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Agrococcus jenensis gen. nov., sp. nov., a New Genus of Actinomycetes with Diaminobutyric Acid in the Cell Wall

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Two strains of a new gram-positive coryneform bacterium isolated from soil and from a sandstone surface are described. Strain 2002-39/1^T (T = type strain) is a coccoid, nonmotile, non-acid-fast, microaerophilic organism. The menaquinones of this strain are MK-12 and MK-11, and the main components of the whole-cell sugars are glucose and rhamnose. No mycolic acids are present. The G+C content of the DNA is 74 mol%. Comparative 16S ribosomal DNA studies and a cell wall analysis revealed that this strain represents a new genus belonging to the group of actinomycetes that have diaminobutyric acid in their peptidoglycans. The second strain, strain ST54, which was isolated from a sandstone surface, had the same characteristic features as strain 2002-39/1^T. The name *Agrococcus jenensis* gen. nov., sp. nov., is proposed for these organisms. The type strain is strain 2002-39/1, which has been deposited in the German Collection of Microorganisms and Cell Cultures as strain DSM 9580.

Actinomycetes are considered an inexhaustible source of chemically diverse secondary metabolites. In addition to the genera *Streptomyces*, *Micromonospora*, and *Actinoplanes*, coryneform and nocardioform bacteria have been used in screening programs for new compounds of medical and biotechnological importance (5, 8).

In the course of a program aimed at isolating actinomycetes that have potential for producing novel bioactive compounds, we isolated a large number of strains from different soils. In about 65 of these strains diaminobutyric acid (DAB) was the diagnostic diamino acid in the peptidoglycan. These strains had morphological and chemotaxonomic characteristics that placed them near the genera *Agromyces* and *Clavibacter*.

In this paper we describe the isolation and characterization of two strains which differed markedly from the members of the genera *Agromyces* (27), *Clavibacter*, and *Rathayibacter* (26) that have been described. On the basis of our morphological, physiological, and biochemical data, as well as the results of our 16S ribosomal DNA (rDNA) analysis, we concluded that these strains belong to a new genus and species, for which we propose the name *Agrococcus jenensis*. These strains have been deposited in the German Collection of Microorganisms and Cell Cultures as strains DSM 9580^T (T = type strain) and DSM 9996.

MATERIALS AND METHODS

Bacterial strains and cultural conditions. Strain 2002-39/1^T was isolated from a sample of frozen compost soil obtained near Jena, Germany, at a depth of about 10 cm. Isolation of this organism involved dilution plating on nutrient agar containing 2% peptone, a pancreatic digest (meat, fish), 0.5% NaCl, and 1.2% agar. Strain ST54 was isolated from the sandstone surface of the Alte Pinakothek building in Munich, Germany. The *Agromyces* and *Clavibacter* type strains which we used in this study are listed in Table 1. General laboratory cultivation was performed on solid medium or in liquid rich (R) medium (25) containing 1% Bacto Peptone (Difco Laboratories), 0.5% yeast extract, 0.5% Casamino Acids, 0.2% meat extract, 0.5% malt extract, 0.2% glycerol, 0.1% $MgSO_4 \cdot 7H_2O$, and 0.005% Tween 80 (pH 7.2) at 28°C.

Morphological and physiological characteristics. Cell morphology was determined by examining cultures of different ages by phase-contrast microscopy. Colony morphology was studied by using a stereomicroscope. For scanning electron microscopy an 18-h-old culture of strain 2002-39/1^T on an agar plate was suspended in a phosphate-buffered salt solution. The cells were fixed with 0.5% glutaraldehyde, washed, and dehydrated in a series containing increasing concentrations of ethanol. After sputter coating with gold-palladium, the cells were observed with a Zeiss model 962 scanning electron microscope. Acid production from carbohydrates was examined by using the method of Hugh and Leifson (9), as modified by Gledhill and Casida (6). Utilization of organic acids was studied

TABLE 1. Strains used

Taxon	Strain[a]	Other designation(s)[a]
Agrococcus jenensis	2002-39/1^T	DSM 9580^T
Agrococcus jenensis	ST54	DSM 9996
Agromyces cerinus subsp. *cerinus*	IMET 11525^T	DSM 8595^T, VKM Ac-1340^T
Agromyces fucosus subsp. *fucosus*	IMET 11529^T	DSM 8597^T, VKM Ac-1345^T
Agromyces ramosus	IMET 11027^T	ATCC 25173^T, DSM 43045^T, NCIB 10801^T
Clavibacter michiganense subsp. *michiganense*	IMET 11518^T	DSM 46364^T, LMG 7333^T, NCPPB 2979^T

