Friedmanniella antarctica gen. nov., sp. nov., an LL-Diaminopimelic Acid-Containing Actinomycete from Antarctic Sandstone

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A gram-positive, aerobic, slowly growing actinomycete was isolated from antarctic sandstone. Packets of spherical cells of this organism form clusters. The diagnostic diamino acid of the peptidoglycan is LL-diaminopimelic acid with glycine in position 1 of the peptide subunit. The major menaquinone is MK-9(H₄), and the main cellular fatty acids are 12- and 13-methyltetradecanoic acids. Only a few organic compounds are metabolized. The DNA base composition is 73 mol% G + C. A 16S ribosomal DNA sequence comparison showed that this isolate is a phylogenetic neighbor of the propionibacteria and related taxa. Its closest relative is *Microthricus phosphovor*. Morphological, physiological, and genotypic characteristics support the description of a new genus and new species, *Friedmanniella antarctica* gen. nov., sp. nov. The type strain is strain AA-1042 (= DSM 11053).

Actinomycete genera which contain LL-diaminopimelic acid (LL-A₂pm) in their peptidoglycan fall in five phylogenetic lineages, as revealed by 16S ribosomal DNA (rDNA) sequence analyses. These are (i) the streptomycetes (39), (ii) the genus *Terrabacter* (5), (iii) the genus *Sporichthya* (30), (iv) the genera *Nocardioides* and *Aeromicrobium* (4, 5, 8, 21, 43), and (v) the genera *Propioniferax* (49), *Luteococcus* (42), and *Microthricus* (24). These genera are distinguished from each other and from other actinomycete genera mainly by chemotaxonomic characteristics and by their 16S rDNA sequences. From antarctic sandstone a single LL-A₂pm-containing strain was isolated, and its 16S rDNA was analyzed. On the basis of morphological, physiological, and 16S rDNA analyses, we concluded that this strain, strain AA-1042^T (T = type strain), belongs to a new genus and species, for which the name *Friedmanniella antarctica* is proposed.

MATERIALS AND METHODS

Bacterial strains. Strain AA-1042^T was isolated from a sandstone sample containing a cryptoendolithic microbial community collected by one of us (P.H.) at 1,600 m above ocean level from Linnaeus Terrace, McMurdo Dry Valleys, Asgard Range, Transantarctic Mountains, Antarctica (15). The sandstone boulder was flat, had an area of approximately 2 m², and had at the sampling side (east side) a pH of 4.8. The stone was quite brittle, and some loosened material was directly sprinkled onto the surfaces of PYGV (41) (pH 6.9) agar plates. The plates were incubated for 5 months at 9°C in dim light. Individual colonies around sand grains were picked and streaked onto the same medium for purification. Resulting single and pure colonies were subcultured after 5 months on PYGV agar slants at 4 to 6°C. Strain AA-1042^T has been deposited in the IFAM Culture Collection (Institut für Allgemeine Mikrobiologie, Kiel, Germany). The strains used for comparison were the following: *Aeromicrobium erythreum* NRRL B-3381^T (= DSM 8599^T), *Aeromicrobium fastidiosum* NCIB 12713^T (= DSM 10552^T), *Intrasporangium calvum* 7 KIP^T (= DSM 43043^T), *Luteococcus japonicus* IFO 12422^T (= DSM 10546^T), *Microthricus phosphovor* JCM 9379^T (= DSM 10555^T), *Nocardioides albus* IMET 7807^T (= DSM 43109^T), *Nocardioides simplex* NCIB 8929^T (= DSM 20130^T), *Nocardioides jensenii* NCIB 9770^T (= DSM 20641^T), *Nocardioides plantarum* NCIMB 12834^T (= DSM 11054^T), *Propioniferax innocua* NCTC 11082^T (= DSM 8251^T), *Sporichthya polymorpha*

IMET 7840^T (= DSM 46113^T), *Streptomyces albus* ATCC 3004^T (= DSM 40313^T), and *Terrabacter tumescens* NCIB 8914^T (= DSM 20308^T).

