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# *Frigoribacterium faeni* gen. nov., sp. nov., a novel psychrophilic genus of the family *Microbacteriaceae*

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**The taxonomic position of five actinobacterial strains isolated from dust, an animal shed, the air inside a museum and soil was investigated using a polyphasic approach. The growth characteristics were unusual for actinomycetes. Optimal growth was at temperatures ranging from 2 to 10 °C. After small-step adaptation (5 °C steps) to higher temperatures, the strains were also able to grow at 20 °C. Cell wall analyses revealed that the organisms showed a hitherto undescribed, new group B-type peptidoglycan [type B2$\beta$ according to Schleifer & Kandler (1972), but with lysine instead of ornithine]. All strains contained menaquinone MK-9. Mycolic acids were not detected. Diphosphatidylglycerol, phosphatidylglycerol and an unknown glycolipid were detected in the polar lipid extracts. The main fatty acids were 12-methyl-tetradecanoic acid (15:0anteiso), 12-methyl-tetradecenoic acid (15:1anteiso), 14-methyl-pentadecanoic acid (16:0iso) and 14-methyl-hexadecanoic acid (17:0iso), as well as an unusual compound identified as 1,1-dimethoxy-anteiso-pentadecane (15:0anteiso-DMA). The G+C content of DNA was approximately 71 mol%. The results of 16S rRNA gene sequence comparisons revealed that the strains represent a new lineage in the suborder *Micrococcineae* and the family *Microbacteriaceae* of the order *Actinomycetales*. On the basis of these results the new genus *Frigoribacterium* gen. nov. is proposed, harbouring the new species *Frigoribacterium faeni* sp. nov. (type strain = 801$^T$ = DSM 10309$^T$).**

**Keywords:** *Frigoribacterium* gen. nov., psychrophilic genus of the family *Microbacteriaceae*, 16S rDNA, chemotaxonomy

## INTRODUCTION

The family *Microbacteriaceae* was proposed by Park *et al.* (1993) to accommodate the Gram-positive genera *Agromyces*, *Aureobacterium*, *Clavibacter*, *Curtobacterium* and *Microbacterium*. On the basis of 16S rRNA sequence data, Stackebrandt *et al.* (1997) redefined the family to accommodate the above-mentioned genera in addition to the genera *Agrococcus* and *Rathayibacter*. The new genera *Leucobacter* (Takeuchi *et al.*, 1996) and *Cryobacterium* (Suzuki *et al.*, 1997) were also shown to represent new branches within the family *Microbacteriaceae*. The type genus *Microbacterium* was redefined by Takeuchi & Hatano (1998), who proposed the unification of the genera *Microbacterium* and *Aureobacterium* on the basis of 16S rRNA sequence data and chemotaxonomic data. A common feature of the eight currently recognized genera within the family *Microbacteriaceae* is the group B-type peptidoglycan. In this type the dicarboxyl amino acid (in most cases D-glutamate or D-hydroxyglutamate) at position 2 is linked with the amino acid at position 4 (D-alanine) via an interpeptide bridge containing a D-amino acid. Very recently, the occurrence of psychrophilic organisms within the family *Microbacteriaceae* has been shown with the

**Abbreviations:** DMA, dimethyl acetal; ECL, equivalent chain length; FAME, fatty acid methyl ester.

The EMBL accession numbers for the 16S rRNA gene sequences of strains 801, NS 4, 227, 301 and 312 are Y18807, AJ243012, AF157478, AF157479 and AF157480, respectively.

description of the genus *Cryobacterium* (Suzuki *et al.*, 1997). In an extensive study of bacteria collected from dust samples and animal sheds (Andersson *et al.*, 1999) four isolates were obtained which revealed atypical growth characteristics. This was also found for a strain isolated from the Sainsbury Center for Visual Arts in Norwich, UK. All of them showed initially good growth at 2–10 °C but only moderate growth at 25 °C. A detailed study of the cell wall type revealed an interesting hitherto undescribed B-type.

In this paper we describe the morphological, physiological, chemotaxonomic and phylogenetic characteristics of these organisms. On the basis of our results and the unique taxonomic properties of the organisms, it can be concluded that they represent a new genus for which we propose the genus name *Frigoribacterium* gen. nov. and the new species *Frigoribacterium faeni* sp. nov.

## METHODS

**Sampling and isolation.** Strains 301, 312 and 801$^T$ were isolated from airborne dust in a cattle barn in Southern Finland. Dusts aerosolized during the distribution and handling of feed and bedding were collected on nuclepore filters as described by Palmgren *et al.* (1986). The cow barn aerosols were collected for 10 min on nuclepore filters with a nominal pore size of 0·2 μm, using a low flow personal sampling pump precalibrated to a flow of 2 l min$^{-1}$. Sampling was carried out at a distance of 0·4 m from the bales of hay and straw opened while the air contained clouds of visible dust. Strain 227 was isolated from settled dust. Settled dusts were collected from horizontal surfaces in the animal sheds > 2 m above the floor. Strain NS 4 was isolated during a sampling campaign for airborne bacteria in the Sainsbury Center for Visual Arts in Norwich, UK. It was cultivated on casein minimal medium (CAS MM; Altenburger *et al.*, 1996) supplemented with cycloheximide and incubated at room temperature. Subcultivation was done on PYES medium (Altenburger *et al.*, 1996).