[a] ATCC, American Type Culture Collection, Rockville, Md.; DSM, Deutsche Sammlung von Mikroorganismen and Zellkulturen GmbH, Braunschweig, Germany; IMET, IMET-National Collection of Microorganisms (now Deutsche Sammlung von Mikroorganismen and Zellkulturen GmbH, Jena Branch), Jena, Germany; LMG, Collection Laboratorium voor Microbiologie, Ghent, Belgium; NCPPB, National Collection of Plant Pathogenic Bacteria, Harpenden, United Kingdom; VKM, All-Russian Collection of Microorganisms, Institute of Biochemistry and Physiology of Microorganisms, Pushchino, Russia.

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TABLE 2. Comparison of physiological properties of *Agrococcus*, *Agromyces*, *Clavibacter*, and *Rathayibacter* strains

Characteristic	*Agrococcus jenensis* 2002-39/1^T	*Agrococcus jenensis* ST54	*Agromyces ramosus* IMET 11027^T	*Agromyces cerinus* subsp. *cerinus* IMET 11525^T	*Agromyces fucosus* subsp. *fucosus* IMET 11529^T	*Clavibacter michiganense* subsp. *michiganense* IMET 11518^T	*Rathayibacter rathayi* VKM Ac-1601^T[a]
Decomposition or hydrolysis of:							
Adenine	−[b]	−	−	−	−	−	
Casein	+	−	−	−	−	+	−
Esculin	+	+	+	+	+	+	
Gelatin	−	−	−	−	−	−	
Hippurate	−	−	ND	−[c]	−[c]	−	−
Hypoxanthine	−	−	−	w	w	−	−
Potato starch	+	+	+	+	+	+	−
Tween 80	−	−	−	−	−	−	−
Tyrosine	−	−	−	+	+	−	
Urea	−	−	−	−	−	+	−
Xanthine	−	−	−	−	−	−	−
Acid produced from:							
L-Arabinose	w	w	+	−	+	+	−
Cellobiose	−	−	−	+	+	+	+
Dextrin	−	−	−	+	+	+	−
Fructose	w	w	+	+	−	+	v
Galactose	−	−	−	+	+	+	−
Glucose	−	−	−	+	+	+	−
Glycerol	w	w	+	+	+	+	+
Inulin	w	w	+	−	−	−	
Lactose	−	−	−	+	−	+	−
Maltose	−	−	−	+	+	+	−
Mannitol	w	w	w	−	−	−	+
Mannose	−	−	−	+	+	+	−
Raffinose	−	−	+	−	−	−	+
L-Rhamnose	w	w	+	+	+	−	−
Ribose	−	−	−	−	−	−	
Salicin	−	−	−	+	+	+	+
D-Glucitol	w	w	−	−	−	−	
Sucrose	−	−	+	+	w	+	−
Potato starch	−	−	−	+	+	+	
Trehalose	−	−	−	−	−	+	+
D-Xylose	−	−	−	−	−	+	+
Utilization of:							
Acetate	−	−	+	+	+	+	+
Aconitate	−	−	−	+	−	−	
Benzoate	−	−	−	−	−	−	
Citrate	−	−	−	+	−	−	+
Formate	−	−	−	−	−	−	+
Malate	+	+	+	+	+	−	
Succinate	+	+	+	−	−	−	+
DL-Tartrate	−	−	−	−	−	−	+
Growth in the presence of:							
2% NaCl	+	+	+	+	+	+	
4% NaCl	+	w	w	+	w	w	
10% NaCl	−	−	−	−	−	−	
Growth at:							
28°C	+	+	+	+	+	+	+
37°C	v	−	+	w	w	+	−
Nitrate reduction	−	−	v	−	+	+	−
Production of H_2S	+	+	+	+	+	+	
Catalase reaction	+	+	−	+	+	+	
Voges-Proskauer test	−	−	−	−	−	−	+
Methyl red test	−	−	−	−	−	−	+
Oxidase test	−	−	−	−[c]	+	w	
Indole test	−	−	−	−	−	−	
Antibiotic susceptibility							
Ampicillin (10 μg)	+	+	+	+	−	+	
Chloramphenicol (30 μg)	+	+	+	+	+	+	
Ciprofloxacin (5 μg)	+	+	+	w	+	+	
Erythromycin (15 μg)	+	+	+	+	+	+	
Gentamicin (10 μg)	+	+	−	+	+	+	
Kanamycin (30 μg)	+	+	+	+	+	+	
Lincomycin (2 μg)	+	+	−	−	−	+	
Neomycin (30 μg)	+	+	+	+	+	+	
Nitrofuran (300 μg)	+	+	w	w	w	+	
Oxacillin (5 μg)	w	+	+	−	−	w	
Oxytetracycline (30 μg)	+	+	+	+	+	+	
Penicillin G (2 IU)	+	+	+	−	−	+	
Polymyxin B (300 IU)	+	+	+	w	+	+	
Rifampin (2 μg)	+	+	+	+	+	+	
Streptomycin (10 μg)	+	+	+	+	+	+	
Sulfonamid (300 μg)	−	−	−	−	−	+	