Media. The following media were used in the liquid state or in the semisolid state (prepared by adding 1.5% [wt/vol] Koble 1 agar [Serva]): (i) PYGV medium (41); (ii) R medium (48); (iii) modified organic medium 79 (29) containing (per liter) 10 g of dextrose, 10 g of Bacto Peptone (Difco), 2 g of Casamino Acids (Difco), 2 g of yeast extract (Serva) and 6 g of NaCl (pH 7.5); (iv) TSB medium (Difco); (v) enriched nutrient agar (Serva) and enriched nutrient broth (Serva) (pH 7.2).

Cultural conditions. The biomass used for all chemotaxonomic studies of strain AA-1042^T was produced on R-agar slant cultures at 22°C and was harvested after 2 to 3 weeks. *Sporichthya polymorpha* was cultivated on nutrient agar at 28°C for 3 weeks. The other organisms were cultivated at 28°C for between 24 and 48 h in 100-ml flasks containing 20 ml of R medium by using a horizontal shaker at 220 rpm. For menaquinone studies organic medium 79 was used. The cell mass used for analyses of cell walls, mycolic acids, and phospholipids and for DNA extraction was obtained in R medium. Fatty acid analyses were performed with cells grown in TSB medium.

The following temperatures were used to study growth, morphology, and pigmentation: 6, 9, 18, 22, 25, 28, and 37°C. The source of light for strain AA-1042^T was daylight near a window without direct exposure to sun.

Staining procedures. Gram staining and acid-fast staining were done as described previously (18).

Morphological studies. Cell morphology and aggregate morphology were determined by phase-contrast microscopy (oil immersion objective; magnification, ×100) of material obtained from surface growth on agar plates, as well as by direct phase-contrast observation of growing cultures on 2-mm agar layers by using ring cultures 50 mm in diameter and plastic film to reduce evaporation.

Physiological and biochemical characterization. All physiological tests were performed at 22°C. Inoculum was taken from R-agar slant or plate cultures. Spreading and dispersing of the inoculum were restricted by its consistency. Cultures were examined after 5, 8, 14, and 21 days of incubation.

The relationship to oxygen was studied with agar stab cultures and, in parallel, slant cultures covered with paraffin oil. Oxidase activity was checked by examining oxidation of *N,N,N',N'*-tetramethyl-*p*-phenylenediamine dihydrochloride (38). Acidification of carbohydrates was verified by a color change from blue to yellow in a medium containing 0.5% (wt/vol) Bacto Tryptone (Difco), 0.4% (wt/vol) Casamino Acids (Difco), 0.07% (wt/vol) (NH₄)₂HPO₄, 0.5% (wt/vol) NaCl, 0.003% (wt/vol) bromocresol purple, and the substrates at a final concentration of 1% (wt/vol). Utilization of organic acids as carbon sources (13) and decomposition of adenine, xanthine, hypoxanthine, and DL-tyrosine were determined as described previously (12). Catalase production was shown by the production of bubbles after a suspension of the organism was mixed with a drop of a 3% hydrogen peroxide solution on a slide. Urease activity, nitrate reduction, esculin hydrolysis, and hydrolysis of Tween 80 were studied by using previously described methods (18). Decomposition of the emulsion of UV-sterilized cinefilm stripes incubated in phosphate buffer was used to verify gelatin hydrolysis. Clear zones in streak-inoculated agar (a mixture of 6 ml of liquefied aqua dest. agar and 2 ml of steamed skim milk) indicated positive casein hydrolysis reactions. DNA hydrolysis was indicated by clear zones in spot-inoculated plates containing Bacto DNase test agar (Difco). Decomposition of hippurate was

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determined as described previously (9). Hydrolysis of starch was shown by the occurrence of colorless zones around colonies grown on inorganic salts-starch agar (37) after the surface was flooded with Lugol's solution containing 0.1% (wt/vol) iodine and 0.2% (wt/vol) potassium iodide. H_2S production was determined as described previously (27). NaCl tolerance was checked on R agar containing NaCl at final concentrations of 2, 4, and 10% (wt/vol).