Bacteria in settled and airborne dusts were resuscitated by a method described previously (Andersson *et al.*, 1999) and cultured on tryptic soy agar at 13–16 °C. The medium and diluent for serial plating were obtained from Difco unless otherwise stated.

**Morphological characteristics.** Cell morphology was examined by phase-contrast microscopy with a light microscope (Leitz). Motility was studied by the hanging drop method. Cell dimensions were measured with an ocular (× 10) and an objective (× 100/1·25). Gram staining was performed by using Hucker's modification (Gerhardt *et al.*, 1994). Colony morphology was studied by using a stereo microscope (Olympus model SZ 11). For electron microscopy the cells were grown on tryptic soy agar for 3 d at room temperature. Thin sections were prepared as described previously (Andersson *et al.*, 1995).

**Physiological characteristics.** The effects of different growth temperatures were determined on Bacto Nutrient Agar incubated at 0, 2, 5, 10, 20, 28, 37, 45 and 50 °C. Minimal growth temperature was determined on tryptic soy agar plates. The plates were incubated for 2 weeks and growth was registered as visible colonies. Physiological tests in microtitre plates were done as described earlier (Kämpfer *et al.*, 1991, 1997). Tests were read after 7 d at 20 °C.

**Chemotaxonomy.** Preparation of cell walls and determination of peptidoglycan structure were carried out by the methods described by Schleifer & Kandler (1972) with the modification that TLC on cellulose sheets was used instead of paper chromatography. Briefly, 1 mg freeze-dried cell walls were hydrolysed in 0·2 ml 4 M HCl at 100 °C for 16 h (total hydrolysate) or 45 min (partial hydrolysate). Diamino acids were identified from total hydrolysate by one-dimensional chromatography in the solvent system methanol/pyridine/water/10 M HCl (320:40:70:10 by vol.). Amino acids and peptides from partial hydrolysate were identified after two-dimensional chromatography in the systems described by Schleifer & Kandler (1972) by their mobilities and staining characteristics with ninhydrin spray. The resulting 'fingerprints' could be compared with known peptidoglycan structures. The configuration of lysine was determined by using L-lysine decarboxylase (Sigma; L 0882). Cell wall acyl type was determined by the method of Uchida & Aida (1977).

**Extraction and analysis of isoprenoid quinones and polar lipids.** Menaquinones were extracted and analysed as described by Tindall (1990). Polar lipids were extracted and analysed by TLC according to Ventosa *et al.* (1993).

**Preparation and analysis of fatty acids and fatty acetals.** Fatty acid methyl esters (FAMEs) were determined according to Kämpfer *et al.* (1997), except that the cells were grown at 28 °C. DMAs (dimethyl acetals) were preliminarily identified from the extracts prepared for FAME analysis using the MIDI anaerobic library (BHIBLA, Version 3.8). The identity of the DMA in *Frigoribacterium* strains was confirmed by GC-MS analysis using an HP 5MS capillary column, an HP 6890 gas chromatograph and an HP 6890 mass selective detector set at the ionization energy of 70 eV. The temperature was increased from 170 to 270 °C at a rate of 5 °C min$^{-1}$; the inlet temperature was 250 °C. The Wiley 138K and NIST/EPA/NIH mass spectral libraries were used for reference.

**Base composition of DNA and DNA–DNA reassociation studies.** DNA was isolated by chromatography on hydroxyapatite by the procedure of Cashion *et al.* (1977). The base composition of DNA and the calculation of the G+C ratio was determined as described by Mesbah *et al.* (1989) and Tamaoka & Komagata (1984). DNA–DNA reassociation experiments were performed as described by De Ley *et al.* (1970), with the modifications described by Huss *et al.* (1983), by using a Gilford System model 2600 spectrophotometer equipped with a model 2527-R thermoprogrammer and plotter (Gilford Instruments). Renaturation rates were computed with the TRANSFER.BAS program (Jahnke, 1992).

**16S rRNA gene sequence determination and phylogenetic analyses.** The extraction of genomic DNA, PCR amplification of the 16S rRNA gene and sequencing of the purified PCR products were carried out as described previously (Rainey *et al.*, 1996). Sequence reaction products were purified by ethanol precipitation and electrophoresed with a model 373A or a model 310 Genetic Analyzer (Applied Biosystems). The 16S rRNA sequences obtained in this study were aligned against previously determined actinobacterial sequences available from the public databases using the ae2 editor (Maidak *et al.*, 1994). The method of Jukes & Cantor (1969) was used to calculate evolutionary