[a] Data from reference 26.
[b] −, negative; +, positive; w, weakly positive; v, variable; ND, not determined.
[c] Data from reference 27.

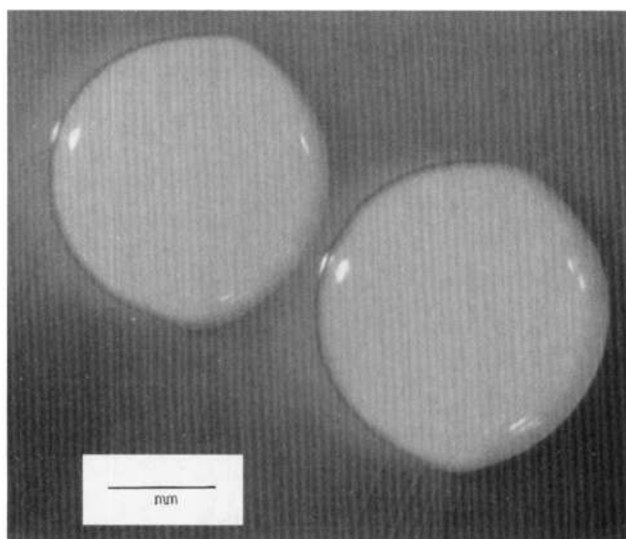

FIG. 1. Single colonies of strain 2002-39/1^T grown on solid R medium at 28°C for 5 days. Bar = 1 mm.

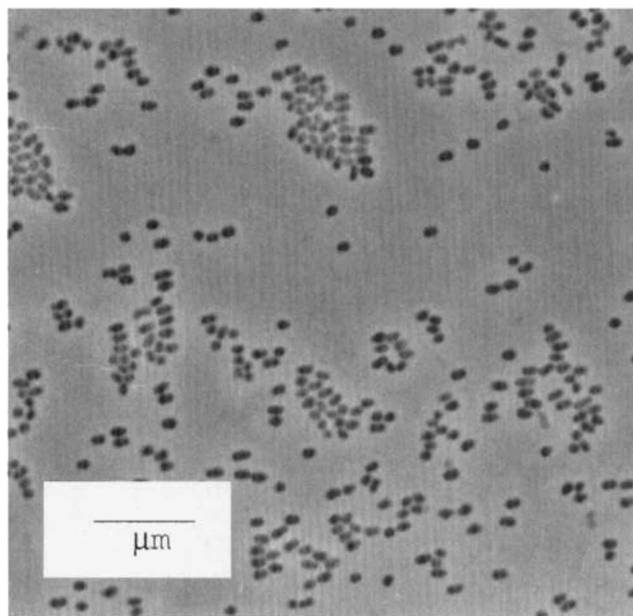

FIG. 2. Micrograph of strain 2002-39/1^T cells grown in liquid R medium at 28°C for 24 h. Bar = 10 µm.

in the medium described by Cowan and Steel (3) by adding sodium salts of organic acids to a final concentration of 0.2%. Nitrate reduction, urease activity, indole production, hydrogen sulfide production, hydrolysis of Tween 80 and gelatin, and the methyl red and Voges-Proskauer reactions were studied as described by Lanyi (11). Catalase production and hydrolysis of casein and starch were studied by the methods of Gledhill and Casida (6). Decomposition of adenine, hypoxanthine, xanthine, and tyrosine was studied by using the method recommended by Gordon et al. (7). Oxidase activity was studied by monitoring the oxidation of a 1% tetramethyl-*p*-phenylenediamine solution on filter paper discs (3). Hydrolysis of hippurate was studied on hippurate agar (3). Growth was tested at 28 and 37°C, and tolerance to NaCl was investigated at concentrations between 2 and 10% on R medium. Susceptibility to antibiotics was studied by placing antibiotic discs (Oxoid) on R medium plates seeded with suspensions of the test strains. Oxygen requirements were studied with the Generbag microaer incubation system (bioMerieux).