Color determination. Colors of agar cultures were determined by using a color code (26) as applied in the International *Streptomyces* Project (37).

Cell wall analysis. Purified cell wall preparations were obtained by previously described methods (35). Amino acids and peptides of cell wall hydrolysates were analyzed by two-dimensional ascending thin-layer chromatography on cellulose plates by using the solvent systems described previously (35). Cell wall sugars were determined as alditol acetates (32) by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). Molar ratios of amino acids were determined by GC and GC-MS of *N*-heptafluorobutyl amino acid isobutyl esters (19). The instruments used for GC and GC-MS have been described previously (14). The glycolate content of bacterial cells was determined by the colorimetric method described by Uchida and Aida (44).

Lipid analysis. Cellular fatty acid methyl esters were separated by GC and GC-MS as described previously (14). They were identified by comparison with the Bacterial Acid Methyl Esters CP Mix (catalog no. 1114; Matreya Inc., Pleasant Gap, Pa.) and with fatty acid methyl ester extracts from *Dermacoccus nishinomiyaensis* DSM 20448^T containing unsaturated iso and anteiso methyl-branched acids (40). 2-Hydroxyhexadecanoic acid methyl ester was identified on the basis of its characteristic fragment ion having a mass of 227 by GC-MS. Menaquinones were extracted (7) and analyzed by high-performance liquid chromatography as described previously (14). Polar lipids extracted as described by Minnikin et al. (23) were identified by two-dimensional thin-layer chromatography and spraying with specific reagents (6). The absence of mycolic acids was demonstrated as described previously (22).

Analysis of DNA base composition. DNA was isolated and its G + C content was determined by high-performance liquid chromatography as described previously (14).

16S rDNA sequence determination and analysis. Genomic DNAs were extracted from the strains investigated in this study and were used for PCR-mediated amplification of 16S rDNA (31). The purified PCR products were directly sequenced by using previously described protocols (31), and the sequence reaction mixtures were electrophoresed with a model 373A automatic DNA sequencer (Applied Biosystems, Foster City, Calif.).

The 16S rDNA sequences were manually aligned with the sequences of members of the order *Actinomycetales* by using the ae2 editor (20). Evolutionary distances were calculated as described previously (16). Phylogenetic dendrograms were reconstructed by using treeing algorithms contained in the PHYLIP package (11). Bootstrap analyses were used to evaluate the tree topologies of the neighbor-joining data by performing 1,000 resamplings (10).

Nucleotide sequence accession numbers. The 16S rDNA sequences determined in this study have been deposited in the EMBL database under the following accession numbers: strain AA-1042^T (= DSM 11053^T), Z78206; *Aeromicrobium fastidiosum* DSM 10552^T, Z78209; *I. calvum* DSM 43043^T, Z78214; *L. japonicus* DSM 10546^T, Z78208; *Microlunatus phosphovorus* DSM 10555^T, Z78207; *Nocardioides albus* DSM 43109^T, Z78213; *Nocardioides jensenii* DSM 20641^T, Z78210; *Nocardioides plantarum* DSM 11054^T, Z78211; and *Nocardioides simplex* DSM 20130^T, Z78212. The accession numbers of the sequences of the reference strains used in the phylogenetic analyses are as follows: *Actinoplanes philippinensis* DSM 43019^T, X93187; *Aeromicrobium erythreum* NRRL B-3381^T, M37200; *Arthrobacter globiformis* DSM 20124^T, M23411; *Atopobium minutum* ATCC 33267^T, M59059; *Microbacterium lacticum* DSM 20427^T, X77441; *Nocardia asteroides* DSM 43757^T, X80606; *Propionibacterium acnes* DSM 1897^T, X53218; *Propionibacterium freudenreichii* DSM 20271^T, X53217; *Propioniferax innocua* DSM 8251^T, S93388; *T. tumescens* DSM 20308^T, X83812; *Saccharothrix australiensis* ATCC 31497^T, X5192; *Sporichthya polymorpha* DSM 46113^T, X72377; and *Streptomyces albidoflavus* DSM 40455^T, Z76676.

