***Modestobacter multiseptatus* gen. nov., sp. nov., a budding actinomycete from soils of the Asgard Range (Transantarctic Mountains)**

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Oligotrophic PYGV medium, inoculated with soils from Linnaeus Terrace (1600 m, Antarctica), yielded four aerobic actinomycetes with short rods, multiple and irregular septa and often motile buds. Cells were 1.0–2.8 × 1.0–3.0 µm and colonies were beige to pink. The isolates were nearly identical in physiological and biochemical tests. Three strains grew from 0 °C to 25–28 °C, but one was psychrophilic with a maximum growth temperature of 20 °C. Carbon sources utilized were D-glucose, D-galactose, lactose, sucrose or mannitol; malate, succinate, fumarate, pyruvate or glutarate were decarboxylated aerobically. Peptone and yeast extract were the preferred nitrogen sources. Nitrate was reduced aerobically or anaerobically. Cell walls contained meso-diaminopimelic acid, glutamate, alanine, glycine, galactose, glucose and ribose. Major fatty acids of strains AA-802, -824, -825 and -826^T were n18:1, i16:0 and ai17:0. Major respiratory quinones were MK-9(H₄) and MK-8(H₄). Polar lipids were diphosphatidylglycerol, phosphatidylethanolamine and phosphatidylinositol. Phosphatidylglycerol was found in most strains. The DNA G+C contents were 68–70 mol%. In 16S rDNA analyses, similarity values obtained for 500 nucleotides from the 5' terminus were >99.5%. Almost complete sequences from AA-826^T and -825 were 99.9% similar. Strain AA-826^T belonged to a novel cluster of desert soil and rock isolates within the *Geodermatophilaceae* and was equidistantly related to members of *Geodermatophilus* and to a *Blastococcus* lineage. The four isolates represent a new genus, *Modestobacter* gen. nov., with *Modestobacter multiseptatus* sp. nov. as the type species. The type strain, *Modestobacter multiseptatus* AA-826^T, was deposited in the DSMZ as DSM 44406^T

Keywords: *Modestobacter*, budding bacteria, actinomycetes, Antarctic soils

INTRODUCTION

The ice-free McMurdo Dry Valleys of Antarctica cover a region of about 7000 km² and consist of alternating mountain ranges and glacial valleys. This area has all the characteristics of a hostile, dry and cold desert (Nienow & Friedmann, 1993). The relative humidity is low, and small amounts of snow, which rarely fall, melt quickly into soils and rocks or are rapidly blown away. Soil-forming processes are extremely slow, and fine-granular soil material often

collects locally when blown around by wind and must be considered as regolithic because it is almost devoid of organic components. Summer temperatures normally vary between –15 °C and 0 °C, and winter temperatures can drop as far down as –60 °C (Friedmann, 1980). However, some conditions allowing for microbial growth do exist in special locations: McKay & Friedmann (1985) found summer temperatures underneath sandstone surfaces reaching +17.3 °C, and Behling (1971) observed soil surface temperatures exceeding +20 °C.

For many years, this cold 'Ross Desert' was considered to be sterile (Horowitz *et al.*, 1972) because it lacks visible plant or animal life (apart from a few

The GenBank/EMBL accession number for the 16S rDNA sequence of *Modestobacter multiseptatus* AA-826^T is Y18646.

lichens). Micro-organisms found in soils (Boyd *et al.*, 1966; Cameron, 1971) were thought to have come from human activities or to have arrived with coastal air as 'contaminants'. Microbial communities within the sandstone pore space were first reported by Friedmann & Ocampo (1976). This cryptoendolithic ecosystem was studied more thoroughly by an international research group (Friedmann, 1980, 1985; Kappen *et al.*, 1981; Hirsch *et al.*, 1988; Nienow & Friedmann, 1993). However, endemic microbial life in these hostile habitats and possible *in situ* activity has been supported by relatively few reports. Uydess & Vishniac (1976) studied Dry Valley soil bacteria with transmission electron microscopy and suspected that these bacteria had grown locally. They also found possibly new, multiseptate bacterial forms. Kappen & Friedmann (1983) measured active CO₂ gas exchange of cryptoendolithic lichens, and P. Hirsch & C. A. Gallikowski (unpublished results) observed growth of bacterial microcolonies on glass slides that had been exposed for one year to soils of Linnaeus Terrace (Asgard Range, 1600 m).