Cell wall analysis. Purified cell wall preparations were obtained by the method of Schleifer and Kandler (20). The amino acids and peptides in cell wall hydrolysates were analyzed by two-dimensional ascending thin-layer chromatography on cellulose plates by using the solvent systems described by Schleifer and Kandler (20). Whole-cell sugars were determined as alditol acetates by gas chromatography and gas chromatography-mass spectrometry as described by Saddler et al. (19) by using a type FFAP fused silica capillary column (25 m by 0.25 mm [inside diameter]) and a program in which the temperature increased from 220 to 230°C at a rate of 1°C/min. The molar ratios of amino acids were determined by gas chromatography and gas chromatography-mass spectrometry of *N*-heptafluorobutyryl amino acid isobutyl esters as described by MacKenzie (12). Gas chromatography analyses were performed by using a model GC-14A Chromatopac gas chromatograph (Shimadzu Corp., Tokyo, Japan) equipped with a flame ionization detector and an integrator. For the gas chromatography-mass spectrometry analysis we used a model GCMS-QP2000 mass spectrometer (Shimadzu Corp.). The glycolate content of bacterial cells was determined by the colorimetric method of Uchida and Aida (24) by using a model 901 UV-visible spectrophotometer (Büchi Laboratoriums-Technik AG, Flawil, Switzerland).

Lipid analysis. Cellular fatty acid methyl esters obtained by the method described by Stead et al. (21) were separated by gas chromatography on polar (type FFAP) and nonpolar (type OV-1) capillary columns (25 m by 0.25 mm [inside diameter]) installed in the model GC-14A gas chromatograph by using He as the carrier gas at a linear velocity of 200 mm/s and a temperature of 190°C. Menaquinones were extracted as described by Collins et al. (2) and were analyzed by high-performance liquid chromatography (HPLC). The HPLC system which we used consisted of a model LC-9A solvent delivery module, a model DGU-3A online degasser, a model CTO-10AC column oven, a model SIL-9A automatic sample injector, and a model SPD-6A UV spectrophotometric detector connected to a model C-R4AX Chromatopac integrator (Shimadzu Corp.). Menaquinones were eluted from a type RP 18 column (25 cm by 4.6 mm [inside diameter]) by using a solution containing acetonitrile and 2-propanol (65:35, vol/vol) at a flow rate of 1.3 ml/min and a temperature of 20°C. The detection wavelength was 269 nm. Polar lipids extracted by the method of Minnikin et al. (16) were identified by two-dimensional thin-layer chromatography and spraying with specific reagents as described by Collins and Jones (1). We proved that no mycolic acids were present by performing thin-layer chromatography as described by Minnikin et al. (15).

DNA base composition. DNA was isolated by using a modification of the Marmur method (13). After purification by treatment with proteinase K, the DNA was degraded to nucleosides by using P1 nuclease and bovine intestinal mucosa alkaline phosphatase as described by Mesbah et al. (14). The nucleosides were separated by reversed-phase HPLC by using the HPLC system described above and the methods described by Tamaoka and Komagata (23). The G+C content of the DNA was calculated from the ratio of deoxyguanosine to thymidine.

16S rDNA sequence determination. Genomic DNA was extracted, and PCR-mediated amplification of the 16S rRNA genes was performed as described previously (17). Purified PCR products were sequenced directly by using a *Taq* DyeDeoxy terminator cycle sequencing kit (Applied Biosystems, Foster City, Calif.) as recommended by the manufacturer. The purified sequence reaction mixtures were electrophoresed by using an Applied Biosystems model 373A DNA sequencer. The sequence which we determined was manually aligned with previously published sequences available from public databases. Evolutionary distances calculated by the method of Jukes and Cantor (10) were used to construct a phylogenetic tree by the least-squares method of De Soete (4).