RESULTS

Cultural characteristics. R agar, 22°C, and diffuse daylight were found to be optimal for growth of strain AA-1042^T as measured by the diameter of the colonies. The following results were obtained under these conditions. Visible growth appeared 4 days after R agar was inoculated from 2-week-old cultures on this medium. The colonies reached the maximum size within 2 to 3 weeks. The raised colonies (diameter, 0.5 to 2.2 mm) had dull and crumbly surfaces. The edges and surfaces were irregular and depended on the internal structure of the colonies. The color was bright orange (color code [37], Oc 2r-O) but was less intense when the organism was grown in the dark. In aging cultures the color changed from orange to yellow (color code, Co 3a-Cr) and finally to faint yellow; this was

accompanied by a decline in viability and even a loss of viability. Growth was slower and there was less growth on PYGV agar than on R agar; the orange color was less intense (color code, Oc 3a), and the colony surface became smooth and shiny.

The temperature range for growth on R agar was 18 to 25°C, and the optimum temperature was 22°C. Growth was slow and poor at 9°C, and growth did not occur at 6, 28, or 37°C. Addition of 2% (wt/vol) NaCl to R agar resulted in restricted growth, while no growth occurred in the presence of 4% (wt/vol) NaCl. The optimal pH was 6.0 to 7.2. At pH 8.1 growth was very poor, and no growth occurred at pH 5.1 and 8.7.

Growth was slow and very poor in submerged cultures. Growth could be improved by aeration. Shaken cultures turned faintly turbid, and the material sedimented rapidly. There was no growth in stab cultures and on agar slant cultures covered with paraffin oil. There was less growth and cluster formation on PYGV agar than on R agar, and the color was yellow to orange.

Maintenance. Serial transfers at 2-week intervals on R-agar slant cultures at 22°C under diffuse daylight kept the culture viable. Strain AA-1042^T was lyophilized in PYGV medium and was subcultured successfully after 27 months of storage at 4°C. Maintenance in the vapor phase of liquid nitrogen resulted in good viability when the organism was recultivated after 33 months. Permanent cultures in glycerol were not prepared since the two other methods proved to be successful.

Morphological characteristics. On PYGV medium single cells were spherical or nearly spherical and ranged from 1.2 to 1.5 µm in diameter. Occasionally larger cells that were 2.0 to 2.2 µm in diameter were observed. On R agar the cells varied from 0.5 to 2.2 µm in diameter. Generally, the cells occurred in packets (Fig. 1A and B) which resulted from cell division in three perpendicular planes. The packets adhered to one another, forming clusters (Fig. 1A and B). The clusters were more regular on PYGV medium (Fig. 1A) than on R medium (Fig. 1B). On R agar, dividing cells had an ellipsoidal appearance (Fig. 1C). Motile cells and formation of spores were never observed.

Staining reactions. Strain AA-1042^T stained gram positive, and no lysis was observed following treatment with 3% (wt/vol) KOH. The cells were not acid fast.

Physiological characteristics. Strain AA-1042^T grew strictly aerobically. The oxidase test was negative. *Microlunatus phosphovorus*, which was described as weakly oxidase positive (24), was found in this study to be distinctly oxidase positive. Catalase and urease were produced. Nitrate was not reduced to nitrite. Acid was produced from D-ribose, was produced weakly from L-arabinose, and was produced very weakly from D-xylose. No acid was produced from L-rhamnose, D-glucose, D-fructose, D-mannose, D-galactose, maltose, lactose, sucrose, D-cellobiose, D-trehalose, D-raffinose, glycerol, D-mannitol, and myo-inositol. Starch and esculin were hydrolyzed, while Tween 80 and DNA were only weakly hydrolyzed. Casein and gelatin were not hydrolyzed. Sodium formate was utilized as a carbon source. Sodium acetate, sodium aconitate, sodium benzoate, sodium citrate, disodium succinate, and potassium hydrogen tartrate were not utilized. Hypoxanthine, xanthine, adenine, DL-tyrosine, and sodium hippurate were not decomposed. H_2S was produced.