Research on such soils and on the cryptoendolithic ecosystem in sandstone prompted attempts to isolate heterotrophic bacteria from rocks and surrounding soils. The use of oligotrophic media and mostly low incubation temperatures resulted in a wealth of different bacterial morphotypes many of which could be isolated (Hirsch *et al.*, 1985, 1988; Gallikowski & Hirsch, 1988). Some of these isolates were identified: Siebert & Hirsch (1988) characterized 15 coccid bacteria, Dawid *et al.* (1988) demonstrated the presence, in soil samples, of psychrophilic myxobacteria, and Schumann *et al.* (1997) described *Friedmanniella antarctica*, a new actinomycete that came from a sandstone sample. Recently, *Hymenobacter roseosaliarius* was described as a relatively fast-growing bacterium from Ross Desert soils and sandstone samples (Hirsch *et al.*, 1998). The present work concerns the description of four soil isolates as members of a new actinomycete genus and determines their phylogenetic position.

METHODS

Sampling, enrichment culture conditions and isolations. Soil samples were obtained aseptically by using sterile spoons and sterile Whirl-Pack plastic bags. All samples were stored and transported continuously frozen until studied in the Kiel laboratory. Oligotrophic medium PYGV (Staley, 1968) was used for enrichment cultures and isolations. It contained (l⁻¹): 250 mg each of Bacto peptone (Difco), Bacto yeast extract and glucose, as well as 20 ml Hutner's basal salts medium (Cohen-Bazire *et al.*, 1957) and 10 ml Vitamin solution no. 6 (Van Ert & Staley, 1971). Enrichment culture and isolation conditions are listed in Table 1. Pure cultures were kept on PYGV agar slants or lyophilized or frozen at -71 °C.

Microscopy. For phase-contrast light microscopy and size measurements, preparations were made of young, liquid-grown cells on agar-coated slides (Pfennig & Wagener,

1986). Motility was observed in hanging drop preparations. Cells for transmission electron microscopy were washed with 0.5 M ammonium acetate, dried and C/Pt-shadowed at an angle of 35°, and observed with a Philips EM 300 electron microscope at 80 kV.

Physiological and biochemical characterizations. For physiological tests, the cells were incubated at 20 °C, but strain AA-824 grew better at 9 °C. Growth was determined as turbidity (OD₆₂₀) after homogenizing the aggregated cultures, or as protein increase using the Bio-Rad assay (Labrenz *et al.*, 1998). All experiments were carried out in triplicate. Utilization of sugars or sugar alcohols (1%, w/v) was tested after 2–6 weeks cultivation on mineral salts medium as described by Shirling & Gottlieb (1966). For the study of acid production from sugars or sugar alcohols, the strains were cultivated and evaluated as previously described (Gordon *et al.*, 1974). Tests for hydrolysis of polymers (casein, gelatin, starch, hippurate, urea) followed directions of Gordon *et al.* (1974). DNase, lipase and phosphatase activities were tested as described by Smibert & Krieg (1981). Degradation of xylan, arbutin, aesculin, adenine, hypoxanthine and xanthine was studied according to Horan & Brodsky (1986). The following enzyme activities were tested as described in the literature: catalase (Smibert & Krieg, 1981), cytochrome oxidase (Deibel & Evans, 1960), sialidase (Potier *et al.*, 1979), and type II restriction endonuclease (Whitehead & Brown, 1985). Temperature tolerance for growth was studied with an aluminium temperature gradient block with four parallel borings for test tubes arranged in 1 °C steps between 0 and 35 °C. Evaluation of growth was done by turbidity measurement (OD₆₂₀) of homogenized cells. NaCl tolerance for growth was tested in triplicate cultures with medium PYGV to which up to 20% (w/v) NaCl was added in steps of 0.5–1.0%. Growth was evaluated as turbidity after 2–8 weeks. For pH tolerance experiments, medium PYGV was adjusted to pH 2–13 in steps of 0.5 units by adding NaOH or HCl, respectively. Between pH 6 and 8, the buffer capacity was increased by the addition of 0.05 g (l⁻¹) each of KH₂PO₄ and Na₂HPO₄·2H₂O. Antibiotic resistance was tested on PYGV agar plates which were inoculated with 100 µl young culture. A 1-cm-diameter sterile filter disc was treated with 100 µl antibiotic solution (2 mg ml⁻¹) and placed onto the agar plate centre. The antibiotics chloramphenicol, kanamycin, penicillin G, streptomycin and tetracycline were also added to liquid medium PYGV in concentrations of 10, 50, 100, 500 and 1000 µg ml⁻¹. Growth was evaluated after 10–30 d by protein determination and compared to growth in the absence of antibiotics. Nitrate reduction was tested according to Smibert & Krieg (1981). Formation of H₂S from cystine was checked with lead acetate paper. Formation of ammonia from arginine or from peptone/yeast extract was tested with medium PYGV to which in the former case 3 g arginine l⁻¹ was added (Skerman, 1967).