Nucleotide sequence accession number. The 16S rDNA sequence of strain 2002-39/1^T has been deposited in the EMBL database under accession number X92492.

RESULTS

Morphological and cultural characteristics. During isolation and during laboratory cultivation strain 2002-39/1^T grew

TABLE 3. Chemotaxonomic characteristics of the two *Agrococcus* strains and other coryneform taxa that have DAB in their peptidoglycans

Taxon	G+C content (mol%)	Fatty acid types[a]	Major menaquinone(s)	Polar lipids[b]
Agrococcus	74	S, A, I	MK-12, MK-11	PG, DPG
Agromyces	71–76	S, A, I	MK-11, MK-12	DPG, PG, GL
Clavibacter	67–78	S, A, I	MK-10, MK-9	DPG, PG, GL
Rathayibacter	63–72	S, A, I	MK-10	PG, DPG, PL

[a] S, straight-chain saturated; A, anteiso-methyl branched; I, iso-methyl branched.

[b] DPG, diphosphatidylglycerol; PG, phosphatidylglycerol; GL, glycolipid; PL, unknown phospholipid (glycosyldiacylglycerol).

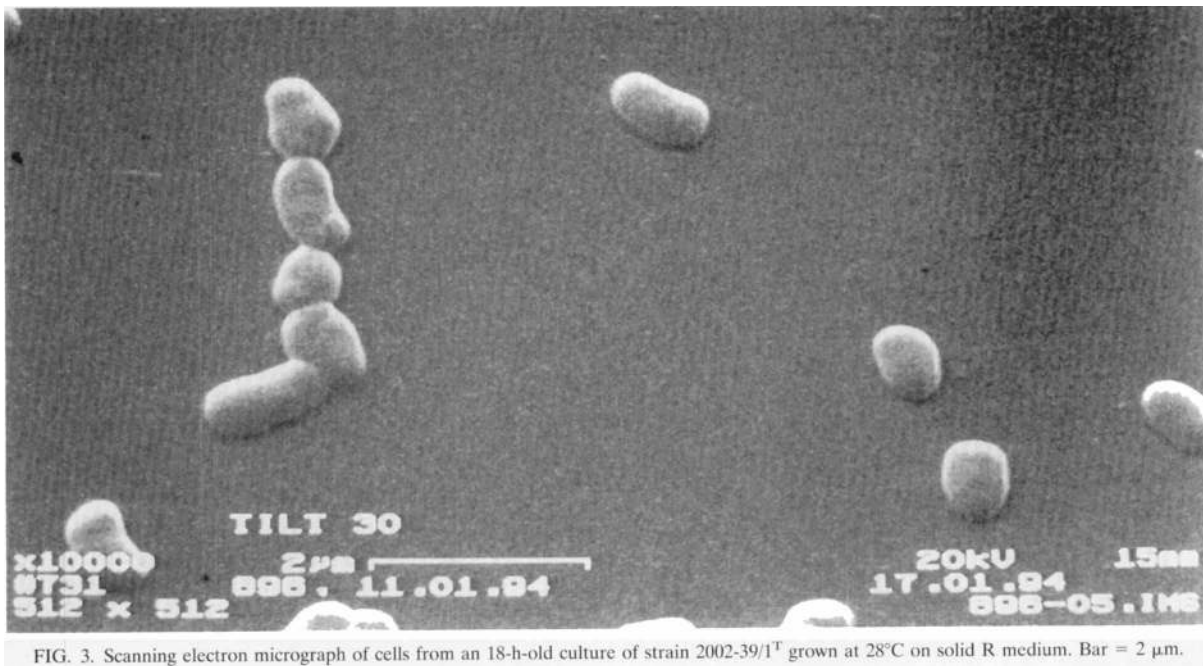

FIG. 3. Scanning electron micrograph of cells from an 18-h-old culture of strain 2002-39/1^T grown at 28°C on solid R medium. Bar = 2 μm.

as uniformly round, slightly convex, smooth colonies that were mainly orange and 2 to 4 mm in diameter (Fig. 1). Each culture consisted of small, irregular, coccoid to short rodlike cells. The cells multiplied without any mycelial phase. They occurred as single cells, in pairs, in short flexible chains of about three to six cells, and in small clusters (Fig. 2 and 3). The cells were 0.7 to 1.0 by 0.7 to 1.7 μm. Sometimes larger cells (length, about 2 to 2.6 μm) appeared in the population. Strain 2002-39/1^T grew well on complex organic media at 28°C. At 37°C growth was variable and depended on the culture medium and the physiological state of the cells used as the inoculum. No growth occurred at 50°C. The maximum concentration of NaCl tolerated was 8%. Prolonged storage of strains 2002-39/1^T and ST54 at 4°C should be avoided, and maintenance in liquid nitrogen is recommended.

