4.0 | 2.6 |
| References | This study | Männistö et al. (2000) | Männistö et al. (2000) | Si et al. (2017) |
described *Subtercola* species (Männistö et al. 2000; Si et al. 2017). The metabolic characteristics that differentiate strain DB165\textsuperscript{T} from *S. boreus* and *S. frigoramans* are utilisation of methyl-β-D-xylopyranoside, arbutin salicin, α-melezitose and gentobiose as carbon sources and the enzymatic activities of trypsin, α-galactosidase, tryptophanase deaminase and tryptophanase, as well as the production of acetoin. In contrast to strain DB165\textsuperscript{T}, *S. frigoramans* exhibited as unique features arginine dihydrolase and N-acetyl-β-glucosaminidase. *S. frigoramans* and *S. boreus* can use citrate as unique carbon source and showed the enzymatic activities of alkaline phosphatase and α-mannosidase. *S. boreus* showed β-glucoronidase enzymatic activity and can use D-arabinose, D-ribose, and D-lactose, carbon sources that strain DB165\textsuperscript{T} and *S. frigoramans* cannot use. The fatty acid profile of strain DB165\textsuperscript{T} is similar to those of the type strains of other *Subtercola* species. Major differences were observed in the content of iso-C16:0, with 17% in strain DB165\textsuperscript{T} compared to 4.2–10.2% in the other *Subtercola* species and a low content of 3% anteiso-C15:0 DMA, compared to 9.7–11.0% in the other *Subtercola* species (Table 2).

The phylogenetic analysis of the 16S rDNA gene sequence clearly shows the close relationship of strain DB165\textsuperscript{T} to *Subtercola* species rather than to *Agreia* and *Frondihabitans* species. The genus *Agreia* forms a sub-cluster within *S. boreus* (this study and Si et al. 2017) and different chemotaxonomic traits have been proposed to distinguish the two genera (Schumann et al. 2003). Though both genera have a cross-linkage

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**Fig. 2** Maximum-likelihood phylogenetic tree based on 16S rRNA gene sequence comparison of strain DB165\textsuperscript{T} and species of the family *Microbacteriaceae* with *Cellulomonas carbonis* KCTC 19824\textsuperscript{T} as outgroup. Numbers at the nodes represent bootstrap support (%) based on the analysis of 1000 bootstrap replications, asterisks indicate branches of the tree that were also recovered using neighbour joining algorithm. Only bootstrap values ≥ 50% are indicated. Genbank accession numbers are given in parentheses. Bar indicates 0.01 substitutions per site.
between the amino acids in the peptidoglycan of type B2γ, in the case of Subtercola species, the cross-linkages have DAB, while Agreia species have L-DAB connected to D-Orn. Frondihabitans species can be clearly distinguished by their peptidoglycan, which is of the B2β type (Zhang et al. 2007). Fatty acids play an important role in the differentiation of the genera. The presence of 1,2 dimethyl acetals (iso-C16:0 DMA and anteiso-C17:0 DMA) is observed in all Subtercola species, while Agreia only contains iso-C15:0 DMA in low proportions (< 4%) (Schumann et al. 2003; Behrendt et al. 2002). Frondihabitans species have a fatty acid profile very distinct from those of Subtercola and Agreia species, having C18:1 and C14:0 2-OH as major fatty acids but lacking 1,2 dimethyl acetals. The major menaquinones of Subtercola species, including the strain DB165T, comprise MK-9 and MK-10, while in Agreia bicolorata DSM 14575T MK-10 and in Agreia pratensis DSM 4246T MK-10 and MK-11 are dominant, and in Frondihabitans spp. MK-8 and MK-7. The presence of MK-9 as a major component can be used as a marker to differentiate these three genera (Table 3). It should be mentioned that A. pratensis, which was originally classified as Subtercola pratensis, contains MK-10 (51%) and MK-11 (21%) as major menaquinones, but in addition 13% of MK-9 (Behrendt et al. 2002, Evtushenko et al. 2001). Irrespective of the problematic taxonomic position of Agreia species and the similarity of 16S rRNA gene sequences with Frondihabitans, the phylogenetic relationships (Fig. 2) and chemotaxonomic criteria clearly support the classification of strain DB165T as a member of the genus Subtercola (Table 3). Based on the phenotypic and genetic analyses presented in this work, strain DB165T is considered to represent a new species of the genus Subtercola, for which the name Subtercola vilae sp. nov. is proposed. The Digital Protologue database (Rosselló-Móra et al. 2017) TaxoNumber for strain DB165T is TA00217.