Chemotaxonomy. Cell wall preparations followed the rapid screening method of Schleifer & Kandler (1972). The diamino acid type and isomer were identified with two-dimensional TLC (Harper & Davis, 1979). Molar ratios of cell wall amino acids were determined after derivatization with the PITC method (Pharmacia-LKB). Separation of derivatives occurred on a reverse-phase column (25 × 4.6 cm; Bischoff) with Spherisorb RP18, 5 µm, as stationary phase. Retention time calculations and integration of peak areas were done with an IBM PS/2 model 30 computer using chromatography software (Nelson; model 2600 Rev. 5.0). For identification of cell wall sugars with the system of

Table 1. Origin, enrichment and isolation conditions for the bacterial strains

The soil samples used for inoculation were collected aseptically from Linnaeus Terrace (1600 m, Asgard Range). Medium PYGV (Staley, 1968) was used throughout.

	AA-802	AA-824	AA-825	AA-826 ^T
Samples:				
Number	845/246	845/247	845/247	845/247
Depth (cm)	0–5	5–7	5–7	5–7
pH (0.1 M KCl)	5.2	5.6	5.6	5.6
Liquid enrichments:				
Inoculum (g 50 ml ⁻¹)	3	3	—	—
Temperature (°C)	9	9	—	—
pH	8.0	8.0	—	—
Light condition	Dark	Dark	—	—
Agar direct inoculation:				
Inoculum (g plate ⁻¹)	—	—	0.9	0.9
Temperature (°C)	—	—	4	9
pH	—	—	6.5	6.5
Light condition	—	—	Dark	Dim light*
First subculture				
After months	12	12	5	1

* Dim light: 2–4 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$.

Staneck & Roberts (1974), the cellulose-covered aluminium plates were developed three times. The plates were sprayed with an acid aniline-phthalate solution and heated to 105 °C.

Respiratory lipoquinones were extracted and purified as previously described (Collins *et al.*, 1977). Their TLC separation followed the protocol of Collins *et al.* (1980) using reversed-phase thin-layer plates (HPTLC, RP18; Merck). Lipoquinone extracts (1 ml) were quantified with a reversed-phase RP18 column with acetonitrile:tetrahydrofuran (70:30, v/v) as mobile phase and the elution was done at 30 °C at a flow rate of 1 ml min⁻¹. MS (Finnegan Mat) at 200–230 °C and with an ionization voltage of 70 eV was used for identifying the quinones.

Polar lipids were separated and identified as described by Minnikin *et al.* (1984), using 6.6 × 6.6 cm TLC silica gel 60 F₂₅₄ plates (Merck). The solvent systems were chloroform:methanol:water (65:25:4, v/v, dimension 1) and chloroform:acetic acid:methanol:water (40:7.5:6:2, v/v, dimension 2). Presence of free mycolic acids was tested according to Mordarska *et al.* (1972) with *Nocardia asteroides* IFAM 450 as positive and *Amycolata saturnea* IFAM 71 as negative controls. Tests for mycolic acids, which are only released by acid methanolysis, followed methods of Minnikin *et al.* (1975), with *Mycobacterium phlei* IFAM 437 as the positive control. The extraction, purification and methylation of fatty acids from 150–180 mg lyophilized cell material followed methods of Eckhardt *et al.* (1979) and Hirsch *et al.* (1988). Fatty acid methyl esters were separated with a GC (Packard model 419 Becker) and an FFAP-CB-1.0 capillary column (0.53 mm × 25 m; Macherey-Nagel) using a temperature programme of 190–280 °C (2.2 °C min⁻¹), and of 160–210 °C (2 °C min⁻¹), respectively, with nitrogen as the carrier gas (7 ml min⁻¹). The GC was equipped with an FID and Autolab Digital Integrator 6300/02. Peaks were identified by comparison with analytical grade fatty acid methyl esters (Sigma).

Chemotaxonomic data for strain AA-825 were obtained following methods of Groth *et al.* (1996).