Physiological characteristics. The physiological properties of isolates 2002-39/1^T and ST54, as well as the type strains of *Agromyces* species, *Clavibacter michiganense*, and *Rathayibacter rathayi*, are summarized in Table 2.

Stain reactions. Gram staining of the cells gave variable results. The coccoid cells were gram positive when ethanol was used for decolorization. When commercially available solutions (bioMerieux) containing ethanol and acetone (50:50) were used, the coccoid cells appeared to be gram negative. The cells were not acid fast as determined by Ziehl-Neelsen staining (11).

Chemotaxonomic characteristics. The chemotaxonomic characteristics that differentiated strains 2002-39/1^T and ST54 from the genera *Agromyces*, *Clavibacter*, and *Rathayibacter* are summarized in Table 3. The fatty acid compositions of strains 2002-39/1^T and ST54 are shown in Table 4. The cell wall Ala-Gly-Thr-DAB-Asp-Glu ratios for strains 2002-39/1^T and ST54 were 3.0:2.0:1.0:0.9:1.0:1.0 and 3.1:2.0:0.8:0.7:1.3:1.0, respectively. The acyl type was acetyl. The main components of the whole-cell sugars were glucose and rhamnose; in addition, minor amounts of ribose, mannose, and galactose were present. One component of the sugar pattern could not be identified. The phospholipids were phosphatidylglycerol, diphosphatidylglycerol, two unknown glycolipids, and one unknown phospholipid. The menaquinones were MK-12, MK-11, MK-10, MK-13 (peak area ratio, 49:37:7:5) in strain 2002-39/1^T and MK-12, MK-11, MK-13, MK-10 (peak area ratio, 41:41:13:4) in strain ST54. No mycolic acids were present. The G+C content of strain 2002-39/1^T DNA was 74 mol%.

Phylogenetic analysis. We determined the almost complete sequence of strain 2002-39/1^T, as was a partial sequence (~1,000 nucleotides) of strain ST54. The partial sequence of strain ST54 was identical to the comparable region of the strain 2002-39/1^T sequence. The phylogenetic dendrogram in Fig. 4 shows that strain 2002-39/1^T belongs to the radiation of actinomycetes that contain group B peptidoglycans. We found that strain 2002-39/1^T is a member of this phylogenetic group and represents a distinct lineage. It forms a branch at the base of the *Microbacterium-Aureobacterium* cluster. We found that as determined by 16S rDNA similarity values (Table 5) strain

TABLE 4. Fatty acid compositions of *Agrococcus jenensis* 2002-39/1^T and ST54

Strain	Fatty acid composition (%)[a]												
	i-$C_{14:0}$	$C_{14:0}$	i-$C_{15:0}$	i-$C_{15:1}$	ai-$C_{15:0}$	ai-$C_{15:1}$	$C_{15:0}$	i-$C_{16:0}$	$C_{16:0}$	i-$C_{17:0}$	ai-$C_{17:0}$	$C_{18:0}$	$C_{18:1}$
2002-39/1^T	0.6	0.3	12.2	1.9	57.8			12.6	2.0	1.9	9.3	0.1	0.1
ST54			11.9	0.7	56.5	5.3	0.1	11.8	1.0	2.0	9.9		

[a] The abbreviations for fatty acids are illustrated by the following examples: $C_{16:0}$, hexadecanoic acid; $C_{18:1}$, octadecenoic acid; i-$C_{15:0}$, 13-methyltetradecanoic acid; ai-$C_{15:0}$, 12-methyltetradecanoic acid.