### Description of Subtercola vilae sp. nov.

Subtercola vilae (vi’lae, of Vila, named in honour of Irma Vila, a Chilean limnologist with outstanding contributions to the microbiology and ecology of lakes in the Chilean Altiplano and Atacama Desert).

Cells are short, irregular rods 0.5 μm wide and 1.0–1.2 μm long. Colonies are golden yellow, circular convex. Growth occurs chemoheterotrophically under oxic conditions. Optimum growth is at 10–15 °C (range from 5 to 28 °C), at pH 7 (range from pH 5 to 8)
and in the absence of NaCl. Cells produce esterase C4, esterase lipase C8, leucine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α- and β-glucosidase, tryptophan deaminase, tryptophanase and acetoin. Weak activity is observed for valine arylamidase, cysteine arylamidase, trypsin, and α- and β-galactosidase. Carbon sources used under oxic conditions include inositol, D-sorbitol, D-sucrose, D-melibiose, glycerol, L-arabinose, D-xylene, methyl-β-D-xylopyranoside, D-galactose, D-glucose, D-fructose, D-mannose, L-rhamnose, D-mannitol, amygdalin, arbutin, esculin, salicin, D-cellobiose, D-maltose, D-sucrose, D-trehalose, D-melezitose, gentiobiose and D-turanose. The cell-wall peptidoglycan is type B2γ with DAB as the diagnostic amino acid and 3-hydroxy-glutamic acid instead of glutamic acid. Major menaquinones are MK-9 and MK-10. Polar lipids comprise phoshatidylglycerol, diphasatidylglycerol, 5 unidentified glycolipids, 2 unidentified phospholipids and 5 unidentified lipids. The major cellular fatty acids are anteiso-C15:0, iso-C16:0, anteiso-C17:0, and iso-C14:0, while C14:0 and C16:0 are found only in traces. Major dimethylacetals are iso-C16:0 DMA, anteiso-C15:0 DMA and anteiso-C17:0 DMA, while C16:0 DMA and iso-C15:0 DMA are present in trace amounts. The G + C content of the DNA of the type strain is 65.0 mol%.

The type strain DB165T (= DSM 105013T = JCM 32044T) was isolated from Llullaillaco volcano lake in Chile. The GenBank/EMBL/DDBJ accession number for the 16S rRNA of strain DB165T is MF276890.

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Compliance with ethical standards

Conflict of interest The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

Bankevich A, Nurk S, Antipov D et al (2012) SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol 19:455–477

Behrendt U, Ulrich A, Schumann P, Naumann D, Suzuki K (2002) Diversity of grass-associated Microbacteriaceae isolated from the phyllosphere and litter layer after mulching the sward; polyphasic characterization of Stubercula pratesis sp. nov., Curtobacterium herbarum sp. nov. and Plantibacter flavus gen. nov., sp. nov. Int J Syst Evol Microbiol 52:1441–1454

Bligh EG, Dyer WJ (1959) A rapid method of total lipid extraction and purification. Can J Biochem Physiol 37:911–917

Bolger AM, Lohse M, Usadel B (2014) Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30:2114–2120

Christner BC, Mosley-Thompson E, Thompson LG, Zagorodnov V, Reeve JN (2007) Isolation and identification of bacteria from ancient and modern ice core archives. In: Casassa G, Sepúlveda FV, Sinclair RM (eds) The Patagonian icefields: a unique natural laboratory for environmental and climate change studies. Springer, New York, pp 9–15

Evtushenko LI (2012) Family XI. Microbacteriaceae. In: Whitman W, Goodfellow M, Kämpfer P, Busse HJ, Trujillo M, Ludwig W, Suzuki KI, Parte A (eds) Bergey’s manual of systematic bacteriology, volume 5: the actinobacteria. Springer, Berlin, pp 807–994

Evtushenko LI, Dorofeeva LV, Dobrovolskaya TG et al (2001) Diversity of grass-associated Microbacteriaceae isolated from the phyllosphere and litter layer after mulching the sward; polyphasic characterization of Stubercula pratesis sp. nov., Curtobacterium herbarum sp. nov. and Plantibacter flavus gen. nov., sp. nov. Int J Syst Evol Microbiol 52:1441–1454

Gärtner A, Wiese J, Imhoff JF (2008) Amphireia atlantica gen. nov., sp. nov., a gammamproteobacterium from the Logatchev hydrothermal vent field. Int J Syst Evol Microbiol 51:2073–2079