Determination of DNA base composition. Approximately 1 g wet cells was suspended in 0.1 M Tris/HCl pH 8.0, treated with 1 mg lysozyme ml⁻¹ (crystalline, 15000 U mg⁻¹) and incubated for 30 min at 60 °C. This was followed by the addition of proteinase K and SDS (both 0.5%, w/v) until the viscosity reached a maximum. The SDS concentration was then raised to 2% (w/v) and the resulting suspension was incubated for 20 min at 60 °C before 5.5 ml 5 M NaClO₄ was added (Holst, 1985). The DNA preparation was purified according to Marmur (1961) and the purity determined in a spectrophotometer 250 (Gilford) as described by Chandra & Appel (1973). The base composition of nine replicates was determined as *T_m* using a Gilford spectrophotometer with a thermoprogrammed cuvette (Mandel & Marmur, 1968).

16S rDNA sequence determination and analysis. The following strains were included in 16S rDNA analyses: almost complete sequences were obtained for strains AA-826^T and AA-825 while a stretch of 500 nucleotides of the 5' terminus was determined for strains AA-802, -824 and -826^T. Extraction of genomic DNA, PCR amplification of the 16S rDNA, and sequence analysis of the purified PCR products were as described previously (Rainey *et al.*, 1996) and the sequence reactions were electrophoresed using a model 373A automatic DNA sequencer (Applied Biosystems). Sequences were aligned manually first with sequences of the class *Actinobacteria* and subsequently with those of the suborder *Frankineae* (Stackebrandt *et al.*, 1997) published previously (Normand *et al.*, 1996; Eppard *et al.*, 1996; Rainey & Stackebrandt, 1993; Rainey *et al.*, 1993; Yoshimi *et al.*, 1996). Similarity values were transformed into phylogenetic distance values that compensate for multiple substitutions at any given site in the sequence (Jukes & Cantor, 1969). The algorithm of DeSoete (1983) (Maidak *et al.*, 1997) and the neighbour-joining method contained in the PHYLIP package

(Felsenstein, 1993) were used in the construction of phylogenetic dendrograms. All analyses were done on a SUN SparcII workstation.

Accession numbers. The accession numbers of the 16S rDNAs of reference strains included in Fig. 3 were: *Geodermatophilus obscurus* subsp. *obscurus* DSM 43160^T, X92356; *G. obscurus* subsp. *dictyosporus* DSM 43161^T, X92355; *G. obscurus* subsp. *utahensis* DSM 43162^T, X92357; *Sporichthya polymorpha* DSM 46113^T, X72377; *Acidothermus cellulolyticus* ATCC 43068^T, X70494; *Microsphaera multipartita* JCM 9543, D50066; *Frankia* strain Ar14, L11307; strain A7S, X92360; strain G6, X92366; strain 4S, X92361; strain B-SWPS, X92363; strain 33S, X92362. The EMBL accession numbers of 16S rDNA of *Blastococcus aggregatus* DSM 4725^T and strain AA-826^T are Y18647 and Y18646, respectively.

RESULTS

Enrichments, isolation of bacteria and their morphology

Oligotrophic liquid medium PYGV (50 ml) was adjusted to pH 8.0, inoculated with 3 g soil and incubated in the dark at 9 °C for 12 months (Table 1). Also, PYGV agar plates (pH 6.5) were inoculated directly with soil particles (0.9 g) and incubated for 1–5 months at 4 or 9 °C. A large variety of bacterial morphotypes multiplied (Hirsch *et al.*, 1988). Among these were irregularly shaped, short rods or cocci with cross walls and often longitudinal septa. These cells produced slender buds (Fig. 1a, b) which eventually were motile after separation, although flagella were not found with the electron microscope. Corresponding morphotypes could be isolated from four additional soil samples and from a greenish powder inside a cracked sandstone sphere. Four isolates (AA-802, -824, -825 and -826^T) were similar, although their enrichments and/or isolations varied (Table 1). All

Fig. 1. Phase-contrast light micrographs. (a) Strain AA-802 grown 4 d on PYGV (pH 8.0) at 16 °C. (b) Strain AA-825 grown 6 d on PYGV agar at 16 °C. Bars, 5 µm.

Fig. 2. Transmission electron micrographs, C/Pt shadowed. The isolates were grown on medium PYGV at 16 °C; bars, 2 µm. (a) Strain AA-824 after 39 d. (b) Strain AA-826^T after 13 d.

were Gram-positive, short rods or cocci with a tendency to aggregate. Their cell size ranges were 1.0–2.8 × 1.0–3.0 µm with mean sizes of 1.7 × 1.6–1.9 µm. New cell formation was by cross and longitudinal wall growth as well as by budding with the production of motile swarmer cells, but in isolate AA-825, motility could not be detected. Electron microscopy showed that new polar buds were often produced on still attached, small buds (Fig. 2a). Flagella were not found. Colonies were irregularly shaped, increasingly shiny, initially beige and later pinkish.