Chemotaxonomic characteristics. The fatty acid profile of strain AA-1042^T (Table 1) is characterized by the predominance of anteiso- $C_{15:0}$ and iso- $C_{15:0}$ and the occurrence of iso-branched tetra- and hexadecanoic acids, iso-hexadecenoic acid, and octadecadienoic acid. Since the results of fatty acid analyses vary to some degree between laboratories and the methods used differ to some extent, the fatty acid profiles of

FIG. 1. Phase-contrast photomicrographs of strain AA-1042^T. (A) Clusters grown on PYGV broth for 4 weeks at 20°C. Magnification, $\times 1,280$. (B) Cluster grown on R medium for 5 days at 22°C. Magnification, $\times 1,500$. (C) Single cells, partly dividing. Magnification, $\times 1,500$.

the type strains of related taxa were determined under standardized conditions (Table 1). The peptidoglycan of strain AA-1042^T contains LL-A₂pm, and its interpeptide bridge consists of a single glycine residue. Position 1 of the peptide subunit is occupied by glycine. The molar ratio of the peptidoglycan amino acids Gly, Ala, Glu, and LL-A₂pm is 2.1:0.5:1.0:1.4. On the basis of these results strain AA-1042^T represents peptidoglycan type A3 γ' as described by Schleifer and Seidl (36). The acyl type is acetyl. Glucose and minor amounts of ribose, mannose, and rhamnose occur as cell wall sugars. The isoprenoid quinones are MK-9(H₄) and MK-9(H₂); the

TABLE 1. Cellular fatty acid compositions of strain AA-1042^T and the type strains of related taxa

Taxon	Fatty acid composition (%) ^a																			
	i-C _{14:0}	C _{14:0}	i-C _{15:0}	i-C _{15:1}	ai-C _{15:0}	C _{15:0}	C _{15:1}	i-C _{16:0}	i-C _{16:1}	C _{16:0}	C _{16:1}	i-C _{17:0}	ai-C _{17:0}	i-C _{17:1}	ai-C _{17:1}	C _{17:0}	C _{18:0}	C _{18:1}	C _{18:2}	TBSA
AA-1042 ^T	2.3		23.2		54.5			2.4	2.9										6.0	
<i>Microtholunatus phosphovorus</i>	4.0	2.9	22.4		43.5			6.2		7.5		2.2	6.7	1.3	2.9	2.4	48.1	3.2		5.0
<i>Propioniferax innocua</i>	3.2		20.9	1.2	46.5			7.3	4.5							2.4	2.1	3.0	18.9	
<i>Luteococcus japonicus</i>		1.0				5.6	11.7			4.8	20.5					2.4		2.7	20.2	15.8
<i>Aeromicrobium erythreum</i> ^b		3.1				3.1				47.0	5.2					2.4		2.7		
<i>Aeromicrobium fastidiosum</i> ^c		1.1				1.4				20.6	5.4									
<i>Nocardiothex albus</i>	2.3		1.7			1.5		70.8		3.0	1.9	2.1				2.1	6.0	2.1	2.6	1.9
<i>Nocardiothex simplex</i>	4.6		2.6					41.8	1.1	3.8	5.0	1.9				5.2			13.1	5.2
<i>Nocardiothex jensenii</i>	1.7		16.7			1.3		28.6	2.8	4.3	2.3	9.6	1.0			3.9	6.5	1.5	10.3	2.4
<i>Nocardiothex plantarum</i>			3.4			1.0		26.0		2.9	1.0	5.4	16.8			6.1	4.0	1.8	15.7	1.9
<i>Leishmania tarentolae</i>	24.2		35.4	1.5	3.7	1.1		13.4	8.6		1.4					2.4				
<i>Intrasporangium calvum</i>	5.0		37.8	3.5	12.6	2.7	1.5	12.3	3.1	1.2		1.7	3.9	3.7		1.0				
<i>Sporichthya polymorpha</i>		4.5				6.1		8.9		24.4	9.9	6.7	10.9	1.1	1.7	8.7	20.9	7.5	5.3	
<i>Streptomyces albus</i>	5.3		16.3		25.6			18.7	6.7	1.7	1.0									

^a Values less than 1% not shown. i, iso; ai, anteiso; TBSA, tuberculoheptanoic acid (10-methyl octadecanoic acid).

