Dietzia, a New Genus Including *Dietzia maris* comb. nov., Formerly *Rhodococcus maris*

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Sequencing of the 16S ribosomal DNAs (rDNA) of two strains of *Rhodococcus maris* was performed to determine the relationship of this species to other mycolic acid-containing actinomycetes. For this purpose we also determined the 16S rDNA sequences for the type species of the genus *Rhodococcus*, *Rhodococcus rhodochrous*, and for *Mycobacterium chlorophenolicum* (formerly *Rhodococcus chlorophenolicus*), *Rhodococcus erythropolis*, *Gordona bronchialis*, and *Gordona terrae*, for which only partial sequence data have been available previously. The sequences of the two strains of *R. maris* were identical. The results of a distance matrix analysis indicated that *R. maris* is not a member of the genus *Rhodococcus* but is located between members of the genus *Corynebacterium* and members of the *Rhodococcus-Nocardia-Mycobacterium-Gordona-Tsukamurella* cluster. The finding that *R. maris* is phylogenetically isolated is supported by the presence of *N*-acetyl residues in the glycan moiety of the peptidoglycan and the lack of phosphatidylinositol and phosphatidylinositol mannosides, characteristics which distinguish this taxon from related taxa. On the basis of our results and previous findings, we propose that *R. maris* should be reclassified in a new genus, *Dietzia*. The type species is *Dietzia maris* comb. nov.

Rhodococcus maris was originally known as “*Flavobacterium maris*” (9) but later was classified as a species of the genus *Rhodococcus* (17). The reason for this transfer was the presence of morphological and chemotaxonomic characteristics of the genus *Rhodococcus*, including gram-positive cells, lack of an aerial mycelium, cell wall chemotype IV, mycolic acids of the *Rhodococcus erythropolis* type, MK-8(H₂) as the major isoprenolog, fatty acids that included straight-chain saturated and monounsaturated fatty acids and tuberculostearic acid, and a DNA G+C content of 73.2 mol%. Differentiation from other *Rhodococcus* species was based on the results of physiological reactions. In this study we found that on the basis of additional chemotaxonomic data and phylogenetic evidence, *R. maris* cannot be considered an authentic member of the genus *Rhodococcus*.

MATERIALS AND METHODS

Bacterial strains and cultivation. *R. maris* DSM 43672^T (T = type strain) and DSM 46102, *R. erythropolis* DSM 43066^T, *Rhodococcus rhodochrous* DSM 43241^T, *Gordona bronchialis* DSM 43247^T, *Gordona terrae* DSM 43249^T, and *Mycobacterium chlorophenolicum* (formerly *Rhodococcus chlorophenolicus*) DSM 43826^T were grown on TSB agar (3% [wt/vol] Trypticase soy broth [BBL], 1.5% [wt/vol] Bacto Agar [Difco]) for 4 days at 28°C.

16S rDNA sequencing. Genomic DNAs were extracted from two strains of *R. maris*, *R. rhodochrous*, *R. erythropolis*, *M. chlorophenolicum*, *Corynebacterium glutamicum*, *G. bronchialis*, and *G. terrae* and the 16S ribosomal DNAs (rDNAs) were amplified as described previously (20). PCR products were sequenced directly by using a Taq DyeDeoxy Terminator Cycle sequencing kit (Applied Biosystems) according to the manufacturer's instructions. The sequence reaction products were electrophoresed by using an Applied Biosystems model 373A DNA sequencer. Bootstrap values, which were based on an analysis of 1,000 trees of 860 polymorphic sites, were calculated by using the programs NJFIND and NJBOOT.

Our sequences were manually aligned with previously published sequences for representatives of the main actinomycete sublines of descent included in the Ribosome Database Project (15) and for the organisms which we studied.

Pairwise evolutionary distances were computed by using the correction of Jukes and Cantor (12). Phylogenetic analyses were carried out by using the algorithm of DeSoete (5).

Determination of the acyl type of murein. The acyl type of murein of *R. maris* was determined by a modification of the colorimetric method of Uchida and Aida (21). In contrast to the original procedure, our whole-cell hydrolysate was neutralized by passing it through an ion-exchange column (Analytichem Bond Elut SCX; Varian).