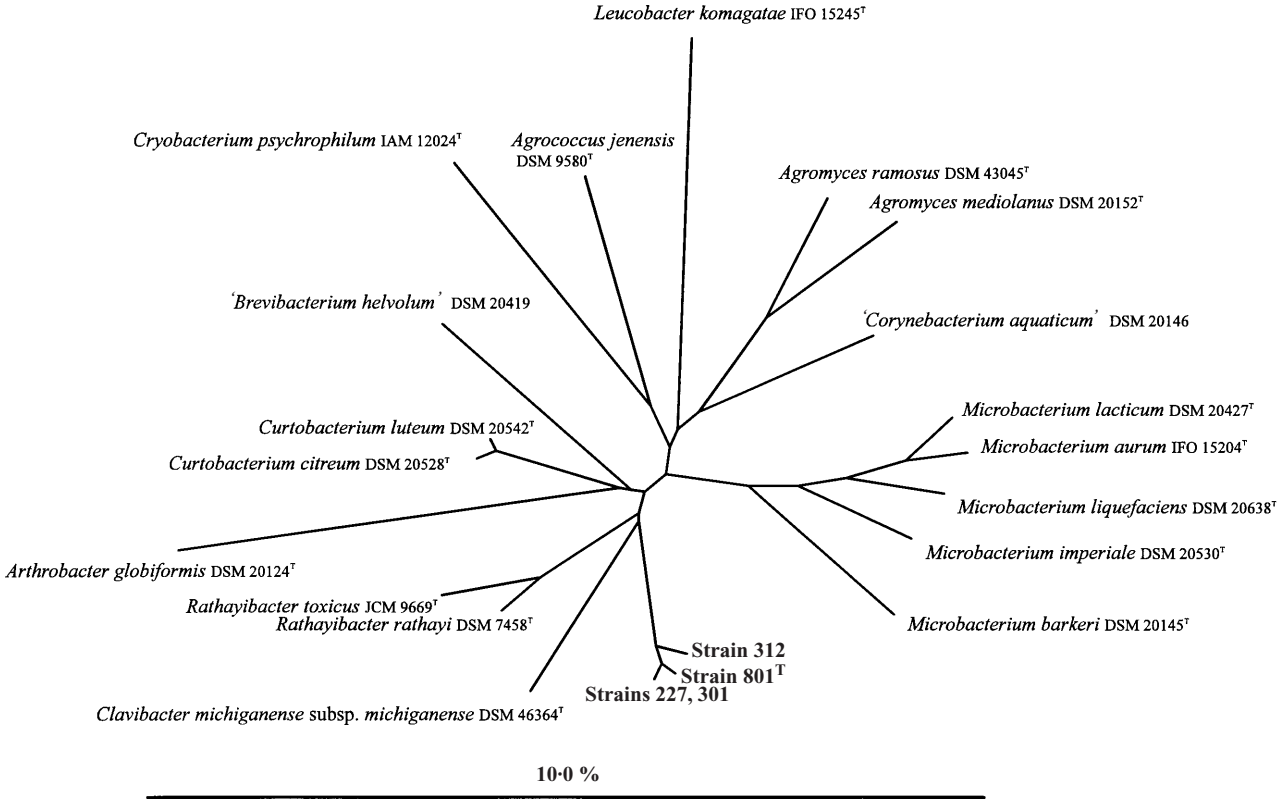


***Fig. 1.*** Phylogenetic tree based on 16S rDNA sequence comparison demonstrating the phylogenetic position of four strains of the genus *Frigoribacterium*. Scale bar indicates 10 nt substitutions per 100 nt.

distances. Phylogenetic dendrograms were generated using various treeing algorithms contained in the PHYLIP package (Felsenstein, 1993). For strain NS 4, the 16S rRNA gene was amplified by PCR using universal primers 27f and 1492r (Lane, 1991). Amplification products were purified by precipitation with polyethylene glycol (Wieser *et al.*, 1999). Purified PCR products were directly sequenced (starting at *Escherichia coli* position 142) at the Service Department at the Vienna Biocentre, Austria (MIG-BASE), on a LI-COR 4000 L, as outlined by Middendorf *et al.* (1992). The following fluorescently labelled primers were used: 27f, 926f, 530f (Lane, 1991). The derived sequence was aligned and compared with those of other bacterial 16S rDNA sequences available in the EMBL database using the University of Wisconsin Genetics Computer Group (GCG) 1995 program package (version 8.1).

**Nucleotide sequence accession numbers.** The strain designations and accession numbers of the reference 16S rRNA gene sequences used in the phylogenetic analyses are as follows: *Agrococcus jenensis* DSM 9580$^T$ (X92492), *Agromyces ramosus* DSM 43045$^T$ (X77447), *Agromyces mediolanus* DSM 20152$^T$ (X77449), *Arthrobacter globiformis* DSM 20124$^T$ (M23411), '*Brevibacterium helvolum*' DSM 20419 (X77440), *Clavibacter michiganense* subsp. *michiganense* DSM 46364$^T$ (X77435), '*Corynebacterium aquaticum*' DSM 20146 (X77450), *Cryobacterium psychrophilum* IAM 12024$^T$ (D45058), *Curtobacterium citreum* DSM 20528$^T$ (X77436), *Curtobacterium luteum* DSM 20542$^T$ (X77437), *Leucobacter komagatae* IFO 15245$^T$ (D17751), *Microbacterium aurum* IFO 15204$^T$ (D21340), *Microbacterium barkeri* DSM 20145$^T$ (X77446), *Microbacterium imperiale* DSM 20530$^T$ (X77442), *Microbacterium lacticum* DSM 20427$^T$ (X77441), *Microbacterium liquefaciens* DSM 20638$^T$ (X77437), *Rathayibacter rathayi* DSM 7458$^T$ (X77439) and *Rathayibacter toxicus* JCM 9669$^T$ (D84127).