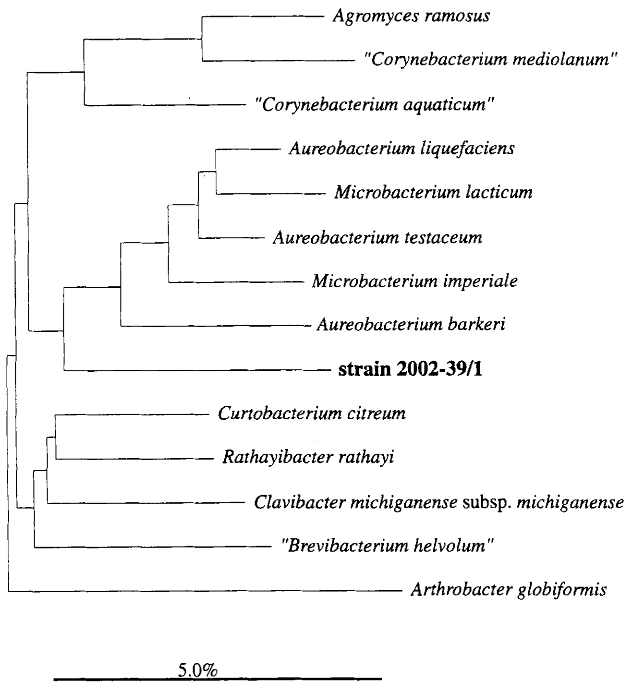

FIG. 4. Phylogenetic dendrogram based on the results of a 16S rDNA sequence comparison. Bar = 5 nucleotide substitutions per 100 nucleotides.

2002-39/1^T is equidistant from all lines made up of group B peptidoglycan-containing strains.

DISCUSSION

The occurrence of gram-positive, branched, filamentous bacteria as a predominant part of the soil microflora was first described by Gledhill and Casida in 1969 (6). As the colony morphology and cell morphology of strains belonging to the genus *Agromyces* were found to change depending on the culture conditions and culture age, chemotaxonomic markers, such as the presence of DAB in the peptidoglycan and the presence of type MK-12 menaquinones, are more reliable for identifying new strains at the genus level. Despite the absence of a mycelial growth phase, strain 2002-39/1^T was initially assigned to the genus *Agromyces* because DAB was present in the cell wall and type MK-12 menaquinones were detected. When strain 2002-39/1^T cells were compared with the cells of some other recently isolated *Agromyces* strains, the coccoid or short rod-shaped cells of strain 2002-39/1^T were similar in size and shape to the coccoid fragments that appeared in old cultures of *Agromyces* strains. Attempts to induce a mycelial phase in the growth cycle by changing the culture conditions failed. Strain 2002-39/1^T multiplied only in the coccoid form, and no rough and wrinkled colonies were detected on any of the solid media tested. Therefore, we concluded that a mycelial growth phase does not occur in this strain.

A comparison of the 16S rDNA sequence data obtained for strain 2002-39/1^T with the sequence data published previously by Rainey et al. (18) and Takeuchi and Yokota (22) confirmed that strain 2002-39/1^T and *Agromyces* species are not related and showed that strain 2002-39/1^T represents a new lineage within the actinomycetes that contain group B peptidoglycans.

When we compared the amino acids in the peptidoglycan of strain 2002-39/1^T with the currently described cell wall types listed in the German Collection of Microorganisms and Cell Cultures catalog of strains (4a), we found that the peptidoglycan of strain 2002-39/1^T has a structure that has not been described previously. The same structure was detected in strain ST54, which had an identical 16S rDNA sequence in the 1,000 nucleotides compared. Strain ST54 was considered a member of the same species as strain 2002-39/1^T on the basis of the morphological, physiological, chemical, and genomic characteristics which we determined. On the basis of the characteristics described above and in view of the uniqueness of

TABLE 5. Levels of 16S rDNA sequence similarity between *Agrococcus jenensis* 2002-39/1^T and related strains