^b Also contains 4.6% 2-hydroxyhexadecanoic acid.

^c Also contains 20.3% 2-hydroxyhexadecanoic acid.

TABLE 2. Differential characteristics of strain AA-1042^T and related taxa that have LL-A₂pm in the cell wall^a

Taxon	Cell morphology	Relationship to oxygen	Major menaquinone(s)	Peptidoglycan interpeptide bridge	Amino acid at position 1 of peptide subunit	Polar lipids	G + C content (mol%)	Reference(s)
AA-1042 ^T	Cocci in packets	a	MK-9(H ₄)	Gly	Gly	PI, PG, DPG, PL	73	
<i>Microthunatus</i>	Cocci	a	MK-9(H ₄)	Gly ^b	Gly ^b	PI, PG, DPG, PL	67.9	24
<i>Luteococcus</i>	Cocci	f	MK-9(H ₄)	Gly ^b	L-Ala ^b	PI, PG, DPG, GL	66-68	42
<i>Propioniferax</i>	Rods	f	MK-9(H ₄)	Gly	L-Ala	PE, PG, PL, GL ^{b,c}	59-63	45, 49
<i>Aeromicrobium</i>	Rods	a	MK-9(H ₄)	Gly	L-Ala	PE, PG	71-73	21, 43
<i>Propionibacterium propionicus</i>	Pleomorphic	f	MK-9(H ₄)	Gly	Gly	ND	63-65	34, 46
<i>Nocardioides albus</i>	Hyphae	a	MK-8(H ₄)	Gly	L-Ala	PG, DPG, PL, PG-OH	66.5-68.6	5, 25, 28
<i>Nocardioides simplex</i>	Rods, cocci	a	MK-8(H ₄)	Gly	L-Ala	PG, DPG, PL, PG-OH	71.7	5, 25
<i>Terrabacter</i>	Rods, cocci	a	MK-8(H ₄)	Gly ₃	L-Ala	PE, PI, DPG, PL	69.8-73.4	5, 35
<i>Intrasporangium</i>	Hyphae	a	MK-8	Gly ₃ ^b	L-Ala ^b	PI, PIM, PG, DPG	68.2	17
<i>Sporichthya</i>	Hyphae	a	MK-9(H ₆), MK-9(H ₈)	Gly ^b	L-Ala ^b	PI, PG, DPG, PL	70.1	30
<i>Streptomyces</i>	Hyphae	a	MK-9(H ₆), MK-9(H ₈)	Gly	L-Ala	PE, PI, PIM, DPG	69-73	47

^a Abbreviations: a, aerobic; f, facultatively anaerobic; Gly, glycine; L-Ala, L-alanine; DPG, diphosphatidylglycerol; PG, phosphatidylglycerol; PG-OH, phosphatidylglycerol containing 2-hydroxy fatty acids; PI, phosphatidylinositol; PIM, phosphatidylinositol mannosides; PE, phosphatidylethanolamine; PL, unknown phospholipid(s); GL, unknown glycolipid(s); ND, not determined. Abbreviations for menaquinones are exemplified by MK-9(H₄): menaquinone with two of nine isoprene units hydrogenated.

^b Our data supplementing previously published descriptions of the respective genera.

^c Strain DSM 8251^T contained five unknown glycolipids.

peak area ratio of these quinones is 63:8. The polar lipid pattern consists of phosphatidylglycerol, diphosphatidylglycerol, phosphatidylinositol, and one unknown phospholipid. Mycolic acids are absent. The DNA base composition is 73 mol% G + C.

Since the previously published chemotaxonomic data for some of the type species listed in Table 1 were found to be incomplete, previously undetermined characteristics are given in Table 2. Moreover, nearly all of the data available for these taxa were examined and confirmed. Except for *I. calvum*, no significant differences were found between the data determined in this study and the data published previously. The data reported previously for the *I. calvum* fatty acid profile, structure of the interpeptide bridge, and phospholipid pattern (17) were not confirmed by our reexamination. The chemotaxonomic characteristics of strain AA-1042^T and the other LL-A₂pm-containing actinomycete genera are summarized in Table 2.