## RESULTS AND DISCUSSION

### 16S rRNA gene sequence comparison

Almost complete 16S rRNA gene sequences of between 1458 and 1467 nt were determined for four isolates and 1301 nt of the 16S rRNA gene sequence of strain NS 4. Phylogenetic analyses based on a data set comprising 1310 unambiguous nt between positions 38 and 1478 [*E. coli* positions (Brosius *et al.*, 1978)] showed the new isolates to cluster together as a distinct lineage within the radiation of the actinomycete genera with group B peptidoglycan that comprise the family *Microbacteriaceae* (Stackebrandt *et al.* 1997) (Fig. 1). Strain NS 4 was not included in the final dendrogram because only a partial sequence was available. The five strains share 99·3–100 % 16S rRNA gene sequence similarity, strains 227 and 301 showing identical sequence. The new isolates show highest 16S rRNA gene sequence similarity with species of the genera *Rathayibacter* (96·1–97·1 %) and *Clavibacter* (96·9–97·1 %), while species of these two previously described genera share 96·3–96·9 % 16S rRNA gene sequence

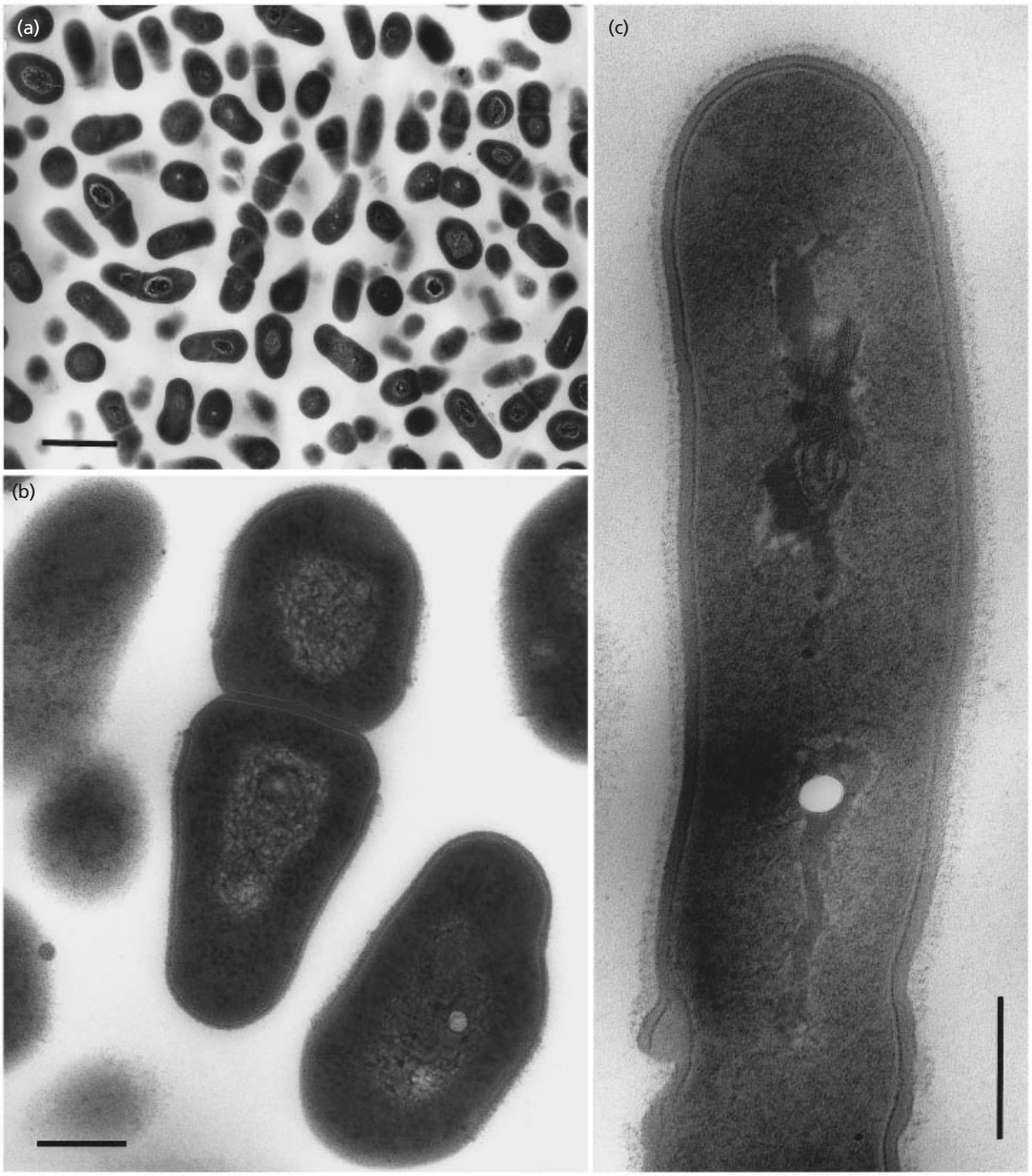


***Fig. 2.*** Electron micrographs of thin sections of strain 801$^T$ grown on TSA plates for 72 h at room temperature. The cells appear coccoid (a) or pleiomorphic (b). At high magnification (c) the cell wall appears hairy and a membranous collar was seen at the expected location of cell division (see Fig. 4c). Bars, 1 (a) and 0·2 μm (b, c).

similarity. The bootstrap analyses indicate no significance in the branching pattern of the lineage comprising the new isolates and any previously described generic lineage of this family (Fig. 1).