Culture and growth characteristics

All isolates grew well on medium PYGV, but growth was better on PYGV-8, containing peptone, yeast extract and glucose at the eightfold concentration of 2 g l⁻¹. Good growth was also observed on DSMZ medium 65. Three of the strains grew at 0 °C, they were facultatively psychrophilic and grew between 0 and 25–28 °C. AA-824 was a true psychrophile with a growth optimum at 11–13 °C and a maximum at 20 °C. The temperature optima of the other three isolates were in the range of 19–22 °C (Table 2). There were some differences in the pH optimum for growth (Table 2), but all strains survived between pH 3–4 and 11–12. The maximum tolerance of NaCl varied between 2 and 8% (w/v), and all strains grew well without NaCl.

Physiological and biochemical characteristics

The four strains showed identical reactions in most physiological and biochemical tests. They exhibited catalase, cytochrome oxidase and phosphatase activities, but were negative for lipase (with triolein) and sialidase. Restriction endonuclease activity was found in AA-824 and AA-826^T, but not in AA-802 or -825. All isolates utilized D-glucose, D-galactose, lactose, sucrose or mannitol for growth, but did not grow with lyxose, cellobiose or inositol. The following carbon sources were used by some of the strains: L-arabinose,

Table 2. Differences between the four Antarctic isolates

Character	AA-802	AA-824	AA-825	AA-826 ^T
Colony colour	Pale pink	Pale pink	Beige to grey	Pale pink
C-utilization (1 %, w/v):				
Maltose	+	+	—	w
D-Mannose	+	+	—	w
Melibiose	+	+	ND	—
Fucose	—	—	ND	+
D-Ribose	+	w	ND	—
Sorbitol	+	w	ND	—
L-Rhamnose	—	w	ND	w
N-Acetylglucosamine	—	w	—	w
Aerobic decarboxylations (2 %, w/v):				
Benzoate	—	—	—	+
Citrate	—	w	ND	+
Glycolate	—	w	—	—
Acid from glucose	+	—	ND	—
Hydrolysis:				
Aesculin	—	—	—	+
Gelatin	—	—	+	—
Temperature for growth (°C):				
Maximum	27	20	25	28
Optimum	20–22	11–13	20	19–21
pH optimum (growth)	7.5	7.5	7.5	8.0–8.5
pH tolerance (survival)	4–12	4–12	ND	3–12
NaCl maximum (%, w/v)	8	4	2	8
DNA (mol % G + C)	69.4*	68.4*	69.9†	69.9*

ND, Not determined; w, weak positive reaction.

* Determination as T_m .

† Determination with HPLC (Groth *et al.*, 1996).

erythritol and salicin. Except for isolate AA-824, all strains hydrolysed starch, but none of the strains hydrolysed adenine, hypoxanthine, xanthine, hippurate, cellulose, chitin, dextrin, xylan, arbutin or casein. Nitrogen sources used by all strains were peptone and yeast extract, AA-825 grew also with nitrate and ammonium. The isolates showed aerobic and anaerobic nitrate reduction. Ammonia formation from arginine, peptone or yeast extract was negative. The strains did not form H_2S from cystine or sulfate. They were sensitive to $10 \mu\text{g ml}^{-1}$ of ampicillin, carbenicillin, tobramycin or trimethoprim, and all strains showed sensitivity to $50 \mu\text{g lysozyme ml}^{-1}$. Sensitivity to cystathionine, leucomycin, neomycin or tyrocidine was observed in some of the strains. The following organic acids were decarboxylated aerobically: fumarate, malate, succinate, pyruvate, glutarate and lactate. Differences among the strains (Table 2) concerned reactions to some carbon sources and tolerances of specific growth conditions.

Chemotaxonomy

The pattern of fatty acids is shown in Table 3. The major fatty acids in strains AA-802, -824, -825 and -826^T were n18:1, i16:0 and ai17:0 but some quan-

titative differences were found in the case of cy19:0. The strains had MK-9(H_4) and MK-8(H_4) as major respiratory quinones (Table 4) and MK-9(H_6) was found in small amounts, but not in AA-825. Mycolic acids were not present in any of the four strains. Polar lipids observed were diphosphatidylglycerol, phosphatidylethanolamine and phosphatidylinositol. Phosphatidylglycerol was also present but absent from AA-824 and -825 (Table 4). The cell walls contained *meso*-diaminopimelic acid, glutamate and alanine. Strains AA-824 and -826^T also had significant molar percentages of serine (Table 5). Cell wall hydrolysates of all strains contained galactose, glucose and ribose. DNA G+C contents of the isolates ranged from 68 (AA-824) to 70 (AA-826^T) mol %.