16S rDNA sequence analysis. Nine almost complete 16S rDNA sequences comprising between 1,460 and 1,484 nucleotides (>95% of the *Escherichia coli* sequence [1]) were determined in this study. New sequences were determined for a number of reference strains (see above) due to numerous ambiguities in the sequences previously deposited in the public databases. The phylogenetic dendrogram shown in Fig. 2 was reconstructed from evolutionary distances by the neighbor-joining method (33). A total of 1,136 nucleotides present in all of the strains between position 39 and position 1313 (*E. coli* positions [1]) were used for this analysis. The phylogenetic dendrogram (Fig. 2) shows that strain AA-1042^T falls within the radiation of the cluster comprising the genera *Microthunatus*, *Luteococcus*, *Propioniferax*, and *Propionibacterium*. This cluster is recovered in 94% of bootstrap analyses. Strain AA-1042^T represents a distinct lineage and exhibits levels of 16S rDNA sequence similarity to the other members of the cluster ranging from 89.1 to 93.9%. The branch point of strain AA-1042^T and its closest relative, *Microthunatus phosphovorius*, was supported by 99% recovery in the bootstrap analysis. A comparison of the sequences of strain AA-1042^T and *Microthunatus phosphovorius* at 1,457 unambiguous nucleotide positions

FIG. 2. 16S rDNA sequence-based phylogenetic dendrogram reconstructed from evolutionary distances by using the neighbor-joining method (33). Scale bar = 5 inferred nucleotide substitutions per 100 nucleotides. The numbers at the branch points are bootstrap values.

showed that these organisms exhibited 94.0% similarity. The phylogenetic position of *I. calvum* DSM 43043^T was also determined in this study. This species clusters with *T. tumescens* (96% sequence similarity) (Fig. 2) and *Sanguibacter* species (data not shown).

DISCUSSION

Our phylogenetic analysis identified the antarctic bacterial isolate AA-1042^T as a neighbor taxon of *Micrococcus phosphovorus*. The genomic relatedness of these taxa is also expressed at the epigenetic level, as demonstrated by very similar chemotaxonomic properties. The isoprenoid quinone composition, polar lipids, and amino acids of the peptide subunit of group A peptidoglycan (35) are identical in these two taxa. The chemotaxonomic properties clearly distinguish this pair of organisms from the other actinomycete genera containing LL-A₂pm in the peptidoglycan and glycine in the interpeptide bridge. Strain AA-1042^T and *Micrococcus phosphovorus* are both characterized by the occurrence of glycine at position 1 of the peptide subunit. This property has also been found in *Propionibacterium propionicum* (46), but this organism is biochemically, morphologically, and phylogenetically a true member of the genus *Propionibacterium* (2, 3). The occurrence of glycine at this position has also been found in members of the family *Microbacteriaceae*; however, these organisms are characterized by the rare group B peptidoglycan (35). In contrast to the previously published data for *Propionibacterium innocua* (49), L-alanine and not glycine occupies position 1 in the peptide subunit (45). Despite striking similarities between strain AA-1042^T and *Micrococcus phosphovorus* JCM 9379^T in several chemotaxonomic properties, differences occur in the primary structure of the 16S rDNA, in morphology, in physiological properties, in the base composition of DNA, and in fatty acid composition. Generally, cells of strain AA-1042^T do not separate from each other after cell division, but packets develop and these packets aggregate to form irregular clusters. In contrast, cells of *Micrococcus phosphovorus* occur singly, in pairs, or in some cases in clusters (24). In submerged cultures *Micrococcus phosphovorus* grows well, while strain AA-1042^T grows very slowly and poorly. Strain AA-1042^T is oxidase negative and uses a very narrow range of organic compounds as carbon sources, while *Micrococcus phosphovorus* is oxidase positive and metabolizes a wide range of organic compounds. Strain AA-1042^T does not tolerate 4% (wt/vol) NaCl in the growth medium, while *Micrococcus phosphovorus* grows in the presence of 6% (wt/vol) NaCl. Strain AA-1042^T does not reduce nitrate to nitrite, whereas *Micrococcus phosphovorus* does. Saturated straight-chain fatty acids with 14, 16, and 18 carbon atoms are found in *Micrococcus phosphovorus* but have not been found in strain AA-1042^T. The fatty acid profile of strain AA-1042^T contains 6% octadecadienoic acid, a compound not found in the other LL-A₂pm-containing organisms included in this study.