Lipid analysis. Free lipids were extracted from dry *R. maris* cell material (ca. 50 mg) as described by Minnikin et al. (16) and were analyzed by two-dimensional thin-layer chromatography, using the method of Collins et al. (3). To analyze whole-cell fatty acids, fatty acid methyl esters were prepared from wet cells (40 to 70 mg) as described previously (14). A 0.3-ml portion of the extract was passed through a silica gel column (Analytichem Bond Elut; Varian) to trap the mycolic acids, and the resulting preparation was then used for a gas chromatographic analysis of the fatty acid methyl esters. Trimethylsilylated derivatives of the free mycolic acids were prepared by mixing another aliquot of the extract with 0.1 ml of a solution containing *n*-methyl-*n*-(trimethylsilyl)-heptafluorobutyramide and trimethylchlorosilane (10:1, vol/vol; Macherey & Nagel, Düren, Germany).

The mixtures of fatty acid methyl esters and trimethylsilylated derivatives of the free mycolic acids were analyzed by capillary gas chromatography, using a model 5898A Microbial Identification System apparatus (Microbial ID, Newark, Del.). For the fatty acid methyl ester analysis standard Microbial Identification System conditions were used. The trimethylsilylated derivatives of the mycolic acids were analyzed by high-temperature gas chromatography with a model HP 5790A gas chromatograph (Hewlett Packard) equipped with a flame ionization detector and a 12-m type HT5 column (part no. 051385; SGE, Victoria, Australia), using H₂ as the carrier gas at a flow rate of 30 ml/min. The oven temperature was increased from 210 to 400°C at a rate of 10°C/min, and the preparation was kept at the final temperature for 7 min. Peaks of the derivatives were identified by comparing their retention times with the retention times of standards and by a gas chromatography-mass spectrometry analysis performed with a KRATOS MS50 spectrometer (ion source temperature, 200°C; ionization energy, 70 eV).

Nucleotide sequence accession numbers. The 16S rDNA nucleotide sequences which we determined have been deposited in the EMBL database under accession numbers X79286 through X79292.

RESULTS AND DISCUSSION

The almost complete 16S rDNA sequences of *R. maris* DSM 43672^T and DSM 46102, consisting of 1,475 and 1,473 nucleotides, respectively, were compared with the sequences of three other *Rhodococcus* species, three *Corynebacterium* species, two *Nocardia* species, two *Gordona* species and *Tsukamurella paurometabolum*, as well as 10 additional species of

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FIG. 1. Phylogenetic tree based on 16S rRNA gene sequences for *R. maris* (described in this paper as *D. maris*), members of other mycolic acid-containing genera, and other actinomycetes. The numbers are the estimated confidence levels, expressed as percentages, for the positions of the branches, as determined by a bootstrap analysis. Bar = 10% difference between sequences, as determined by measuring the lengths of the horizontal lines connecting two species.

actinomycetes that do not belong to the phylogenetically defined group of mycolic acid-containing taxa. In this study the members of each genus for which more than one species was analyzed formed an individual coherent cluster (Fig. 1). The transfer of *R. chlorophenolicus* to the genus *Mycobacterium* that was based on morphological and chemotaxonomic grounds (8) was supported by the results of the phylogenetic analysis (1). The species of each cluster exhibited 16S rDNA homology values of more than 94.9% (Table 1). Two species which, despite their generic assignments, did not group with other members of their genus were *R. rhodochrous* and *R. maris*. *R. rhodochrous*, the type species of the genus *Rhodococcus*, branched off slightly deeper than the other members of the genus *Rhodococcus* and the two *Nocardia* species. The corresponding 16S rDNA homology values ranged from 95.8 to 97.0%. However, the bootstrap value of 43 obtained for the five species examined was statistically insignificant, and the branching point of *R. rhodochrous* may change when more organisms belonging to these or other genera are included in phylogenetic analyses. The phylogenetic position of *R. maris*, on the other hand, can be regarded as statistically more significant (level of confidence, 80%). The two strains of this species examined, which had identical 16S rDNA sequences,

branched off after the *Corynebacterium* line of descent, but before the main radiation that includes members of the genera *Tsukamurella*, *Mycobacterium*, *Gordona*, and *Nocardia* and the authentic *Rhodococcus* species. The 16S rDNA homology values for *R. maris* ranged from 93.2 to 95.9%. The phylogenetic position of *R. maris* remained stable when the analysis was extended to include 15 *Rhodococcus* species, 10 *Nocardia* species, and four *Gordona* species (19). The uniqueness of the 16S rDNA of *R. maris* was expressed by the presence of a few signature nucleotides that were not shared by other organisms included in the analysis. These nucleotides were found at positions (*Escherichia coli* nomenclature [2]) 418 to 425 (U-A), 614 to 626 (U-G), 837 to 849 (G-C), and 987 to 1218 (A-U).