### Phenotypic and chemotaxonomic characteristics

All five strains were Gram-positive, motile, rod-shaped organisms. Cells were 0·2–0·3 μm wide and 1·0–1·5 μm long. On nutrient agar, the organisms formed rough, round, convex, pale yellow colonies. Optimal growth was detected at 8 and 4 °C on tryptic soy agar, but good growth was also detected at 0 °C (moderate growth even at −2 °C, 14 d). It was interesting to note that the strains also grew well at 20 °C and moderately well at 25 °C after a small-step adaptation (5 °C steps) up to these temperatures. For this reason the physiological tests were performed at 20 °C. The ultrastructure of strains 227, 301 and 801$^T$ was very similar and is shown for strain 801$^T$ in Fig. 2. The shape of the

***Table 1.*** Physiological characteristics of the *Frigoribacterium* strains

Test results were read after 72 h incubation at 20 °C. +, Positive; −, negative; (+), weak positive. Acid formation from the carbohydrates lactose, sucrose, D-mannitol, dulcitol, salicin, inositol, sorbitol, L-arabinose, raffinose, rhamnose, maltose, D-xylose, trehalose, cellobiose, methyl D-glucoside, erythritol, melibiose, D-arabitol and D-mannose was negative for all strains. All strains utilized the following substrates as sole source of carbon: *N*-acetylglucosamine, L-arabinose, *p*-arbutin, D-cellobiose, D-fructose, D-galactose, gluconate, D-glucose, D-mannose, D-maltose, α-D-melibiose (weak), D-ribose, sucrose, salicin, D-trehalose, D-xylose, maltitol and D-mannitol. All strains were negative for the utilization of acetate, propionate, *trans*-aconitate, adipate, 4-aminobutyrate, azelate, glutarate, 3-hydroxybutyrate, itaconate, DL-lactate, L-malate, mesaconate, oxoglutarate, pyruvate, suberate, L-alanine, β-alanine, L-histidine, L-leucine, L-phenylalanine, L-serine, L-tryptophan, 3-hydroxybenzoate, 4-hydroxybenzoate and phenylacetate. All strains were positive for hydrolysis of aesculin, pNP-β-D-galactopyranoside (weak), pNP-β-D-glucopyranoside, pNP-α-D-glucopyranoside, pNP-β-D-xylopyranoside, L-alanine-pNA and L-proline-pNA. None of the strains hydrolysed: pNP-β-D-glucuronide, Bis-pNP-phosphonate, pNP-phosphoryl-choline, 2-deoxythymidine-5′-pNP phosphate and L-glutamate-γ-3-carboxy-pNA (pNP, *para*-nitrophenyl; pNA, *para*-nitroanilide).

| Test | 801$^T$ | 227 | 301 | 312 | NS 4 |
|---|---|---|---|---|---|
| Acid produced from: | | | | | |
| Glucose | (+) | − | − | − | − |
| Adonitol | (+) | − | − | − | − |
| Assimilation of: | | | | | |
| L-Rhamnose | + | + | − | + | − |
| Adonitol | + | + | − | − | − |
| i-Inositol | − | + | + | + | + |
| D-Sorbitol | + | + | + | − | − |
| Putrescine | − | + | − | − | − |
| *cis*-Aconitate | − | (+) | (+) | (+) | − |
| Citrate | + | + | + | − | − |
| Fumarate | + | + | + | − | (+) |
| L-Aspartate | + | (+) | + | − | (+) |
| L-Ornithine | + | (+) | + | − | − |
| L-Proline | (+) | (+) | (+) | − | − |

cells was often irregular, such as pleomorphic, curved or asymmetric rods. The plane of cell division was not symmetrical. At low magnifications the cell walls were typically Gram-positive, but at higher magnifications some cells seemed to have an additional hairy layer on their surface (Fig. 2b and c). This hairy layer was missing from the point of cell division (Fig. 2b). A membranous collar was observed sometimes near the plane of division (Fig. 2c).

The biochemical characteristics of the strains are summarized in Table 1. All strains were able to assimilate a wide variety of sugars. Only a few acids and amino acids were utilized as sole source of carbon. The chemotaxonomic features are summarized in Table 2. The amino acids found in the cell wall hydrolysate were glycine, alanine, homoserine and lysine (1:1:1:1 molar ratio). The structure of the peptidoglycan is depicted in Fig. 3. This peptidoglycan belongs to the B2β group, according to Schleifer & Kandler (1972), and is similar to that found in *Curtobacterium*; however, ornithine is replaced by D-lysine. Until now this type has not been found in any organism in the peptidoglycan B group.