On the basis of these differences we concluded that strain AA-1042^T should not be considered a new species of the genus *Micrococcus*. Consequently, a new genus and species, *Friedmanniella antarctica* gen. nov., sp. nov., is proposed, and a description of this taxon is given below.

Description of *Friedmanniella* gen. nov. *Friedmanniella* (Fried. man.ni.el'la. M. L. dim. ending -ella; M. L. fem. n. *Friedmanniella*, named after E. Imre Friedmann, an American microbiologist, in recognition of his contributions to antarctic microbiology). Spherical to ellipsoidal cells that occur mostly in more or less regular packets which develop through cell division in three perpendicular planes. The packets aggregate,

forming clusters. Gram positive. Nonmotile. No spores are formed. Not acid fast. Strictly aerobic. Oxidase negative. Catalase positive. Chemoorganotrophic. Only a few carbohydrates, organic acids, and other carbon sources are metabolized.

The LL-A₂pm-containing group A3γ' peptidoglycan contains glycine in position 1 of the peptide subunit and a single glycine residue as an interpeptide bridge. The major menaquinone is MK-9(H₄), and the main fatty acids are 12- and 13-methyltetradecanoic acids (anteiso-C_{15:0} and iso-C_{15:0}). Mycolic acids are absent. The phospholipid pattern includes phosphatidylglycerol, diphosphatidylglycerol, phosphatidylinositol, and one unknown phospholipid. The DNA base composition is 73 mol% G + C.

The type species is *Friedmanniella antarctica*.

Description of *Friedmanniella antarctica* sp. nov. *Friedmanniella antarctica* (ant 'arc.ti.ca. M. L. fem. adj. *antarctica*, isolated from Antarctica). The spherical cells are 0.5 to 2.2 μm in diameter. The cells are arranged in more or less regular packets, which adhere to one another to form clusters. Growth in submerged standing and shaking cultures is slow and poor. Colonies on R agar are up to 2 mm in diameter and raised and have irregular edges and shapes. The colony surface is crumbly and dull to smooth and shiny depending on the growth medium. The orange color of colonies may become more intense when the organism is cultured in diffuse daylight. The optimum growth temperature is 22°C. The temperature range is approximately 9 to 25°C; no growth occurs at 6 and 28°C. The optimal pH range for growth is 6.0 to 7.2.

Catalase positive. Oxidase negative. Urease positive. Does not reduce nitrate to nitrite. Acid is produced from D-ribose, is produced weakly from L-arabinose, and is produced very weakly from D-xylose. No acid is produced from L-rhamnose, D-glucose, D-fructose, D-mannose, D-galactose, maltose, lactose, sucrose, D-cellobiose, trehalose, D-raffinose, glycerol, D-mannitol, and myo-inositol. Starch and esculin are hydrolyzed, and Tween 80 and DNA are only weakly hydrolyzed. Casein and gelatin are not hydrolyzed. Sodium formate is utilized as a carbon source. Sodium acetate, sodium aconitate, sodium benzoate, sodium citrate, disodium succinate, and potassium hydrogentartrate are not utilized. Hypoxanthine, xanthine, adenine, DL-tyrosine, and sodium hippurate are not decomposed. H₂S is produced. The DNA base composition is 73 mol% G + C.

The organism was isolated from a cryptoendolithic microbial community in sandstone on Linnaeus Terrace, McMurdo Dry Valleys, Antarctica.

The type strain is strain AA-1042. It has been deposited in the DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH as strain DSM 11053^T.

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