The distinct position of *R. maris* outside the radiation of the mycolic acid-containing bacteria indicates that this species merits genus status. In order to compare chemotaxonomic properties that are known to be of taxonomic value for members of this group, we determined the acyl type of murein, the fatty acid composition, the polar lipids, and the numbers of carbons in the parent mycolic acids for *R. maris* (Table 2). The fatty acid spectrum of *R. maris* is similar to the spectra found for all of the other mycolic acid-containing taxa except the genus *Corynebacterium*; the fatty acids include mainly straight-chain saturated types (6% 15:0, 33% 16:0, 6% 17:0 [percentages of the total fatty acids]) and monounsaturated types (13% 16:1, 15% 17:1, 18% 18:1) (6, 7). The polar lipids are phosphatidylethanolamine, phosphatidylglycerol, and diphosphatidylglycerol. Phosphatidylinositol and phosphatidylinositol mannosides are not present (Fig. 2), which distinguishes *R. maris* from all other related taxa. The presence of phosphatidylethanolamine distinguishes *R. maris* from *Corynebacterium* species. The ability to synthesize tuberculostearic acid (12% of the total fatty acids) is shared with members of the genera *Nocardia*, *Rhodococcus*, *Gordona*, and *Tsukamurella*. The presence of this fatty acid has been reported in only a few *Corynebacterium* species (10, 18). The numbers of carbon atoms in the mycolic acids fell in a relatively narrow range (34 to 38 carbon atoms; 14% C₃₄, 21% C₃₅, 35% C₃₆, 16% C₃₇, 9% C₃₈); these mycolic acids are shorter than those reported for the other members of the genus *Rhodococcus*, whose mycolic acids have 34 to 52 carbon atoms (4, 6). *R. maris*, as well as *Corynebacterium* species, contains the common *N*-acetylmuramic acid residue, while all other members of the related taxa contain the rare compound *N*-glycolylmuramic acid (7). On the basis of phylogenetic (Fig. 1) and chemotaxonomic evidence (Table 2), the description of a new genus for *R. maris* appears to be justified. Below the generic name *Dietzia* is proposed to accommodate this species. With the description of *Dietzia maris* and the recent exclusion of *R. chlorophenolicus* (now *M. chlorophenolicum* [1, 8]) and *Rhodococcus aichiensis* (now *Gordona aichiensis* [13]), the genus *Rhodococcus* no longer contains several formerly misclassified species, which should eventually facilitate unambiguous definition of this genus of ecological, biotechnological, and medical importance.

Description of *Dietzia* gen. nov. *Dietzia* (Diet'zi.a. M.L. dim. ending -ia; M.L. fem. n. *Dietzia*, honoring Alma Dietz, an American microbiologist). Gram-positive, non-spore-forming cocci that germinate into short rods. Aerobic and chemoor-ganotrophic. Catalase positive. Rod-shaped cells exhibit a morphogenetic cycle. The diagnostic amino acid of the peptidoglycan is meso-diaminopimelic acid (variation Aly); the glycan moiety of cell walls contains *N*-acetyl residues (*N*-acetylmuramic acid). The major cell wall sugars are arabinose and galactose. Short-chain mycolic acids are present (overall size, 34 to 38 carbon atoms). The long-chain cellular fatty acids are predominantly straight-chain saturated and monosat-

TABLE 1. 16S rDNA sequence similarity matrix for *R. maris*, reference organisms belonging to mycolic acid-containing genera, and other actinomycetes