No glycolate was found in the acid hydrolysate of bacterial cells, which suggested that muramic acid occurred in the *N*-acetyl form. The major isoprenoid quinone was MK-9 (Table 2), found in all five strains. Diphosphatidylglycerol, phosphatidylglycerol and an unknown glycolipid were predominantly detected in the polar lipid extracts of isolate NS 4. The glycolipid displayed the same chromatographic behaviour as the one designated by Collins & Jones (1980), glycolipid 2 ($G_2$). In addition, three unknown lipids were found which were not stained by any of the spray reagents, α-naphthol, molybdenum blue or ninhydrin (Table 3). The lack of additional glycolipids, which are present in the phylogenetic neighbours *Clavibacter* and *Rathayibacter* (Collins & Jones, 1980), clearly distinguishes the strains from the representatives of these two genera. The detection of the polar lipids from strains 801$^T$, 227, 301, 312 (two spots) and NS 4 revealed a homogeneous profile concerning the predominant compounds (Table 3). The reinvestigation of the polar lipids from *Clavibacter michiganense* ICMP 2550$^T$ demonstrated that the unknown glycolipid displayed the same chromatographic behaviour as the one designated glycolipid 2 ($G_2$) by Collins & Jones (1980). Although the overall polar lipid patterns of strains 801$^T$ and 227 were nearly identical, they could be distinguished from each other by the lack of the glycolipids GL1, GL2 and GL3 in strain 801$^T$ and quantitative differences in lipid L2. Both strains differed from the other three strains by the presence of spots 1 and 2. A characteristic of strain 301 was the lack of lipid L1 (pigment 2) and in strain 312 the lack of pigment 3 allowed differentiation from the other four strains. Before staining with any of the spray reagents the two-dimensional chromatographic separation of the polar lipid extracts of strains 227 and 801$^T$ showed a visible identical distribution of the yellow pigment components (eight spots), whereas the pigment profiles were unique for each of strains 301 (six spots), 312 (two spots) and NS 4 (five spots) (results not shown).

The major fatty acids were 15:0anteiso, 16:0iso and 16:0 when grown near the maximum tolerable temperature for these strains, 28 °C, the standard for the MIDI system used (Table 4). When grown at lower temperatures the 15:0anteiso content of the strains (NS 4 was not tested) moderately decreased and that of 16:0 drastically decreased (to $<4$% at $<4$ °C), but increased amounts of 15:1anteiso were generated (as

***Table 2.*** Differentiating characters of the genera of the family *Microbacteriaceae*

Data from Davis *et al*. (1984), Groth *et al*. (1996), Suzuki *et al*. (1997) and Takeuchi & Hatano (1998). ND, Not determined.

| Genus | Cell wall* | Diamino acid‡ | Acyl type | G+C (mol%) | Fatty acid type§ | Major MK† | Polar lipids‖ |
|---|---|---|---|---|---|---|---|
| *Agrococcus* | B | DAB | Acetyl | 74 | S,A,I | MK-11,12 | PG, GL |
| *Agromyces* | B | DAB | Acetyl | 71–76 | S,A,I | MK-11,12 | PG, GL |
| *Clavibacter* | B | DAB | Acetyl | 67–78 | S,A,I | MK-9,10 | PG, GL |
| *Cryobacterium* | B | DAB | ND | 65 | S,A,I (12H) | MK-10 | PG, GL |
| *Curtobacterium* | B | D-Orn | Acetyl | 68–75 | S,A,I (H) | MK-9 | PG, GL |
| *Frigoribacterium* | B | D-Lys | Acetyl | 71·7 | S,A,I¶ | MK-9 | PG, DPG# |
| *Leucobacter* | B | DAB | Acetyl | 66·2 | S,A,I | ÛMK-11 | PG, GL, DPG |
| *Microbacterium* | B | L-Lys/D-Orn | Glycolyl | 65–76 | S,A,I | MK-11,12,13,14 | PG, GL |
| *Rathayibacter* | B | DAB | ND | 63–72 | S,A,I | MK-10 | PG, GL |

* Designation of Schleifer & Kandler (1972).

† DAB, diaminobutyric acid; Lys, lysine; Orn, ornithine.

‡ S, straight-chain saturated; A, anteiso-methyl-branched; I, iso-methyl-branched; (H), cyclohexyl fatty acids sometimes present; 12H, 12-methyl-hexadecanoic acid.

§ Designation of Collins & Jones (1981).

‖ GL, glycolipid; PG, phosphatidylglycerol; DPG, diphosphatidylglycerol.

¶ *Frigoribacterium* strains (NS 4 not tested) contain unsaturated anteiso fatty acids when grown at low temperature (<10 °C).

# For details see Table 3.

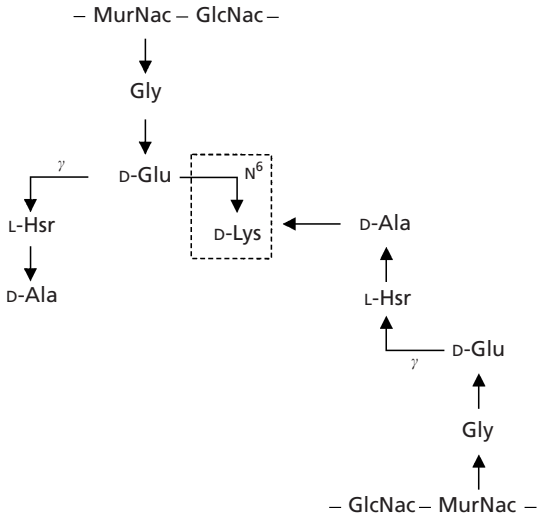


***Fig. 3.*** Fragment of peptidoglycan structure of the genus *Frigoribacterium* gen. nov. showing cross-linkage between positions 2 and 4 of two peptide subunits. MurNac, *N*-acetylmuramic acid; GlcNac, *N*-acetylglucosamine; L-Hsr, L-homoserine.

shown in Fig. 4a). This is different from the mode of cold adaptation of most Gram-positive bacteria, such as *Bacillus* spp., which adapt by changing their ratio of anteiso to iso fatty acids (Kaneda, 1991).