Phylogenetic analysis

Blastococcus aggregatus DSM 4725^T and the Antarctic strains were subjected to 16S rDNA analysis. *B. aggregatus* was found to be closely related to strain G1S from a Canadian landfill leachate (Eppard *et al.*, 1996), while the nearest neighbours of *B. aggregatus* DSM 4725^T were the three subspecies of *Geoderma-tophilus obscurus* (94.8–95.4 % similarity). Similarity

Table 3. Fatty acids (%) of the Antarctic isolates

Data < 1% were omitted.

Fatty acid	AA-802	AA-824	AA-825*	AA-826 ^T
i14:0	1.2	1.1		1.9
n14:1	1.5	3.5	4.2	2.6
n14:0	1.7	2.4	2.5	3.4
i15:0	3.3	7.0	4.8	3.3
ai15:0	5.3	6.5	2.7	7.8
n15:0	1.4	1.8	1.5	2.1
i15:1			6.3	
n15:1			1.2	
i16:0	18.8	10.9	9.8	13.8
n16:1	5.3	5.6	10.0	6.2
n16:0	5.7	5.7	6.4	7.9
i16:1			5.0	
i17:0				
ai17:0	12.7	13.6	12.2	9.2
n17:1			1.9	
n18:1	17.1	23.8	19.8	21.4
n18:0	1.7	2.7		2.2
n19:0		1.1	3.5	
cy19:0	10.1	4.9	3.4	8.5
Unidentified	11.6	6.4		7.0
Total	97.4	97.0	95.2	97.3

* Data obtained by capillary GC according to Groth *et al.* (1996).

Table 4. Respiratory quinones (%) and phospholipids of the Antarctic isolates

	AA-802	AA-824	AA-825	AA-826 ^T
Respiratory quinone:				
MK-9 (H ₄)	87	88	69	87
MK-8 (H ₄)	9	10	9	12
MK-9 (H ₆)	4	2	—	2
Phospholipids:*				
DPG	+	+	+	+
PG	+	—	—	+
PE	+	+	+	+
PI	+	+	+	+
PL 1	—	—	+	—
PL 2	—	+	—	—

* DPG, Diphosphatidylglycerol; PG, phosphatidylglycerol; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PL 1, unidentified phospholipid 1; PL 2, unidentified phospholipid 2.

values obtained for 500 nucleotides from the 5' terminus revealed a high interstrain relationship (>99.5% similarity), and consequently almost complete sequences were generated from only two strains, AA-826^T and -825. Their sequences were 99.9% similar. With similarity values ranging between 96.9

Table 5. Cell wall amino acids of three Antarctic isolates observed with TLC and their molar ratios to glutamic acid as determined with HPLC

Ala, Alanine; Glu, glutamic acid; mDAP, *meso*-diaminopimelic acid; Gly, glycine; Ser, serine; Arg, arginine; Leu, leucine.

Isolate	Ala	Glu	mDAP	Gly	Ser	Arg	Leu
AA-802	1.59	1.00	0.78	0.52	0.01	0.13	0.40
AA-824	1.98	1.00	0.73	0.42	0.26	0.17	0.19
AA-826 ^T	1.75	1.00	0.85	0.66	0.56	0.33	0.15

Fig. 3. Phylogenetic tree showing the position of isolate AA-826^T within the suborder Frankineae, based upon 16S rDNA analysis (DeSoete, 1983; Jukes & Cantor, 1969). The sequence of members of Micrococccineae served as an outgroup sequence. Numbers within the dendrogram indicate the percentages of the branching order in 500 bootstrapped trees. Bar, 2 nucleotide substitutions per 100 nucleotides.

and 97.5% the nearest phylogenetic neighbours of the AA strains were undescribed actinomycete strains isolated from dry soil and monument surfaces (Eppard *et al.*, 1996). These latter strains, for which a formal description has not yet been given, were recently identified as belonging to a novel phylogenetic cluster within the family Geodermatophilaceae in which they formed a 'cluster II' (Eppard *et al.*, 1996). The phylogenetic relatedness of strain AA-826^T and cluster II is supported by high bootstrap values (Fig. 3). Members of this cluster showed an equidistant relationship to members of the genus *Geodermatophilus*, and to the *Blastococcus*-related strain G1S lineage (94–96% similarity).