Organism	% Similarity to:																							
	<i>Rhodococcus maris</i>	<i>Corynebacterium xerosis</i>	" <i>Corynebacterium polymorphus</i> "	<i>Corynebacterium glutamicum</i>	<i>Mycobacterium tuberculosis</i>	<i>Mycobacterium chlorophenolicum</i>	<i>Gordona bronchialis</i>	<i>Gordona terrae</i>	<i>Tsukamurella paurometabolum</i>	<i>Nocardia asteroides</i>	<i>Nocardia oitidiscavarium</i>	<i>Rhodococcus rhodochrous</i>	<i>Rhodococcus fascians</i>	<i>Rhodococcus erythreus</i>	<i>Amycolatopsis methanolica</i>	<i>Actinomyces neuii</i> subsp. <i>anitratus</i>	<i>Propionibacterium acnes</i>	<i>Sporichthya polymorpha</i>	<i>Arthrobacter globiformis</i>	<i>Bifidobacterium bifidum</i>	<i>Acidothermus cellulans</i>	<i>Streptomyces coelicolor</i>	<i>Dactylosporangium thailandense</i>	<i>Atopobium minutum</i>
<i>Rhodococcus maris</i>	100																							
<i>Corynebacterium xerosis</i>	94.1	100																						
" <i>Corynebacterium polymorphus</i> "	93.2	95.8	100																					
<i>Corynebacterium glutamicum</i>	92.3	94.9	94.7	100																				
<i>Mycobacterium tuberculosis</i>	93.2	93.8	92.2	91.2	100																			
<i>Mycobacterium chlorophenolicum</i>	95.0	93.7	92.1	91.8	97.0	100																		
<i>Gordona bronchialis</i>	93.6	93.1	92.1	91.4	93.9	95.0	100																	
<i>Gordona terrae</i>	93.8	92.8	91.9	91.1	94.0	95.0	98.7	100																
<i>Tsukamurella paurometabolum</i>	95.7	93.2	92.5	91.4	93.9	96.1	94.4	94.4	100															
<i>Nocardia asteroides</i>	94.4	92.4	92.4	91.1	94.5	96.0	95.1	94.9	96.0	100														
<i>Nocardia oitidiscavarium</i>	94.6	92.7	93.2	91.4	93.6	95.6	95.9	95.5	95.8	95.8	97.0	100												
<i>Rhodococcus rhodochrous</i>	95.4	93.8	93.5	92.4	94.0	95.9	95.6	95.8	95.8	95.8	95.7	95.9	100											
<i>Rhodococcus fascians</i>	94.5	92.9	92.9	92.1	93.6	94.7	94.4	94.7	94.9	95.7	95.9	95.8	100											
<i>Rhodococcus erythreus</i>	95.9	93.3	93.6	92.0	93.6	95.1	94.4	94.4	95.5	96.6	96.8	96.7	97.6	100										
<i>Amycolatopsis methanolica</i>	92.2	90.5	90.5	90.0	91.3	92.7	92.5	92.3	92.0	93.8	94.1	93.0	92.5	92.8	100									
<i>Actinomyces neuii</i> subsp. <i>anitratus</i>	87.7	88.4	87.7	87.5	87.1	87.7	86.8	86.6	87.5	87.1	86.9	87.1	86.8	86.8	88.2	100								
<i>Propionibacterium acnes</i>	89.2	88.7	88.9	89.0	89.3	90.2	90.3	90.0	89.6	89.8	89.9	90.3	89.7	90.0	88.9	86.6	100							
<i>Sporichthya polymorpha</i>	92.8	90.2	89.5	89.6	89.7	91.7	91.1	90.7	91.8	92.1	92.5	92.4	91.3	92.1	92.6	86.6	88.0	100						
<i>Arthrobacter globiformis</i>	92.6	91.6	91.4	91.5	90.1	92.0	91.6	91.2	92.0	91.5	92.0	92.9	91.2	91.8	90.9	88.8	89.9	91.9	100					
<i>Bifidobacterium bifidum</i>	85.9	86.4	86.7	86.8	85.3	85.9	85.9	85.9	85.6	85.6	86.4	87.1	85.3	85.7	86.3	84.0	85.4	86.2	87.5	100				
<i>Acidothermus cellulans</i>	90.6	89.1	88.9	88.5	89.6	91.1	90.6	90.1	90.7	91.6	91.7	91.1	90.7	91.1	91.1	84.4	87.1	92.3	90.1	84.7	100			
<i>Streptomyces coelicolor</i>	91.4	89.4	89.0	89.4	89.7	91.2	90.9	90.9	91.2	91.7	92.2	92.2	91.7	91.8	92.5	85.6	88.5	92.5	91.4	86.0	89.9	100		
<i>Dactylosporangium thailandense</i>	91.0	89.5	88.6	89.6	89.6	91.9	91.4	90.9	91.2	91.8	92.4	92.1	90.5	91.3	92.7	87.0	89.1	93.4	91.4	86.5	91.4	90.4	100	
<i>Atopobium minutum</i>	84.5	83.5	83.3	83.1	82.3	83.8	83.3	83.0	85.0	84.2	84.3	84.5	83.7	84.6	84.1	81.4	82.3	85.9	84.1	81.9	85.0	84.6	84.3	100

TABLE 2. Differential chemotaxonomic characteristics of the genus *Dietzia* and other mycolic acid-containing genera^a