The MIDI system identified one peak in all strains as the hydroxylated fatty acid 14:0 2OH, with an ECL (equivalent chain length) of −0·008. It was found in relatively high amounts (10–26% of the fatty compounds; Table 4). Mass spectrometric investigations revealed that this component was not a hydroxy FAME. Its mass fragmentation pattern (Fig. 4b) and the mass spectral libraries available (Wiley 138K and NIST/EPA/NIH) proved it was 1,1-DMA. The size ($m/z$) of the mass fragments showed that this DMA had a carbon chain of C15, longer than those recorded in the commercially available mass spectral libraries. The molecular ion ($m/z$ 272) of the 15:0 DMA is not visible in Fig. 4(b). The first loss from the molecular ion was of $m/z$ 31, i.e. methoxyl group, giving an ion of $m/z$ 241. The GC retention time of the C15:0 DMA peak of strain MA227 (= peak at $R_T$ = 6·0 min in Fig. 4a) had an exact match with the ECL of 15:0anteiso-DMA found in the FAME of *Propionibacterium freudenreichii* (3%) and *Propionibacterium jensenii* (2%) (data not shown). One or several of the minor peaks in the total ion chromatogram (Fig. 4b) may present DMAs of shorter carbon chain lengths. In conclusion, we decided that the large peak in the FAME GC of the five strains of *Frigoribacterium*, located between two FAMEs, 15:0anteiso and 16:0iso, was 1,1-dimethoxy-12-methyl-pentadecane (15:0anteiso-DMA). It must be noted that all of the strains can be easily misidentified as sphingomonads based on the yellow pigmentation of the colonies and the signature fatty acid 14:0 2OH which is present in all *Sphingomonas* spp. (Kämpfer *et al*., 1997).

The G+C content of the DNA of strain 801$^T$ was found to be 71·1 mol%. The peptidoglycan type of the

***Table 3.*** Polar lipid composition of *Frigoribacterium* strains

+++, Major spots; ++, minor spots; +, traces detected; ND, not detected. Major spots were visible for PG and DPG and minor spots were visible for pigment 1 and GL1 in all strains.

| Strain | L1 (pigment 2) | L2 | Pigment 3* | Pigment 4* | Pigment 5† | GL2 | GL3‡ | GL4‡ | Spot 1† | Spot 2† |
|---|---|---|---|---|---|---|---|---|---|---|
| 801$^T$ | +§ | ++ | + | ND | ND | ND | ND | ND | + | + |
| 227 | +§ | + | + | ND | ND | + | + | + | + | + |
| 301 | ND | + | + | + | ND | ND | + | + | ND | ND |
| 312 | ++ | + | ND | ND | + | + | + | + | ND | ND |
| NS 4 | +§ | + | + | ND | + | ND | + | + | ND | ND |

* Only visible as a brown spot after detection with α-naphthol.

† Only visible as a grey spot after detection with α-naphthol.

‡ Only visible as a purple spot after detection with α-naphthol.

§ Only visible as a yellow spot before spraying and/or after detection with α-naphthol as a grey spot.

***Table 4.*** Major fatty acid composition of strains of the genus *Frigoribacterium**

All strains were grown on Trypticase soy broth agar at 28 °C for 72 h prior to fatty acid analysis.

| Compound | Total fatty acid (%) | | | | |
|---|---|---|---|---|---|
| | 801$^T$ | 227 | 312 | 301 | NS 4 |
| Saturated fatty acids | 1·4 | 0·5 | 1·8 | 0·7 | |
| 15:0 | 0·4 | | 0·7 | 0·5 | |
| 16:0 | 18·5 | 9·8 | 26·5 | 13·0 | 4·4 |
| 18:0 | | | 0·6 | | |
| Branched fatty acids | | | | | |
| 14:0iso | | | 1·0 | | |
| 15:0iso | 1·8 | 1·4 | 1·8 | 1·8 | 1·3 |
| 15:0anteiso | 39·6 | 37·8 | 38·7 | 48·3 | 46·7 |
| 16:0iso | 14·4 | 15·0 | 10·0 | 11·8 | 16·7 |
| 17:0iso | | 0·4 | | | |
| 17:0anteiso | 8·0 | 14·8 | 7·0 | 10·4 | 8·5 |
| Other | 2·1 | 1·9 | 2·0 | 1·1 | 0·7 |
| DMA | | | | | |
| 15:0anteiso-DMA* | 13·7 | 18·5 | 10·1 | 12·5 | 21·5 |

* 15:0anteiso-DMA = 1,1-dimethoxy-anteiso-pentadecane (see Fig. 4 for mass spectrum). The MIDI system identified this compound as 14:0 2OH.