DISCUSSION

The Antarctic soil isolates were phenotypically very similar and had nearly identical DNA base ratios. Some differences (Table 2) in the carbon utilization spectrum, in the temperature and pH optima, and maximum NaCl tolerance could be due to their

Table 6. Differential characteristics of *Modestobacter multiseptatus* gen. nov., sp. nov. strain AA-826^T and related Gram-positive bacteria

Strains: 1, *Geodermatophilus obscurus* subsp. *obscurus* DSM 43160^T; 2, strain G1S; 3, *Blastococcus aggregatus* DSM 4725^T; 4, Antarctic isolate AA-826^T; 5, strains 4S, B-SWPS, 33S; 6, *Microsphaera multipartita* JCM 9543^T.

Characteristic	1*	2*	3†	4	5*	6‡
Cell shape	Cocci to cuboids	Cocci, rods, filaments	Cocci, rods, vibrios	Cocci, rods	Cocci, rods, filaments	Cocci, never rods
Cell diameter (µm)	0.5–2.0	ca. 0.5–1.0§	0.3–1.5 (2.5)	1.0–2.8 (1.7)	ca. 0.4–1.2§	0.8–3.0
Bud formation	±	+	+	+	+	–
Motility	±¶	–	+	+	–	–
Flagellar insertion	Polar¶		Subpolar			
Colony colour	Brown/black	Orange to pink	Pink	Pale pink	Orange to pink	Cream-coloured
O ₂ requirement	Aerobic	Aerobic	Aerobic/microaerophilic	Aerobic	Aerobic	Aerobic
NaCl tolerance						
Growth at 3%	–	+	+	+	+	+
Growth at 5%	–	+	+	+	–	–
Growth at 6%	–	+	–	+	–	+
Temp. optimum (°C)	24–28	28	25	19–21	28	25
Range for growth (°C)	18–37	18–37	3–40	0–28	18–37	10–35
pH optimum			7.0	8.0–8.5		7.0
Range for growth				4.0–9.5		5.0–9.0
Hydrolysis of starch	+	+	–	+	+	+
Gelatin liquefaction	±	–	–	–	+	–
Casein utilization	–	–	–	–	+	–
Carbon sources:						
Ethanol	–	–	w	–	–	+
D-Glucose	+	–	–	+	–	+
Acetate	–	–	–	+	–	–
Citrate	–	–	–	+	–	–
Nitrate reduction	w	–	w	–	–	–
Major menaquinone	MK-9(H ₄)¶		MK-9(H ₄)¶	MK-9(H ₄)¶		MK-8(H ₄)
Major fatty acids (%)				n18:1 (21.4); i16:0 (13.8) 69.9		i16:0 (19.7); i15:0 (15.7)
DNA (mol % G + C)	72.9–74.6¶					67.5

* Data from Eppard *et al.* (1996).

† Data from Ahrens & Moll (1970).

‡ Data from Yoshimi *et al.* (1996).

§ Measurements determined from micrographs in Eppard *et al.* (1996).

|| Data from *Bergey's Manual of Determinative Bacteriology*, 8th edn (Buchanan & Gibbons, 1974).

¶ Data from Normand *et al.* (1996).

w, Weak but positive reaction.

different origin and enrichment conditions (Table 1). Fatty acid analysis (Table 3) indicated that AA-802, -824, -825 and -826^T were similar with respect to their major fatty acids, n18:1, i16:0 and ai17:0. The phospholipid analysis (Table 4) showed some similarities between strains AA-824 and -825.

Strain AA-826^T best represents this phylogenetically and phenotypically highly related group of Antarctic soil isolates. Phylogenetic analysis clearly indicates that this strain is a member of the family *Geodermatophilaceae*. Its position within the radiation of type species of four genera and a representative *Frankia* strain demonstrates the Antarctic strain group to be closely related to the genus *Geodermatophilus* and *Blastococcus aggregatus* (Fig. 3). Its equidistant position to these two is indicative of a novel genus, and the proposal for a new genus is supported by morphological, biochemical and chemotaxonomic properties which distinguish AA-826^T from the established genera of the family (Table 6).