Genus	Overall no. of carbon atoms in mycolic acids	Fatty acid type ^b	Presence of:			Menaquinone(s)	N-Glycolyl in glycan moiety of wall	Guanine-plus-cytosine content of DNA (mol%)
			Tuberculo-stearic acid	Phosphatidylethanolamine	Phosphatidylinositol and phosphatidylinositol mannosides			
<i>Dietzia</i>	34–38	1B	+	+	–	MK-8(H ₂)	–	73
<i>Corynebacterium</i>	22–36	1A ^c	–	– ^d	+	MK-8(H ₂), MK-9(H ₂)	–	51–67
<i>Gordona</i>	48–66	1B	+	+	+	MK-9(H ₂)	+	63–69
<i>Mycobacterium</i>	60–90	1B	+	+	+	MK-9(H ₂)	+	72–70
<i>Nocardia</i>	44–60	1B	+	+	+	MK-8(H ₄ , ω-cycl) ^e	+	64–72
<i>Rhodococcus</i>	34–64	1B	+	+	+	MK-8(H ₂)	+	63–73
<i>Tsukamurella</i>	64–78	1B	+	+	+	MK-9	+	67–68

^a Data from references 4, 7, and 19.^b Fatty acids types as defined in reference 14.^c Some *Corynebacterium* species have fatty acid type 1B (10, 18).^d Some *Corynebacterium* species contain phosphatidylethanolamine (10, 18).^e Data from reference 11.

urated fatty acids. Tuberculo-stearic acid is present. The major polar lipids are phosphatidylethanolamine, phosphatidylglycerol, and diphosphatidylglycerol. Phosphatidylinositol and phosphatidylinositol mannosides are absent. Menaquinone MK-8(H₂) is the major isoprenolog. The guanine-plus-cytosine content of the DNA is 73 mol% (as determined by the thermal denaturation method).

Phylogenetically, the genus is a member of the mycolic acid-containing group consisting of the genera *Corynebacterium*, *Mycobacterium*, *Nocardia*, *Rhodococcus*, *Tsukamurella*, and *Gordona*. The type species is *D. maris*. The genus *Dietzia* can be readily distinguished from other mycolic acid-containing taxa by the criteria shown in Table 2.

Description of *Dietzia maris* (Nesterenko, Nogina, Kasmova, Kvasnikov, and Batrakov 1982) comb. nov. *Dietzia maris* (mar'is. L. gen. n. *maris*, of the sea). The description below is based on data from references 6, 9, and 17. Coccoid cells germinate into short rods which exhibit snapping division

and produce V forms. Poor to moderate, butyrous, orange growth occurs on glycerol, nutrient, and wort agar media. Circular, raised, butyrous, glistening colonies with entire margins are formed on nutrient agar. Growth in nutrient broth is turbid.

Acetylmethylcarbinol, indole, methyl red, *p*-nitrophenoloxidase, and phosphatase tests are negative. Hydrogen sulfide is not produced.

Acid is produced from fructose, glycerol, and glucose but not from ribitol, arabinose, cellobiose, galactitol, galactose, inositol, lactose, maltose, mannitol, α-methyl-D-glucose, raffinose, rhamnose, salicin, glucitol, sorbose, sucrose, or xylose.

Growth occurs with C₆ to C₁₇, C₁₉, and C₂₃ *n*-alkanes but not with ethane or methane.

Isolated from soil and from skin and intestinal tracts of carp (*Cyprinus carpio*).

The guanine-plus-cytosine content of the DNA is 73 mol% (as determined by the thermal denaturation method).

The type strain is IMV 195 (= DSM 43672 = ATCC 35013 = JCM 6166).

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FIG. 2. Two-dimensional thin-layer chromatogram of phospholipids of *R. maris* DSM 43672^T. The chromatographic conditions were as follows. Silica Gel 60 thin-layer plates (10 by 10 cm) were spotted with 20 µl of a whole-cell lipid extract by using the procedure of Minnikin et al. (16). Chloroform-methanol-water (65:24:4, vol/vol/vol) was used in the first direction, and chloroform-acetic acid-methanol-water (80:15:12:4, vol/vol/vol/vol) was used in the second direction. The plate was sprayed with ninhydrin and then with molybdenum blue reagent to locate and differentiate the phospholipids. Abbreviations: PG, phosphatidylglycerol; DPG, diphosphatidylglycerol; PE, phosphatidylethanolamine.

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