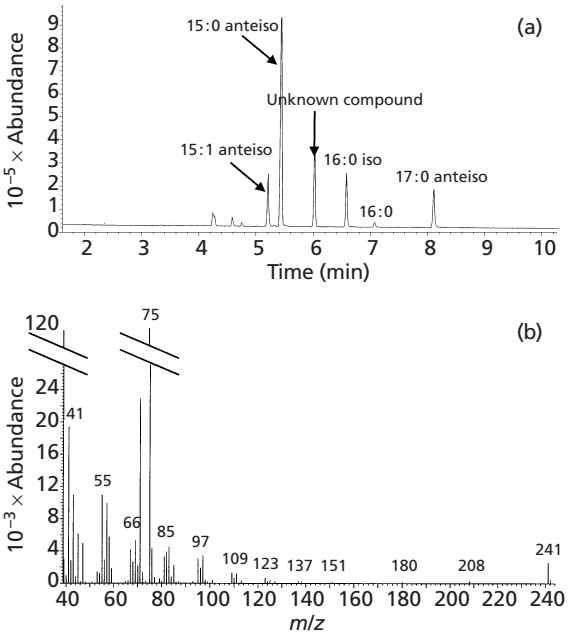


***Fig. 4.*** Identification of the DMA in the FAME extract of *Frigoribacterium* gen. nov. (a) Total ion gas chromatogram of the FAME of strain MA227 grown on TSA plates at 4 °C. (b) Mass fragmentogram of the peak with an $R_T$ of 6·0 min (unknown compound) in (a). The molecular mass of the identified compound, 15:0anteiso-DMA, is 272 Da. The peak at *m/z* 241 of the first product after cleavage of the methoxy group (*m/z* 31) is shown as well as several other diagnostic fragments.

strains is the most striking feature differentiating this organism from members of other genera. The organisms showed an hitherto undescribed, new group B-type peptidoglycan [type B2*β* according to Schleifer & Kandler (1972), but with lysine instead of ornithine]. Characteristics that differentiate the five strains from four other genera with group B-type peptidoglycan are shown in Table 2. The five isolates from different air samples were very similar with respect to the other chemotaxonomic properties. The combination of respiratory quinones, fatty acids and polar lipids indicated that these organisms belong in the family *Microbacteriaceae*. It is interesting to note that 15:0anteiso-DMA was found in considerable amounts in all strains. This compound has also been detected in the anaerobic species *Propionibacterium freudenreichii* and *Propionibacterium jensenii*, confirming a moderate relatedness of *Microbacteriaceae* and *Propionibacteriaceae* (Stackebrandt *et al.*, 1997).

The low levels of similarity of the 16S rRNA gene sequence indicated that the strains represent a distinct lineage within the suborder *Micrococcineae*. On the basis of the distinct peptidoglycan features, the presence of the unusual compound 15:0anteiso-DMA and 16S rDNA sequence analyses, we propose that these strains should be placed in a new genus, *Frigoribacterium* gen. nov. In spite of the great similarity between the strains with regard to physiological and chemotaxonomic properties (Tables 1, 3, 4), DNA–DNA reassociation studies showed that the levels of relatedness (binding rates) between strain 801$^T$ and strains 227 and 301 were 37 and 44 %, respectively, and the level of relatedness (binding rate) between strains 227 and 301 was 52 %. These data indicate that the strains are similar genomovars of one species. This is the first report of the unusual amino acid D-lysine, which is present in the peptidoglycan of *Frigoribacterium*, as a major component of the cell wall of a bacterium.

### Description of *Frigoribacterium* gen. nov.

*Frigoribacterium* (Fri.go.ri.bac′te.ri.um. L. n. *frigor* frost, cold; Gr. n. *bakterion* small rod; N.L. neut. n. *Frigoribacterium* a small rod growing in the cold).

Cells are Gram-positive, non-sporulating, motile, irregular-shaped rods, the cell division zone being thicker than the poles of the cell. No mycelium is produced. Catalase is produced but oxidase is not produced. Growth on complex medium is observed in a temperature range of 2–25 °C, optimum 4–10 °C. The G+C content of the DNA is approximately 71 mol %. Cell wall peptidoglycan contains D-lysine as a diamino acid. The glycan moiety of the peptidoglycan contains acetyl residues. The major isoprenoid quinone is menaquinone MK-9. Mycolic acids are not present. Diphosphatidylglycerol, phosphatidylglycerol and an unknown glycolipid were detected in the polar lipid extracts. The major fatty acids are 15:0anteiso and 16:0iso. As an additional compound 1,1-dimethoxy-anteiso-pentadecane (15:0anteiso-DMA) is produced in considerable amounts (10–30 % of whole-cell fatty substances). Using standardized FAME analysis, the retention time of this compound is almost identical to that of 14:0 2OH. The type species is *Frigoribacterium faeni*.

### Description of *Frigoribacterium faeni* gen. nov., sp. nov.

*Frigoribacterium faeni* (fae′ni. L. n. *faenum* hay; L. gen. n. *faeni* of hay).

Cells are Gram-positive, irregular rods that are 0·2–0·3 μm wide and 1·0–1·5 μm long. The cells are non-sporulating and motile in 2-d-old cultures on nutrient agar. No mycelium is produced. The morphology of the species and its chemotaxonomic characteristics are the same as described for the genus. Aerobic. Good carbon sources are sugars including *N*-acetyl-D-glucosamine, L-arabinose, *p*-arbutin, D-cellobiose, D-fructose, D-galactose, gluconate, D-glucose, D-mannose, D-maltose, L-rhamnose, D-ribose, sucrose, D-trehalose and D-xylose. Only a few organic acids are utilized as sole source of carbon (citrate, fumarate). Details of other physiological properties are given in Table 1. Isolated from hay dust. The type strain, 801$^T$, has been deposited in the Deutsche Sammlung von Mikroorganismen und Zellkulturen as DSM 10309$^T$.

Because of the DNA–DNA similarity data the genus contains at least two further genomovars. The reference strain for genomovar 2 is 227 (= DSM 10310), the reference strain for genomovar 3 is 301 (= DSM 10311). Details of the physiological and chemotaxonomic properties of representatives of these genomovars are given in Tables 1, 3 and 4.

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