Goodfellow M, Fiedler H-P (2010) A guide to successful bio-prospecting: informed by actinobacterial systematics. Antonie van Leeuwenhoek 98:119–142

Kim JS, Lim JM, Ahn JH et al (2014) Description of Galbitalea soli gen. nov., sp. nov., and Frodinhbactins sucicola sp. nov. Int J Syst Evol Microbiol 64:572–578

Kutzner HJ (1981) The family Streptomyctecaceae. In: Starr MP, Stolp H, Trüper HG, Balows A, Schlegel HG (eds) The prokaryotes—a handbook on habitats, isolation and identification of bacteria. Springer, Berlin, pp 2028–2090

Kuykendall LD, Roy MA, O’Neill JJ, Devine TE (1988) Fatty acids, antibiotic resistance, and deoxyribonucleic acid
homology groups of Bradyrhizobium japonicum. Int J Syst Bacteriol 38:358–361

Li HR, Yu Y, Luo W, Zeng YX (2010) Marisediminicola antarctica gen. nov., sp. nov., an actinobacterium isolated from the Antarctic. Int J Syst Evol Microbiol 60:2535–2539

Männistö MK, Schumann P, Rainey FA et al (2000) Subtercola boreus gen. nov., sp. nov. and Subtercola frigoramans sp. nov., two new psychrophilic actinobacteria isolated from boreal groundwater. Int J Syst Evol Microbiol 50:1731–1739

Miller LT (1982) Single derivatization method for routine analysis of bacterial whole-cell fatty acid methyl esters, including hydroxy acids. J Clin Microbiol 16:584–586

Peeters K, Ertz D, Willems A (2011) Culturable bacterial diversity at the Princess Elisabeth Station (Uststeinen, Sør Rondane Mountains, East Antarctica) harbours many new taxa. Syst Appl Microbiol 34:360–367

Pruesse E, Peplies J, Glöckner FO (2012) SINA: accurate high-throughput multiple sequence alignment of ribosomal RNA genes. Bioinformatics 28:1823–1829

Rossello-Móra R, Trujillo ME, Sutcliffe IC (2017) Introducing a digital protologue: a timely move towards a database-driven systematics of archaea and bacteria. Syst Appl Microbiol 40:121–122

Saitou N, Nei M (1987) The neighbour-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406–425

Schaeffer AB, Fulton MD (1933) A simplified method of staining endospores. Science 77:194

Schleifer KH (1985) Analysis of the chemical composition and primary structure of murein. Methods Microbiol 18:123–156

Schumann P (2011) Peptidoglycan structure. Methods Microbiol 38:101–129

Schumann P, Behrendt U, Ulrich A, Suzuki KI et al (2003) Reclassification of Subtercola pratensis Behrendt et al. 2002 as Agreia pratensis comb. nov. Int J Syst Evol Microbiol 53:2041–2044

Si H, Shi F, Zhang L et al (2017) Subtercola lobariae sp. nov., an actinobacterium of the family Microbacteriaceae isolated from the lichen Lobaria retigera. Int J Syst Evol Microbiol 67:1516–1521

Singh P, Hanada Y, Singh SM, Tsuda S (2014) Antifreeze protein activity in Arctic cryoconite bacteria. FEMS Microbiol Lett 351:14–22

Tamura K, Stecher G, Peterson D et al (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. Mol Biol Evol 30:2725–2729

Tindall BJ (1990a) Lipid composition of Halobacterium lacusprofundi. FEMS Microbiol Lett 66:199–202

Tindall BJ (1990b) A comparative study of the lipid composition of Halobacterium saccharovorum from various sources. Syst Appl Microbiol 13:128–130

Tindall BJ, Sikorski J, Smibert RA, Krieg NR (2007) Chapter 15: phenotypic characterization and the principles of comparative systematics. In: Reddy C, Beveridge T, Breznak J, Marzluf G, Schmidt T, Snyder L (eds) Methods for general and molecular microbiology, 3rd edn. ASM Press, Washington, DC, pp 330–393

Zhang L, Xu Z, Patel BKC (2007) Frondicola australicus gen. nov., sp. nov., isolated from decaying leaf litter from a pine forest. Int J Syst Evol Microbiol 57:1177–1182

Zhang DC, Brouchkov A, Griva G, Schinner F, Margesin R (2013) Isolation and characterization of bacteria from ancient Siberian permafrost sediment. Biology 2:85–106