The Antarctic strains are closely related to isolates from Greek marble, limestone from the Negev Desert and rock varnish from the Mojave Desert that were previously analysed (Eppard *et al.*, 1996). Some of the inter-strain 16S rDNA similarity values (97.2–97.9%) are as low as those found for these isolates and strains AA-826^T and -825 (96.9–97.5%). Therefore, strains AA-802, -824, -825 and -826^T are considered to represent a new genus, and the marble strain G6 as well as desert strains 4S, 33S and B-SWPS may be additional species of the same genus. However, these latter strains until now have only been characterized by morphology, pigmentation, some growth characteristics and few biochemical properties. Chemotaxonomy and extensive metabolic traits that would permit their description as novel species have not yet been reported.

The new genus is named *Modestobacter*, and AA-826^T is the type strain for the type species, named *Modestobacter multiseptatus*.

Description of *Modestobacter* gen. nov.

Modestobacter (Mo.des.to.bac'ter. L. adj. *modestus* modest, humble; *bacter* the equivalent of Gr. neut. n. *baktron* a rod or staff; M. L. masc. n.. *Modestobacter* a rod with modest growth requirements).

Gram-positive short rods or cocci with a tendency to remain aggregated. Cells $1.0\text{--}2.8 \times 1.0\text{--}3.0 \mu\text{m}$, showing cross and longitudinal wall growth and multiplying by budding and swarmer formation. Colonies are irregularly shaped, shiny, beige to pink. Does not form spores. Aerobic heterotroph, growing on oligotrophic medium PYGV or on DSMZ medium 65. Growth occurs between $0\text{--}5^\circ\text{C}$ and $20\text{--}28^\circ\text{C}$; a psychrophilic strain grows optimally at $11\text{--}13^\circ\text{C}$. pH tolerance for survival lies between pH 3–4 and 11–12. Positive for catalase, cytochrome oxidase and phosphatase. Some strains show restriction endonuclease activity. Carbon sources utilized for growth are: D-glucose, D-galactose, lactose, sucrose and mannitol. Compounds not hydrolysed are: adenine, hypoxanthine, xanthine, hippurate, cellulose, chitin, dextrin, xylan, arbutin or casein. All strains utilize peptone or yeast extract as nitrogen source and reduce nitrate aerobically or anaerobically. Ammonia is not formed from arginine, peptone or yeast extract. H_2S is not formed from cystine or sulfate. Sensitive to lysozyme. Major fatty acids are n18:1, i16:0 and ai17:0. Major respiratory quinones are MK-9(H_4) and MK-8(H_4); polar lipids of all isolates are diphosphatidylglycerol, phosphatidylethanolamine and phosphatidylinositol. The cell wall contains *meso*-diaminopimelic acid, glutamate, alanine and glycine as well as galactose, glucose and ribose. The type species for the genus is *Modestobacter multiseptatus*.

Description of *Modestobacter multiseptatus* sp. nov.

Modestobacter multiseptatus (mul.ti.sep.ta'tus. L. masc. n. *multus* much; L. adj. *septatus* fenced; M. L. masc. adj. *multiseptatus* much fenced, with many septa).

Cell sizes vary ($1.0\text{--}2.8 \times 1.0\text{--}3.0 \mu\text{m}$) with a mean size of $1.7 \times 1.6\text{--}1.9 \mu\text{m}$. Slender buds may become motile. Colonies irregular, beige to skin-coloured and pinkish. Does not utilize fructose, xylose or trehalose. Utilization of maltose, mannose, melibiose, fucose, ribose, rhamnose, sorbitol or *N*-acetylglucosamine are variable. Growth occurs on succinate or malate. Aerobic decarboxylation of benzoate, citrate, acetate or glycolate is variable. Reactions for amylase and type II restriction endonuclease are positive. Growth occurs at $0\text{--}5^\circ\text{C}$ with a maximum at $20\text{--}28^\circ\text{C}$. pH optimum for growth is $7.5\text{--}8.5$. Major fatty acids are n18:1, ai17:0 and i16:0. The main respiratory quinone is MK-9(H_4), but MK-8(H_4) and MK-9(H_6) may occur in small amounts. Presence of phosphatidylglycerol is variable. The DNA G+C content is 68–70 mol%. Other characteristics are as for the genus. Origin: Antarctic surface soil from Linnaeus Terrace (1600 m) of Asgard Range/Transantarctic Mountains. The type

strain, AA-826^T, was deposited in the DSMZ under accession number DSM 44406^T. Additional isolates of *Modestobacter multiseptatus* are strains AA-825 (= DSM 44405), -824 (= DSM 44404) and -802 (= DSM 44402).

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