Strain	% 16S rDNA similarity												
	Agrococcus jenensis 2002-39/1^T	*Agromyces ramosus* DSM 43045	"*Corynebacterium mediolanum*" DSM 20152	"*Corynebacterium aquaticum*" DSM 20146	*Microbacterium lacticum* DSM 20427	*Microbacterium imperiale* DSM 20530	*Aureobacterium liquifaciens* DSM 20638	*Aureobacterium testaceum* DSM 20166	*Aureobacterium barkeri* DSM 20145	*Clavibacter michiganense* subsp. *michiganense* DSM 46364	*Rathayibacter rathayi* DSM 7485	*Curtobacterium citreum* DSM 20528	"*Brevibacterium helvolum*" DSM 20419
Agromyces ramosus DSM 43045	93.2												
"*Corynebacterium mediolanum*" DSM 20152	92.3	96.6											
"*Corynebacterium aquaticum*" DSM 20146	95.2	95.2	94.8										
Microbacterium lacticum DSM 20427	95.1	92.7	92.5	95.4									
Microbacterium imperiale DSM 20530	93.8	92.2	91.9	94.1	97.0								
Aureobacterium liquefaciens DSM 20638	94.1	93.3	93.4	95.0	97.8	96.6							
Aureobacterium testaceum DSM 20166	94.1	93.8	93.4	94.6	97.3	96.9	98.3						
Aureobacterium barkeri DSM 20145	92.8	92.3	92.4	93.0	94.3	96.2	95.4	96.1					
Clavibacter michiganense subsp. *michiganense* DSM 46364	93.8	93.9	93.2	94.3	93.0	94.2	93.9	93.8	94.1				
Rathayibacter rathayi DSM 7485	94.0	94.8	93.9	94.7	93.5	94.6	93.6	94.2	94.6	95.9			
Curtobacterium citreum DSM 20528	93.5	94.1	93.7	94.1	93.3	94.3	94.1	94.4	95.3	95.6	96.2		
"*Brevibacterium helvolum*" DSM 20419	93.0	93.2	92.7	93.8	92.6	93.8	93.8	94.4	94.5	94.9	94.7	95.2	
Arthrobacter globiformis DSM 20124	90.6	91.9	91.8	91.3	90.8	91.4	91.7	92.0	91.6	92.4	92.1	93.2	92.2

many of these characteristics, we propose that strains 2002-39/1^T and ST54 represent a new genus in the order *Actinomycetales.*

Description of *Agrococcus* gen. nov. *Agrococcus* (Ag.ro.coc'cus. Gr. n. *agros*, field or soil; Gr. n. *coccos*, a grain; M.L. masc. n. *Agrococcus*, a coccus from soil). Cells are irregular, spherical or ovoid to short rods. The cells are 0.7 to 1.0 by 0.7 to 1.7 μm. The cells occur singly, in pairs, in short flexible chains, or in small irregular clusters. They are gram positive, not acid fast, and nonmotile. Endospores are not formed. Colonies are circular, slightly convex, and smooth. Colony diameters range from 2 to 4 mm. The color of the colonies varies between white and orange depending on the culture conditions. Aerobic or microaerophilic. Catalase is produced. Oxidase negative. The cell wall diamino acid is DAB. The acyl type is acetyl. Glucose and rhamnose are the main components of the whole-cell sugars. The phospholipids are phosphatidylglycerol, diphosphatidylglycerol, two unknown glycolipids, and one unknown phospholipid. The main menaquinones are MK-12 and MK-11. No mycolic acids are present. The type species is *Agrococcus jenensis.*

Description of *Agrococcus jenensis* sp. nov. *Agrococcus jenensis* (je.nen'sis. M.L. adj. *jenensis*, referring to the Thuringian town Jena, where the organism was isolated). Cells are irregular, spherical or ovoid to short rods. The cells are 0.7 to 1.0 by 0.7 to 1.7 μm. A few cells may be up to 2.6 μm long. The cells occur singly, in pairs, in short flexible chains of about three to six cells, or in small irregular clusters. Colonies are circular, slightly convex, and smooth. Colony diameters range from 2 to 4 mm. The color of the colonies varies depending on the culture conditions and illumination from whitish to yellow to intense orange. Nitrate is not reduced to nitrite; hydrogen sulfide is produced. Starch and esculin are hydrolyzed. Hydrolysis of casein is variable. Acids are produced only from fructose, glycerol, inulin, mannitol, L-rhamnose, and D-glucitol. Malate and succinate are utilized as carbon sources. Acetate, aconitate, benzoate, citrate, formate, and tartrate are not utilized. Urea, xanthine, hypoxanthine, gelatin, adenine, tyrosine, and Tween 80 are not decomposed or hydrolyzed. Cells are susceptible to ampicillin, chloramphenicol, ciprofloxacin, erythromycin, gentamicin, kanamycin, lincomycin, neomycin, nitrofuran, oxacillin, oxytetracycline, penicillin G, polymyxin B, rifampin, and streptomycin. Cells are resistant to sulfonamide. The G+C content is 74 mol%. The habitat is soil and sandstone surfaces. The type strain is strain 2002-39/1, which has been deposited in the German Collection of Microorganisms and Cell Cultures as strain DSM 9580.

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